The Functional Significance of Colour Polymorphism
in the Lacertid Lizard *Podarcis muralis*

Javier Ábalos

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Supervised by Enrique Font Bisier
and Guillem Pérez i de Lanuza

Doctoral Program in Biodiversity
and Evolutionary Biology

Valencia, January 2021

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VNIVERSITAT DE VALÈNCIA

Institut Cavanilles de Biodiversitat i Biología Evolutiva

Facultat de Ciències Biològiques

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Dr. Enrique Font Bisier, Catedrático de Zoología en el Instituto Cavanilles de Biodiversidad y Biología Evolutiva de la Universitat de València.

Dr. Guillem Pérez i de Lanuza, Investigador contratado Doctor del Instituto Cavanilles de Biodiversidad y Biología Evolutiva de la Universitat de València.

CERTIFICAN que el doctorando Javier Ábalos Álvarez ha realizado bajo nuestra dirección, y con el mayor aprovechamiento, el trabajo de investigación recogido en esta memoria, y que lleva por título "Functional significance of colour polymorphism in the European common wall lizard (*Podarcis muralis*)", para optar al grado de Doctor en Ciencias Biológicas. Y para que así conste, en cumplimiento de la legislación vigente, así lo certificamos en Valencia, a 11 de enero de 2021.

Firmado: Enrique Font Bisier

Enrique Font

Firmado: Guillem Pérez i de Lanuza

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Acknowledgments

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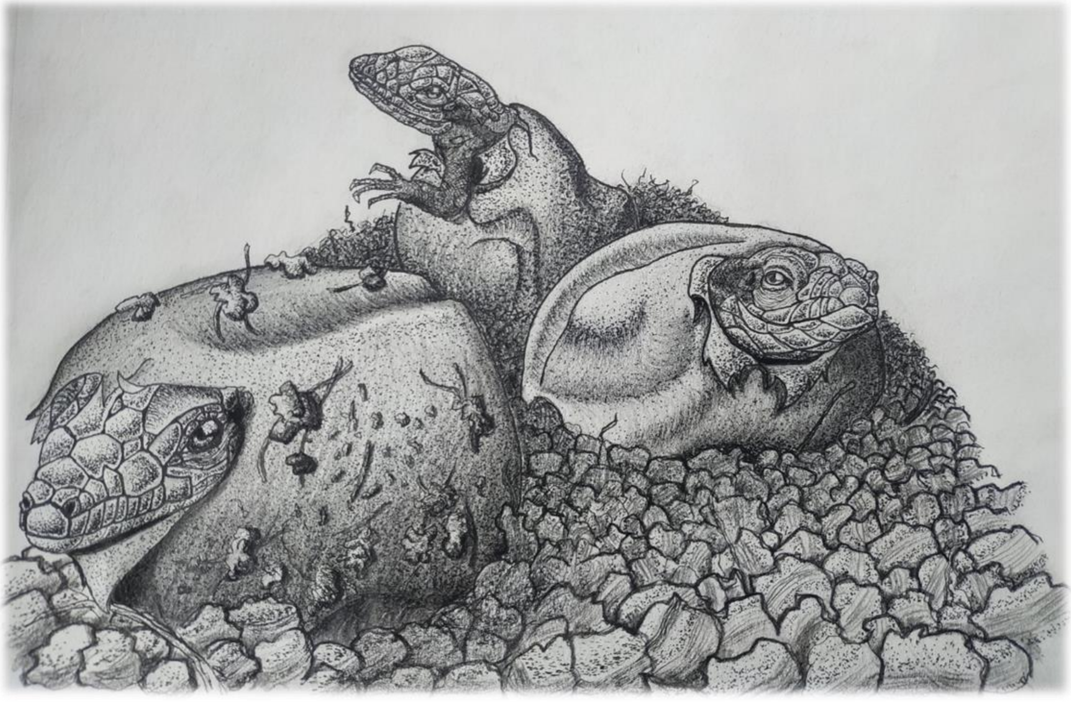

Abstract

Colour polymorphisms, the coexistence of two or more colour morphs of a species within a population, have long fascinated evolutionary biologists interested in the mechanisms generating and maintaining phenotypic variation in nature. The functional significance of colour polymorphisms (i.e. their adaptive value) is often linked to the selective mechanisms responsible for their maintenance over time. In lizards, the hypothesis that colour morphs may reflect alternative reproductive strategies involving differential sociosexual behaviour and/or alternative reproductive strategies has come to dominate the field. Wall lizards (family Lacertidae, genus *Podarcis*), with several geographically distant species that exhibit two or more alternative ventral colour morphs, have often been identified as a group in which alternative reproductive strategies and frequency-dependent selection likely underpin colour polymorphisms. However, several key aspects regarding the link between behaviour, fitness, and lacertid colour polymorphism remain overlooked or inadequately studied. In this thesis we tried to fill these gaps by experimentally addressing some of the most commonly assumed ideas about the functional significance of colour polymorphism in the European common wall lizard (*Podarcis muralis*, Laurenti 1768). In some populations of this species (e.g. eastern Pyrenees), adult lizards of both sexes may show up to three "pure" alternative colour morph (orange, white, yellow), and two mixed morphs consisting of a mosaic of differently-coloured scales (orange-white, yellow-orange). Although colour morphs are frequently thought to function as social signals mediating intraspecific interactions, morph categorization has been never assessed from the viewpoint of the intended receivers (i.e. conspecifics). Here, using a discrimination experiment and visual modelling, we found that lizards likely perceive the alternative colour morphs as chromatically distinct and show evidence of discriminating among them based on hue, rather than luminance. To evaluate the role of male coloration (including alternative colour morphs) in intra-sexual competition), we conducted lab-staged dyadic contests among size-matched males. We found lower fighting ability in lizards showing a smaller fraction of their outer ventral scales covered with dark melanin-based spots, and in lizards with orange ventral colour (which could result from the existence of a subordinate non-territorial strategy in this morph). However, our results in later studies (with a free-ranging population and ten experimental mesocosm enclosures), strongly refute the hypothesis that alternative socio-sexual behaviour or space use strategies characterize colour morphs when lizards interact under more natural conditions. In the free-ranging populations, colour morphs did not differ in inter-annual site-fidelity, home-range size, or male-female overlap. In the mesocosm enclosures, spatial dominance was the prime determinant of male fitness across colour morphs. In a later experiment, we conducted controlled matings among pure colour morphs and found no overall effect of female morph on clutch size or juvenile mass, and no effect of morph combination on offspring viability or prospective fitness. These results refute the existence of alternative reproductive strategies in female morphs and are also in disagreement with predictions from both correlational selection and heterosis. Lastly, by keeping the juveniles of known crosses in outdoor enclosures for a year, we studied the inheritance and ontogeny of *P. muralis* colour polymorphism. Specifically, our results confirmed that orange and yellow colour expression depends on two recessive alleles located at two separate autosomal loci and revealed that the whitish coloration exhibited by newborn lizards is likely perceived by conspecifics as a chromatically distinct colour different from any of the morph colours expressed by adult lizards. Overall, in this thesis we have hopefully presented compelling arguments to revise our perspective on the functional significance of lacertid colour polymorphisms, suggested promising lines of research for future work, and generally contributed to our understanding of the processes maintaining intra-specific variation in natural populations at large.

Chapter I

"I find the style incredibly bad…How I could have written so badly is quite inconceivable".

·Charles Darwin, Letter to John Murray (1858)·

"What you own is your own kingdom, what you do is your own glory,
What you love is your own power, what you live is your own story,
In your heart is the answer, let it guide you all along,
Let your heart be the anchor, and the beat of your own song"

·Rush, Something for Nothing, 2112 (1976)·

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Chapter I:

General introduction

1.1. Definition of colour polymorphism

Life on Earth takes an almost endless variety of forms. Although biologists tend to focus on differences between species or higher taxa, diversity within a single species, the main driver of adaptive evolution, is often striking. Numerous organisms show two or more discrete phenotypic forms (i.e. morphs) among individuals of the same sex and age coexisting within a single interbreeding population, with the rarer morph being too frequent to be solely the result of recurrent mutation (i.e. population polymorphism; Ford 1945; Huxley 1955). Often, this phenotypic variability takes the form of distinct colour morphs that are genetically-inherited, fixed in adults, and not condition-dependent (i.e. colour polymorphism; Galeotti et al. 2003; Roulin 2004; Mckinnon and Pierotti 2010). By this definition, colour polymorphism may coexist with sexual dimorphism or ontogenetic changes, as long as alternative morphs are found within the same age and sex classes. By contrast, when inter-individual differences in coloration are only seasonal, or show either environmental or condition-dependence, the species is not considered to be polymorphic (Roulin 2004). The production of such conditional, discontinuous morphs is referred to as polyphenism, instead of polymorphism (Nijhout 2003). Recent studies describing continuous variation between two or more extreme phenotypes in several polymorphic species has led to the relaxation of the condition that a polymorphic species must always show discrete morphs (Buckley 1987; Roulin 2004).

1.2. Colour polymorphism as a long-standing question in evolutionary biology

For several reasons, colour polymorphism remains an active field of research in evolutionary biology. Colour polymorphism is widely thought to provide the sort of raw material which facilitates sympatric speciation (Gray and McKinnon 2007). Yet, many phylogenetically distant taxa show heritable, life-long stable colour morphs which are maintained at different equilibrium levels over time (e.g. Rohwer and Paulson 1987; Johannesson and Ekendahl 2002; Maan and Cummings 2008; Olsson et al. 2013; Briolat et al. 2018). Selectively neutral polymorphisms can only prevail provided that the population is large enough so that alternative forms are unlikely to disappear through stochastic processes (i.e. genetic drift; Roulin 2004). In contrast, disruptive selection favouring extreme phenotypes of a normally-distributed population, coupled with assortative-mating, might lead to population polymorphism, but only as a transitional phase preluding speciation (Gray & McKinnon, 2007).

Hence, explaining the long-term maintenance of colour polymorphism in natural populations remains a major challenge in evolutionary biology, as it requires some form of selective balance which counteracts the eroding effects of either differential morph fitness or stochastic processes (Galeotti et al. 2003; Roulin 2004; Gray and McKinnon 2007).

1.3. Mechanisms for the maintenance of colour polymorphism

1.3.1 Alternative phenotypic optima (i.e. alternative strategies)

The functional significance of colour polymorphisms (i.e. their adaptive value) is often linked to the selective mechanisms responsible for their maintenance over time (Roulin 2004). Colour morphs often differ in features other than colour (Forsman et al. 2008; Mckinnon and Pierotti 2010). Such co-variations are thought to have originated via disruptive, correlational selection favouring optimal trait combinations representing alternative adaptive peaks (i.e. alternative strategies) (Sinervo and Svensson 2002; Shuster and Wade 2003; Gray and McKinnon 2007). Consider the classical example of the Peppered Moth (*Biston betularia*). During the early part of the XIX century, the darkening of tree trunks and barks caused by pollution conferred a cryptic advantage to dark-coloured moths which resulted in the increased frequency of this morph in detriment of the alternative light-coloured morph. Genetic correlations between colour morph and behaviour (i.e. background selection) would be expected to arise, as individuals that choose backgrounds matching their own colour survive, while those with the wrong preference perish (Kettlewell 1955; Majerus 1998, 2005 ; Sinervo and Svensson 2002; Cook et al. 2012). Such genetic correlations may arise from two non-exclusive mechanisms: linkage disequilibrium (i.e. the non-random association of alleles at two or more loci) and pleiotropy (i.e. which occurs when a single gene influences two or more traits; Sinervo and Svensson 2002; Mckinnon and Pierotti 2010). Discrete colour variation may thus represent either a non-adaptive correlate of selection on other traits (i.e. morphology, physiology, behaviour), or a central feature of the alternative strategies playing a role in a selectively relevant context (e.g. crypsis, mimicry, aposematism, sensory traps, thermoregulation, signalling; Roulin 2004; Roulin et al. 2004; Ducrest et al. 2008; Stevens 2013; Rojas et al. 2014; Twomey et al. 2014; White and Kemp 2015, 2016; Rönkä et al. 2020). Although alternative reproductive strategies (i.e. ARS) have received much attention in the literature, the advantages conferred by each alternative phenotype do not necessarily need to concern reproduction. In fact, a growing body of evidence is documenting the existence of alternative foraging, anti-predator, or physiological strategies in colour polymorphic organisms (Losey *et al.*, 1997; Roulin *et al.*, 2000; Johannesson & Ekendahl, 2002; Galeotti *et al.*, 2003; Calsbeek *et al.*, 2010).

In contrast with conditional strategies, the kind of alternative strategies that are usually linked with colour polymorphism show irreversible genetically-determined expression (i.e. adult lifetime patterns; Brockmann, 2002), which means that individuals showing a particular strategy usually do so for life and cannot change to another depending on context (i.e. they are not condition-dependent, facultative, or phenotypically plastic strategies; Brockmann, 2002; Zamudio & Sinervo, 2003; Roulin, 2004). Genetically fixed strategies are favoured when the environment varies in a coarse-grained manner (i.e. environmental changes are unpredictable and occur at a spatiotemporal scale that exceeds both the typical dispersal distance and life expectancy of the species) so that individuals tend to experience only one selective regime during their lifetime. Under these conditions, individuals will obtain higher fitness payoffs from specializing in alternative solutions to trade-offs in resource allocation (i.e. genetically-determined strategies) rather than being a plastic generalist (Gross 1996; Brockmann 2002; Shuster and Wade 2003; Zamudio and Sinervo 2003; Roulin 2004).

However, the existence of alternative phenotypes that are equally adaptive does not mean that the polymorphism is balanced and stable in time. In fact, the evolution of alternative strategies may give rise to both pre- and post-zygotic barriers among the different morphs (and eventually even lead to speciation) by penalising the production of offspring with sub-optimal trait combinations. Numerous examples in the literature attest how correlational selection (i.e. selection on optimal trait combinations) favours both the emergence of assortative mating and the rise of post-zygotic genetic incompatibilities between morphs (Sinervo and Svensson 2002; Gray and McKinnon 2007; Pryke and Griffith 2009*a*; Mckinnon and Pierotti 2010; Lancaster et al. 2014). Therefore, colour polymorphic strategies can persist over long timescales only if they show the same average fitness over time, which may occur under four main mechanisms of balancing selection: i) when the relative fitness obtained by each strategy is dependent on its frequency in the population (e.g. apostatic, or negative frequency-dependent selection; NFDS), ii) when the fitness of each strategy depends on the absolute number of individuals showing each morph (density-dependent selection), iii) when competitive interactions between the strategies are non-transitive (i.e. akin to a rock-paper-scissors game; RPS), and iv) when environmental heterogeneity causes selection on the different strategies to vary either in space and/or time (i.e. spatiotemporally varying selection). These mechanisms often act in conjunction with other processes (such as source-sink migratory dynamics, morph-biased mating preferences, or genetic incompatibilities), so that starkly different evolutionary scenarios seem to underlie the maintenance of colour polymorphism in different species (Losey et al. 1997; Galeotti et al. 2003; Zamudio and Sinervo 2003; Roulin 2004; Gray and McKinnon 2007; Roulin and Bize 2007; Gosden and Svensson 2009; Mckinnon and Pierotti 2010; Johnston et al. 2013; Wellenreuther et al. 2014; Svensson 2017).

Some representative examples may help clarify these evolutionary dynamics (**Fig. 1.1**). Alternative anti-predator strategies often involve polymorphic coloration. In the pea aphid (*Acyrthosiphon pisum*), green morph individuals suffer higher rates of parasitism by the parasitoid wasp *Aphidius ervi* than red morph conspecifics, whereas the more conspicuous red morph is more likely to be preyed on by ladybird beetles (*Coccinella septempunctata*). Both predatory species show coupled population dynamics with their host/prey, so that fields with high aphid abundance also show greater

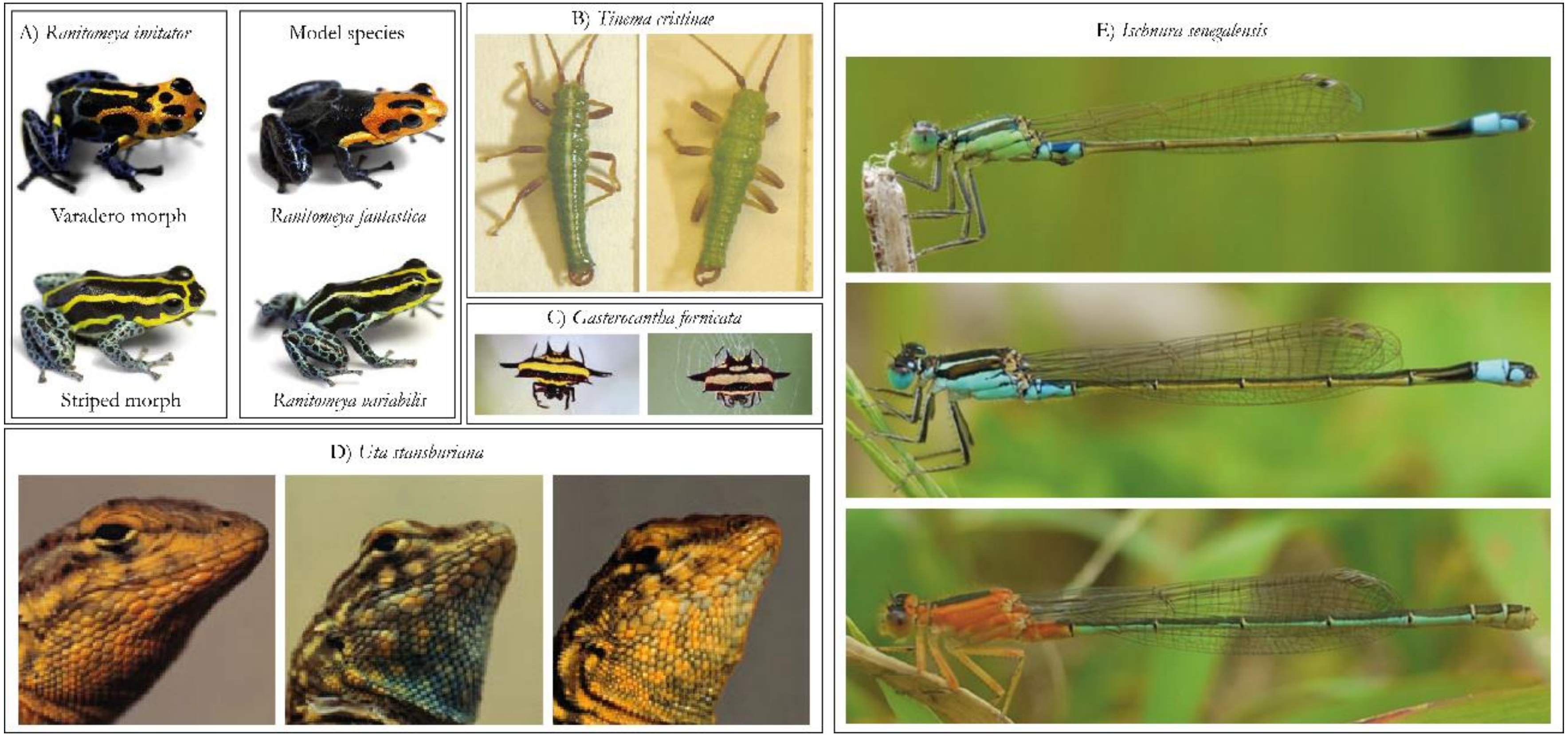


Figure 1.1. Representative examples of polymorphic species where colour morphs reflect alternative phenotypic optima (i.e. genetically-fixed strategies). A) In the mimic poison frog *(Ranitomeya imitator*), selection for Müllerian mimicry has led to the allopatric evolution of several mimetic morphs resembling different model species. B) In walking stick insects (*Timema cristinae*), striped and unstriped morphs are cryptic on alternative host plants. C) In the orb-web spider (*Gasterocantha fornicata*), yellow and white colour morphs may represent alternative colour lures tuned to maximize either chromatic or achromatic contrast and hence attract dypteran prey by exploiting distinct perceptual biases in the receiver. D) In the side-blotched lizard (*Uta stansburiana*), male colour morphs play a cyclical 'rock–paper–scissors' game with correlated mating strategies (i.e. aggressive orange males usurp the smaller territories of blue males, yellow sneakers circumvent orange territory-holders by cuckolding fertilizations, and blue morph lizards avoid losing fertilizations to yellow sneakers by guarding mates). This frequency-dependent male game (which typically lasts 5-6 years) is linked with the two-year cycle of two alternative breeding investment strategies (r/k) in female colour morphs, whose frequency fluctuates with population density. E) Many Odonate species (such as the common bluetail, *Ischnura senegalensis*) show a female-limited colour polymorphism where one morph resembles the conspecific male in body colour and other traits (i.e. andromorph) while one or more other morphs differ from the male (i.e. gynandromorphs). Male mating harassment causes apostatic selection against the most common morph via the formation of male searching images, leading to the long-term maintenance of colour polymorphism through both frequency- and density-dependent selection.

predator densities. Colour morphs can hence be viewed as alternative life-history strategies minimizing mortality from two exploitative species preferentially attacking alternate morphs, and the coexistence of the two morphs may derive from balanced density-dependent mortality from either the parasitoid or predator (Losey et al. 1997). In the cydno longwing butterfly (*Heliconius cydno*), selection for Müllerian mimicry with several co-models has favoured the evolution of alternative mimetic morphs, each mimicking a different unpalatable co-model (Kapan 2001). Morph extinction is prevented by the existence of multiple spatially-segregated co-models (i.e. mimicry rings), which results in colour morphs that can migrate between different patches of habitat where they experience positive frequency-dependent selection when matching the locally-abundant co-model (i.e. geographically varying selection; Joron and Iwasa 2005). Similar mimetic morphs have been observed in poison frogs and other aposematic species (**Fig. 1.1**; Twomey et al. 2014; Briolat et al. 2018). For instance, mimetic morphs inhabiting a narrow phenotypic transition zone in the Peruvian poison frog (*Ranitomeya imitator*) show both assortative mate choice preferences and neutral genetic divergence, suggesting that mimetic radiations can drive reproductive isolation and potentially, speciation (Twomey et al. 2014). Sensory ecology also seems to play a role in favouring the evolution of equally-adaptive polymorphic colorations. For instance, polymorphic warning colours in the dyeing poison frogs (*Dendrobates tinctorius*) may have evolved to maximise detectability by their putative vertebrate predators under varying lighting conditions (Rojas et al. 2014; Lawrence et al. 2019). Similarly, deceptive colour-based lures exploiting perceptual biases in the receiver to attract prey have often found to be polymorphic. This variability is thought to originate from colour morphs representing alternative colour lures either i) aimed at multiple prey receivers, ii) optimised for maximising efficacy at varying lighting conditions, or iii) exploiting different perceptual biases (e.g. chromatic vs. achromatic contrast) in a group of prey receivers (**Fig. 1.1**; White and Kemp 2015, 2016, 2017).

Sexual selection is often involved in the maintenance of colour polymorphism and it is perhaps in this context where the link between colour morphs and alternative strategies has received greater attention (Roulin 2004; Roulin and Bize 2007; Wellenreuther et al. 2014). Alternative reproductive strategies are particularly frequent in males of polygynous (or polygynandrous) species, which experience a high variance in mating success and, thus, intense sexual selection. In these species, the uneven distribution of fertilizations among males playing the conventional strategy promotes the evolution of alternative socio-sexual behaviours adapted to exploit distinct mating niches (e.g., monogynist, satellite, sneaker) which, in turn, may benefit from being signalled through its association with a specific colour morph (Brockmann 2001; Shuster and Wade 2003; Zamudio and Sinervo 2003; Taborsky et al. 2008; Taborsky and Brockmann 2010). One of the best-studied examples is the side-blotched lizard (*Uta stansburiana*). In this species, adult males present one of three alternative throat colours (i.e. orange, blue, yellow), which are associated with different socio-spatial behaviours. Orange-throated males establish large territories overlapping with several females by outcompeting blue-throated males in territorial disputes. These large territories make orange males vulnerable to losing fertilizations in favour of the non-territorial yellow morph, which uses female mimicry to sneak copulations opportunistically. In turn, blue-throated males compensate their competitive disadvantage by guarding females directly and hence securing more fertilizations against the yellow sneaker males (Sinervo and Lively 1996; Zamudio and Sinervo 2000, 2003; Alonzo and Sinervo 2001; Sinervo and Zamudio 2001; Calsbeek and Sinervo 2002*a*; Sinervo and Calsbeek 2006; Sinervo et al. 2006*b*, 2007). This dynamic gives rise to periodic oscillations in the relative frequencies of *U. stansburiana* male colour morphs, in a cyclical "rock–paper–scissors" (RPS) game whereby each colour morph (i.e. strategy), when predominant, is vulnerable to invasion by another morph (Sinervo and Lively 1996; Sinervo et al. 2006*b*, 2010). These frequency-dependent cycles (lasting 5-6 years), are linked with periodic two-year oscillations in female morphs, where orange and yellow throat coloration reflect two alternative breeding strategies differing in their trade-off between offspring quantity and quality (respectively), and whose fitness varies with population density (Sinervo et al. 2000*b*; Sinervo and Zamudio 2001; Calsbeek and Sinervo 2002*a*). Sexual conflict can also act as an agent of selection and promote the evolution of alternative strategies, for example when the benefits of mating with multiple partners outweighs its costs in one sex but not the other (Neff and Svensson 2013; Wellenreuther et al. 2014). This has been particularly well studied in Odonates (such as the blue-tailed damselfly, *Ischnura elegans*), where males often harass multiple females over mating and intense sperm competition has favoured prolonged copulation times (which can last for several hours) to secure female fertilisation (Cordero et al. 1998; Neff and Svensson 2013). This creates a marked sex-imbalance in the fitness returns of multiple matings as evidence from both laboratory experiments and natural populations suggests that male harassment decreases female reproductive success by limiting fecundity and food intake (Cordero et al. 1998; Gosden and Svensson 2009; Takahashi and Watanabe 2010; Takahashi et al. 2010; Neff and Svensson 2013). Interestingly, females of many Odonate species show sex-limited colour polymorphism where one morph resembles the conspecific male in body colour and other traits (i.e. androchromes) while one or more other morphs differ from the male (i.e. gynochromes). Androchromic females are approached by almost three times less males than gynochromes in field experiments, suggesting that male mimicry may lower detection rate and correct categorization as a sexually-mature female by males, thereby reducing costly male harassment (Svensson et al. 2009; Willink et al. 2019). An alternative (though non-exclusive) explanation is that males form a "search image" based on the most common morph at any time in a given population, which would experience higher levels of male mating harassment causing both their fitness and future frequencies to decline. Increasing male search costs in future generations would then make it more profitable for males to switch target female morph, leading to the long-term maintenance of colour polymorphism through both frequency- and density-dependent selection (Svensson and Abbott 2005; Svensson et al. 2005; Gosden and Svensson 2009; Takahashi and Watanabe 2010; Takahashi et al. 2010).

In conclusion, the long-term maintenance of genetic morph-specific strategies requires the build-up of disassortative mating preferences, gene flow and/or balancing selective regimes counteracting the rise of reproductive barriers between the alternative morphs (Sinervo and Svensson 2002; Roulin 2004; Roulin et al. 2004; Chunco et al. 2007; Gray and McKinnon 2007; Roulin and Bize 2007; Mckinnon and Pierotti 2010; Wellenreuther et al. 2014; Svensson 2017; Iversen et al. 2019).

1.3.2 Other mechanisms for the maintenance of colour polymorphism

At least three other mechanisms (apart from the existence of alternative equally-adaptive strategies) have been suggested to explain how colour polymorphisms can be maintained over long timescales: i) heterosis, ii) non-random mating, and iii) balanced source-sink dynamics (Galeotti et al. 2003; Roulin 2004; Wellenreuther et al. 2014).

a) Heterosis

Two or more alternative morphs may persist in sympatry over long timescales if the genetically intermediate (i.e. heterozygote) morph shows a fitness advantage, known as heterosis, overdominance or heterozygote advantage in the literature (Roulin 2004; Roulin and Bize 2007; Johnston et al. 2013; Wellenreuther et al. 2014). The fitness advantage of heterozygous individuals may result from being less inbred than homozygous ones and thus expressing less deleterious recessive traits, which allows them to cope with a wider range of stress-inducing factors (Roulin 2004). Testing this hypothesis in a polymorphic species requires some knowledge of the genetic architecture governing the inheritance and expression of colour morphs, as the fitter heterozygotes could be phenotypically indistinguishable from other genotypes. This was found to be the case in an island population of the Soay sheep *(Ovis aries*), where heterozygous individuals for two polymorphic traits (i.e. coat colour polymorphism and horn size) experience higher reproductive success and survival than their phenotypically identical homozygous counterparts (Gratten et al. 2008, 2010; Johnston et al. 2013). The existence of a heterozygote advantage may have important implications regarding mating behaviour. If heterozygosity at the genes coding for the colour polymorphism (or at genes linked to them) provides a fitness advantage, selection is likely to favour the evolution of disassortative mating preferences as a mean to avoid the production of sub-optimal homozygous progeny. However, if the heterozygote advantage concerns only viability selection (i.e. survival to adulthood), the colour polymorphism may be maintained even if the morphs show random or even maladaptative (i.e. assortative) mating preferences (Krüger et al. 2001; Roulin and Bize 2007).

b) Non-random mating

Sexual selection often contributes to the maintenance of colour polymorphisms through the establishment of non-random mating preferences with respect to morph (Roulin and Bize 2007; Wellenreuther et al. 2014). The attractiveness (i.e. sexual appeal to conspecifics) of individuals from each morph may vary either spatially or temporally, with immigration between habitat patches or seasonal fluctuations occurring with sufficient frequency so as to avoid the depletion of genetic variance in the population (Osawa and Nishida 1992). Alternatively, colour polymorphism may persist in time if individuals mate preferentially with conspecifics displaying an uncommon (or novel) morph (Farr 1977). Antagonic mate choice preferences (i.e. where males prefer to mate with females displaying a given morph whereas females prefer to mate with males showing another morph), can also result in stabilizing selection (Houtman and Falls 1994). Mate choice preferences may also vary among individuals if the expected benefits derived from mating with differently coloured individuals differ depending on the chooser's morph (e.g. genetic compatibility), or if colour polymorphism evolved in allopatry and a non-adaptive assortative mate choice preference is maintained when the morphs meet in secondary contact (Roulin 2004; Roulin and Bize 2007; Mckinnon & Pierotti 2010; Wellenreuther *et al.* 2014).

c) *Balanced source-sink dynamics*

When a morph is selected against in a given population, polymorphism may still be maintained through immigration of the counter-selected morph from neighbouring populations where it is not at a selective disadvantage (Roulin 2004; Chunco et al. 2007; Gray and McKinnon 2007). As exemplified above, mimetic radiations in which a species evolves several alternative morphs (each resembling a different suite of spatially-segregated and unpalatable models) provide a good example of this evolutionary mechanism. Predator generalization on prey search image should result in positive frequency-dependent selection and morph fixation yet, in numerous species, counter-selected morphs have been found to be replenished by recurrent immigration from other patches of habitat (Kapan 2001; Joron and Iwasa 2005; Gordon et al. 2015; Rojas et al. 2015; Rojas 2017; Rönkä et al. 2020).

1.4. Lizard colour polymorphisms

Among the many of species showing colour polymorphism, lizards have received particular attention (Sinervo and Svensson 2002; Zamudio and Sinervo 2003; Olsson et al. 2013; Svensson 2017; Stuart-Fox et al. 2020). Colour polymorphism seems to have evolved multiple times in distantly related families of lizards, providing an opportunity to study convergence and divergence in the design, genetic bases, ontogeny, and functional significance of such alternative colorations (**Fig. 1.2**). Although alternative dorsal or lateral colour patterns have been reported in many lizard species, polymorphic colour variation in lizards typically involves the throat, head, or ventral surface of the animals. Ventral colour polymorphism has been reported in at least seven different families of lizards, both geographically and phylogenetically distant (i.e. Agamidae, Dactyloidae, Lacertidae, Liolaemidae, Phrynosomatidae, Sphaerodactylidae, and Tropiduridae; reviewed in Stuart-Fox et al. 2020). What is more, there seems to be remarkable convergence regarding colour hue among these distantly related groups, with morph diversity in most polymorphic species typically combining three alternative colours: orange/red, yellow, and white/blue (**Fig. 1.2**). Polymorphic lizards may show anywhere from two to six different morphs, usually comprising up to three "pure" morphs showing uniform coloration, and up to three mixed morphs showing either a central coloured patch different from the surrounding colorations in some iguanians and agamids, throat stripes in some iguanians, or a mosaic of differently coloured scales in lacertids (Stuart-Fox et al. 2020). Despite this convergence, the available evidence on the biochemical and cellular basis of lizard colour polymorphism has revealed that equivalent colour morph hues are often produced through different mechanisms in each polymorphic species.

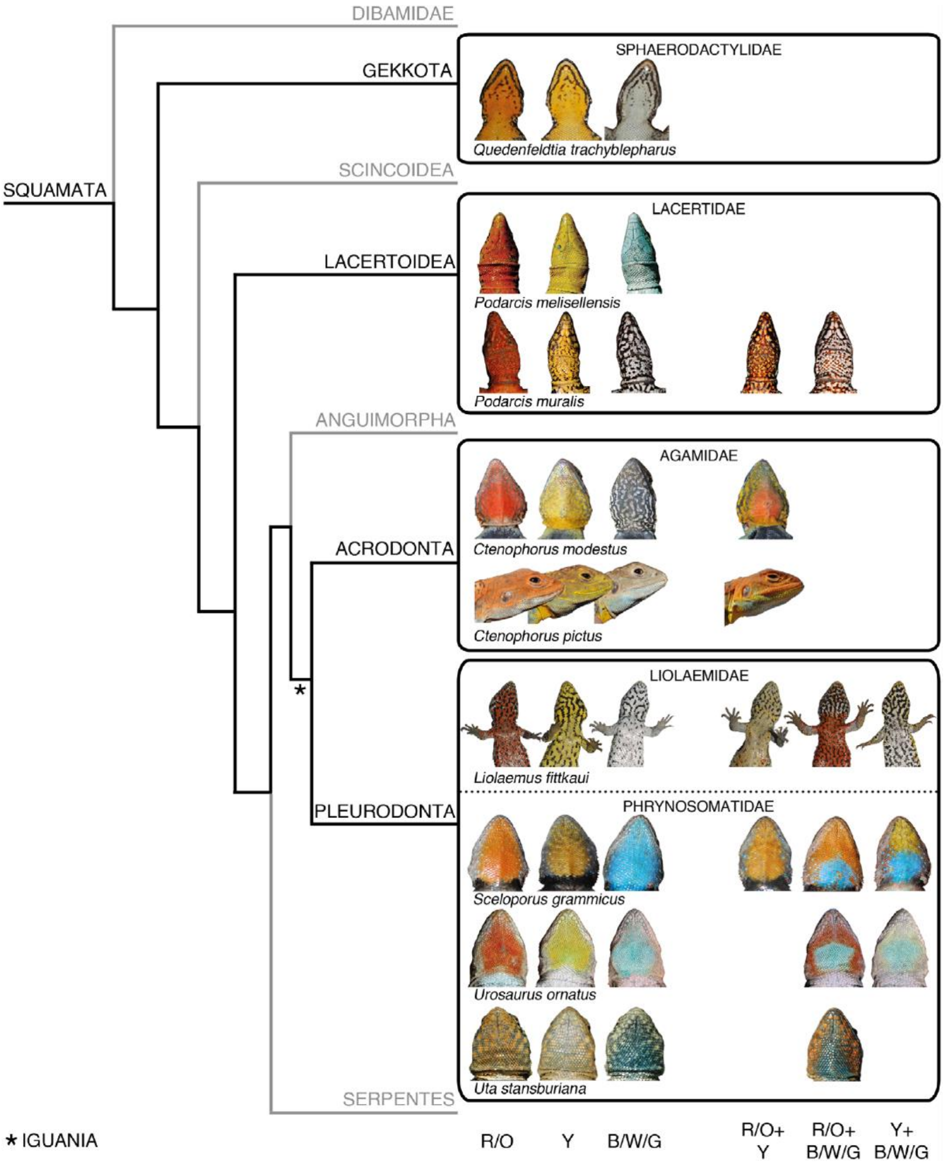


Figure 1.2. Convergence in colour morph hues across the order Squamata (lizards and snakes). Black branches contain species with known throat/head/ventral colour polymorphism while grey branches do not. Phylogenetic tree based on Pyron et al. (2013). R/O = red, orange; Y = yellow; B/W/G = blue, white, grey; R/O = red/orange. Reproduced from Stuart-Fox et al. (2020) with permission from the author.

1.4.1 Mechanisms for colour production in lizards

Lizard coloration depends on the interaction between light and pigment molecules or nanoscale structures present in the integument. Pigments and nanostructures reside inside specialized cells (i.e., chromatophores) located in the dermal layer of the skin. The xantophores are the most superficial chromatophores and contain pigments (i.e., pteridines and/or carotenoids) that absorb short-wavelength light and reflect (or transmit) long wavelengths. Iridophores contain intracellular guanine platelets that scatter the incident light (Bagnara et al. 2007; Haisten et al. 2015; Kuriyama et al. 2017, 2020). Melanophores occupy a basal position in the dermis (although may present dendritic processes extending around and above the overlying iridophores) and contain eumelanin that absorbs all light transmitted by the xantophores and the iridophores (Cooper and Greenberg 1992). Variation in the relative abundance and spatial arrangement of these cell types, as well as in the type and concentration of pigments within them, can produce the great array of skin colours found in lizards (Macedonia et al. 2000; Grether et al. 2004; Bagnara et al. 2007; Olsson et al. 2013; Saenko et al. 2013).

Long wavelength colours (red to yellow) result from two different classes of pigments in the xantophores: pteridines and carotenoids. Carotenoids are hydrophobic molecules that typically absorb visible light primarily in the 400–500 nm range (producing yellow to red colours), and that must be obtained directly from the diet, or metabolically converted from dietary precursors (Hill and McGraw 2006). Pteridines are hydrophilic compounds that primarily absorb light between 340 and 500 nm, can be synthesised within chromatophores from purine molecules, and may appear yellow (xanthopterin and sepiapterin), red (drosopterin or erythro- pterin), or colourless to the human eye (Kayser 1985; Grether et al. 2004). Despite red/orange and yellow morphs being present in most polymorphic lizards, equivalent colour morph hues from different species (or even populations of the same species; McLean et al. 2019) often diverge in their biochemical and cellular basis (Stuart-Fox et al. 2020). In the European common lizard (*Zootoca vivipara*), red to yellow hues are produced exclusively by carotenoids (i.e. orange coloration resulting from higher carotenoid concentrations), with changes in the iridophores also playing a fundamental role modulating the spectral properties of throat reflectance (Fitze et al. 2009; San-Jose et al. 2013). Iridophores influence orange and yellow colour production also in the eastern fence lizard (*Sceloporus undulatus erythrocheilus*), although in this species carotenoid vesicles are absent and long-wavelength colours result solely from pteridines (i.e. orange morph having higher drosopterin concentration; Morrison et al. 1995). In the side-blotched lizard (*Uta stansburiana*), the swift rock dragon (*Ctenophorus modestus*), and the European common wall lizard (*Podarcis muralis*), orange and yellow morphs result both from a combination of pteridines and carotenoids (as well as from the light-scattering properties of the underlying layer of iridophores), but differ in the relative proportions of each pigment family (Haisten et al. 2015; McLean et al. 2017; Andrade et al. 2019). In all three of these species, orange-coloured scales show a relatively high concentration of red/orange pteridines (e.g. riboflavin and drosopterin) while yellow-coloured scales have a relatively high concentration of dietary yellow carotenoids (e.g. zeaxhantin, lutein; Stuart-Fox et al. 2020). Short-wavelength colours (also involved in some lizard polymorphisms), are structural colours produced by light scattering in the iridophores, although these colours also depend on interactions with xanthophores and the underlying layer of melanophores (Bagnara et al. 2007; Saenko et al. 2013; Umbers 2013). Specifically, UV and blue colours are produced when xanthophores contain few or no pigments (allowing almost all wavelengths of the incident light to interact directly with the iridophores), and iridophores contain regularly-spaced guanine crystals producing thin-film interference (Bagnara et al. 2007; Olsson et al. 2013; Umbers 2013; Stuart-Fox et al. 2020). Additionally, variation in the density of underlying melanophores may also influence the saturation of UV and blue colours by absorbing any long-wavelength light transmitted by the upper layers of xanthophores and iridophores (Bagnara et al. 2007; Pérez i de Lanuza and Font 2010; Raia et al. 2010; Saenko et al. 2013; Umbers 2013).

1.4.2 The genetic architecture of lizard colour polymorphism

Polymorphisms are often expected to have a simple genetic basis. Since morph differences often comprise other traits besides coloration, the genetic basis of colour polymorphism is thought to involve either pleiotropic regulation, or tight physical linkage between genes associated with colour production and co-adapted traits (thus resulting in few loci of major phenotypic effects; Roulin 2004; Mckinnon and Pierotti 2010; Jamie and Meier 2020; Stuart-Fox et al. 2020). The rationale behind this idea is that polymorphisms are subject to high levels of gene flow among morphs and therefore, if polymorphisms were based on multiple unlinked genes, inter-morph mating would easily lead to mismatched sub-optimal recombinants (Sinervo and Svensson 2002*a*; Jamie and Meier 2020; see 1.3.1). To maintain adaptive gene combinations, inversions (or other recombination suppressors) are strongly favoured by selection as well as a dominance hierarchy preventing the expression of intermediate phenotypes. Likewise, due to the lower effective population size and enhanced genetic drift inherent to the sex chromosomes, genes coding for colour polymorphism and its co-adapted traits are expected to show autosomal inheritance patterns (Roulin 2004; Mckinnon and Pierotti 2010; Jamie and Meier 2020).

Although few studies have examined the genetic basis of lizard colour polymorphism, the available evidence suggests a rather simple genetic basis (Stuart-Fox et al. 2020). In *Uta stansburiana*, polymorphism seems to be governed by a single autosomal locus with three, codominant alleles (o, b and y). In males, homozygous lizards have uniform throat colours [oo (orange), bb (blue), or yy (yellow)] whereas heterozygotes show throats striped with two colours corresponding to their two different alleles (e.g. orange and yellow in oy lizards). Despite their codominant effects on colour expression, these alleles show a marked dominance hierarchy over male reproductive strategy (see 1.3.1), with the o allele (i.e. usurper) being dominant to the y allele (i.e. sneaker), which is dominant to the b allele (i.e. mate-guarder) (Sinervo 2001; Sinervo and Clobert 2003). A similar genetic basis (i.e. a single autosomal locus with three co-dominant alleles) was assumed to underpin colour polymorphism in the lacertid *Zootoca vivipara* (Sinervo et al. 2007) and could not be rejected in a coetaneous study of *Ctenophorus pictus* due to limited data (Olsson et al. 2007*b*). There are, however, strong reasons to believe that a few (rather than a single) loci govern morph expression in lizards. First, none of these studies considered alternative models of inheritance with more than one locus. Second, the patterns of geographic variation in *Uta stansburiana*, with some populations fixed for orange-blue throats, appear inconsistent with a single autosomal oby locus (Corl et al. 2010; Stuart-Fox et al. 2020). Third, a recent pedigree study on *Ctenophorus modestus* found that the best supported model was one with two bi-allelic loci showing complete dominance of the two alleles coding for orange and yellow coloration (Rankin et al. 2016). A genomic study with *Podarcis muralis* found that the presence of orange and yellow ventral coloration is determined by two recessive alleles at two separate loci (both of them autosomal) associated with the regulatory regions of pteridine [sepiapterin reductase (SPR)] and carotenoid [beta-carotene oxygenase 2 (BCO2)] metabolism (Andrade et al. 2019). Interestingly, alleles at those two loci are shared by at least six other *Podarcis* species exhibiting similar colour polymorphism, and haplotype divergence suggests that both ancestral genetic diversity and introgression by hybridisation underpin between-species variation at these loci (Andrade et al. 2019). Together, these studies confirm that discrete colour polymorphism in lizards is controlled by a few loci of major effects, as expected if correlational selection had favoured the evolution of alternative, morph-specific phenotypic optima.

1.4.3 The functional significance of lizard colour polymorphism

In lizards, the hypothesis that colour morphs may reflect underlying alternative reproductive strategies involving complex suites of morphological, behavioural, physiological, and life-history traits has come to dominate the field since Hover's (1985) pioneer work on the ornate tree lizard (*Urosaurus ornatus*). Morph composition varies greatly across this species' distribution. In some populations only blue-throated adults can be found, whereas in other populations male throat coloration may vary among solid blue, orange or yellow coloration, or two mixed morphs (orange or yellow background with a blue central patch; Carpenter 1995; Meyers et al. 2006). Hover's key observation was that males showing blue throat coloration (solid or in the form of a central patch) outcompeted orange- and yellow-throated males in dyadic territorial contests (Hover 1985). His results were replicated in later studies in which male throat coloration was experimentally altered with dye, and males showing a larger blue throat patch dominated males without blue dye, or with a smaller blue patch (Thompson and Moore 1991). Orange-throated males, which were initially characterized as always nomadic, have been later suggested to show a conditional strategy based on environmental conditions: they seem to maintain small undefended home-ranges (about the size of a female home-range) in humid years but are nomadic in drought years (Moore et al. 1998). Lastly, yellow-throated males have been found to preferentially settle in the vicinity of blue male territories and are therefore thought to play a fixed satellite strategy to sneak copulations (Waltz 1982; Lattanzio and Miles 2016). The level of specialisation required for each of these behavioural strategies is expected to result in further differences between morphs. In *Urosaurus ornatus*, for example, morphs have been found to vary in body size, diet, habitat use, and early-life hormonal (i.e. progesterone) levels (Moore et al. 1998; Lattanzio and Miles 2014, 2016; Paterson and Blouin-Demers 2018).

The most detailed description of a polymorphic lizard showing alternative reproductive strategies is arguably that of the iconic side-blotched lizard (*Uta stansburiana*). In a series of landmark studies, Barry Sinervo and colleagues outlined the functional significance of male colour polymorphism in this species as an easily-scored phenotypic correlate of three different male strategies playing a rock-paper-scissors game (**Fig. 1.3**.; Sinervo and Lively 1996). Briefly, populations dominated by ultra-aggressive orange males (which outcompete and usurp blue male territories) are easily invaded by yellow female-mimicking males which are, in turn, vulnerable to invasion from blue subordinate males playing a mate-guarding strategy (DeNardo and Sinervo 1994; Zamudio and Sinervo 2000; Sinervo and Zamudio 2001; Calsbeek and Sinervo 2002*b*, 2002*c*; Calsbeek et al. 2002; Sinervo and Calsbeek 2006). Apart from differential socio-sexual behaviour and space use, each strategy entails correlated physiological and life-history traits: orange males disperse further from their natal site and show higher levels of testosterone, activity and endurance than blue and yellow males, but also lower levels of inter-annual survival (Sinervo et al. 2000*a*, 2006*a*; Sinervo and Clobert 2003). Encouraged by these results, Zamudio and Sinervo (2003) noted that lizards share several key features in their behavioural ecology which could have favoured the evolution of fixed alternative reproductive strategies. For instance, many lizards occupy isolated patches of habitat where resources are unevenly distributed (promoting territoriality while hindering dispersal), are either short-lived or restricted in their activity to short breeding seasons, and show pronounced sex differences in morphology and behaviour (with males being markedly larger and more aggressive towards conspecifics; Calsbeek et al. 2002; Zamudio and Sinervo 2003; Baird 2013). In these species, intense male-male competition over resource monopolisation and adaptive site-fidelity give rise to mating systems where subordinate males are unlikely to experience more than one social environment during their lifetime (hence promoting the evolution of fixed reproductive strategies in this sex; Brockmann 2001; Shuster and Wade 2003; Taborsky and Brockmann 2010; Neff and Svensson 2013). These ideas ignited research on lizard colour polymorphism, with most studies seeking to detect evidence of multi-trait reproductive and life-history strategies in male colour morphs that could help to clarify the long-term maintenance of discrete colour variation. After nearly three decades of research on the topic there is now abundant evidence documenting the existence of morphological, physiological, behavioural and life-history differences among the colour morphs of many geographically and phylogenetically distant lizards (Huyghe et al. 2007, 2009*a*; Olsson et al. 2007*a*, 2009, 2013; Healey and Olsson 2008; Runemark and Svensson 2012; Bastiaans et al. 2013; San-Jose et al. 2014; Yewers et al. 2016, 2018; Yewers 2017; McDiarmid et al. 2017; Fernández et al. 2018).

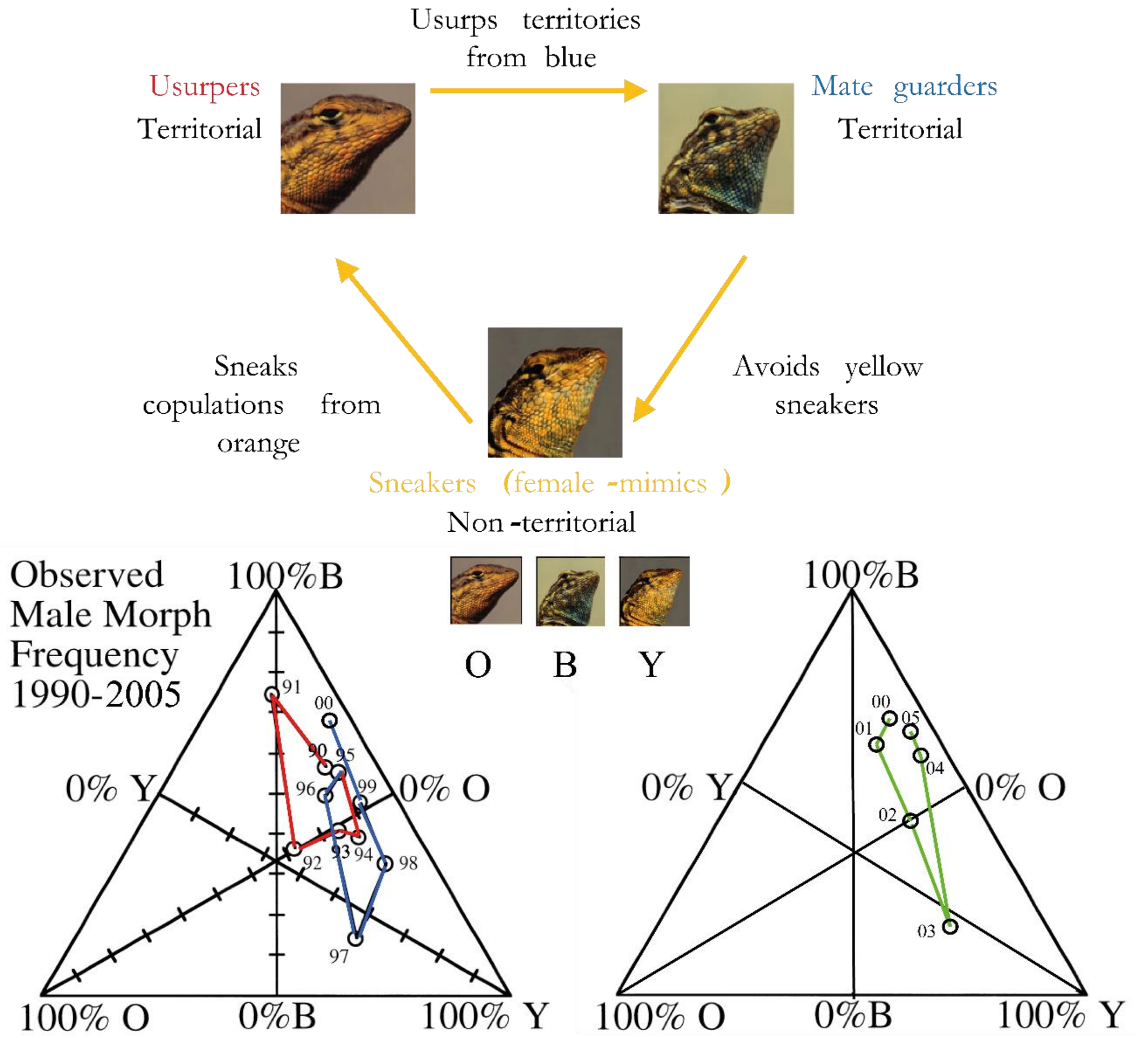


Figure 1.3. Colour polymorphism in *Uta stansburiana* is maintained over time through the association of male colour morphs with three alternative reproductive strategies showing intransitive fitness payoffs akin to a rock-paper-scissors (RPS) game. Due to the short lifespan of this species (1 year), this RPS dynamic gives rise to periodic 5-6 year-long cycles in the relative frequencies of male colour morphs (blue: 1990-1995; red: 1995-2000; green: 2000-2005). Ternary plots modified from Sinervo and Calsbeek (2006) and Sinervo et al. (2007, 2010).

For a number of reasons, morph-specific ARS, morph fluctuations, and rock-paper-scissors dynamics similar to those described in *Uta stansburiana* have been predicted to occur in Eurasian lacertids, particularly in wall lizards (genus *Podarcis*, family Lacertidae; Sinervo et al. 2007; Calsbeek et al. 2010; Mangiacotti et al. 2019). First, ventral colour polymorphisms involving three alternative colours (i.e. orange, white, and yellow) have been documented in adult individuals of at least 13 out of the 24 species currently recognized within the *Podarcis* genus, and are thus thought to have an ancestral origin in this group (Arnold et al. 2002; Galeotti et al. 2007; Huyghe et al. 2007; Sacchi et al. 2007; Runemark et al. 2010; Andrade et al. 2019; Pérez i de Lanuza et al. 2019; Brock et al. 2020*b*; Jamie and Meier 2020). Second, many of these species show high site-fidelity, low inter-annual survival, and occupy habitats where resources relevant to reproduction (e.g. stone-walls) are unevenly distributed (Strijbosch et al. 1980; Barbault and Mou 1988; Edsman 1990, 2001; Carretero 2007; Sinervo et al. 2007; Calsbeek et al. 2010; Font et al. 2012*a*). Third, males of many wall lizards experience strong intra-sexual competition, mainly in the contexts of territorial disputes and sperm competition. Females seem to be attracted to high quality and/or familiar patches of habitat rather than to males with certain phenotypic characteristics (Edsman 1990, 2001; Font et al. 2012*a*). Moreover, behavioural observations and genetic analyses have confirmed that receptive females often mate with more than one male before oviposition, which results in a high incidence of multiple paternity (Oppliger et al. 2007; Uller and Olsson 2008; Heathcote et al. 2016). Consequently, adult males try to secure fertilizations by investing significant time and energy in the defence of territories offering resources valuable to females (such as basking spots, shelters, optimal egg-laying sites, etc.) against other males (Edsman 1990; Font et al. 2012*a*; Baird 2013). The outcome of these territorial disputes is crucial to male reproductive success, and patterns of shared paternity have often been found to reflect spatial and social dominance among males (Oppliger et al. 2007; Uller and Olsson 2008; While et al. 2015; MacGregor et al. 2017*b*). For these reasons, alternative colour morphs in many wall lizards are often believed to represent the visible mark of heritable ARS involving differential socio-spatial behaviours in males (Huyghe et al. 2007; Sinervo et al. 2007; Calsbeek et al. 2010; Pérez i de Lanuza et al. 2017; Andrade et al. 2019). Although alternative reproductive strategies have been more commonly described in males, this bias could simply result from female alternative strategies having been traditionally overlooked. While the ecological factors generating variance in male fitness are relatively easy to identify and quantify (i.e. number of partners), the factors generating fitness variance among females are less well understood (e.g. condition, fecundity, survival, direct or indirect costs and benefits of mating; Neff and Svensson 2013). However, females often vary in fitness and several studies suggest the existence of alternative breeding strategies in polymorphic lizards (including lacertids) whereby one female morph conceives many, small offspring (r-strategists) while another produces fewer, large offspring (K-strategists; Zucker and Boecklen 1990; Sinervo et al. 2000*b*, 2000*a*; Svensson et al. 2001, 2002; Comendant et al. 2003; Vercken et al. 2007; Galeotti et al. 2013).

1.5. Study species and area

1.5.1 The European common wall lizard (*Podarcis muralis*)

Among the numerous species showing colour polymorphism within the genus *Podarcis,* the European wall lizard (*Podarcis muralis*, Laurenti 1768) has been the most studied. This species shows the widest distribution in the genus *Podarcis,* with populations spreading across Europe (from northern Iberia to the west coast of the Black Sea and North-Western Anatolia), and several sub-lineages corresponding to southern refugia in the Mediterranean islands and peninsulas (Arnold et al. 2002; Salvi et al. 2013). *Podarcis muralis* typically inhabits sunny semi-open areas with abundant rocky outcrops interspersed with vegetation, but it can also be found using human structures in anthropized areas (e.g. stone-walls between abandoned agricultural fields). Its diet consists mainly of small arthropods with arachnids and some insect groups (i.e. Diptera, Homoptera, Orthoptera, Coleoptera, Lepidoptera) usually composing over 75% of prey, although plant consumption (i.e. flowers and berries) has also been reported (Arnold et al. 2002; Rasilla 2009; Scali et al. 2016). Its natural predators include ophidians (e.g. *Coronella girondica, C. austriaca, Vipera seoanei, V. aspis, Hierophis viridiflavus, Zamenis longissimus*), birds of prey (e.g. *Falco tinnunculus, Buteo buteo, Circaetus gallicus, Tyto alba, Strix aluco, Pica pica*), and mammals (e.g. *Martes martes, Mustela nivalis, Crocidura russula),* especially domestic cats (*Felis s. domesticus*) (Martin and López 1990; Arnold et al. 2002; Rasilla 2009).

While lizards are active throughout the year under mild weather conditions, in mountain and northernmost populations they hibernate for 10-14 weeks before the onset of the spring breeding season. Males precede females in coming out of hibernation and engage in contests with other males to secure a territory including resources valuable to females. Aggression between females is uncommon and groups of females are frequently observed basking in close vicinity (often accompanied by a single male; Edsman 1990, 2001; Pérez i de Lanuza et al. 2013). Despite this, females often copulate with more than one male during the ovarian cycle and multiple paternity affects more than 87% of clutches in natural populations (Oppliger et al. 2007). Females typically produce two to three clutches per year, laying more and larger eggs in earlier clutches (Van Damme et al. 1992; Ji and Braña 2000). Newborn lizards hatch in late summer and take at least a year to reach sexual maturity, with some populations in cold climates reportedly taking up to 2-3 years (Eroğlu et al. 2018; Kolenda et al. 2020). Using both capture-re-capture methods and skeletochronological analysis, maximum life expectancy in *P. muralis* populations from cold climates has been estimated to be seven-eight years (Castanet and Smirina 1990; Vollono and Guarino 2002; Eroğlu et al. 2018; Kolenda et al. 2020).

1.5.2 Colour polymorphism in the European common wall lizard (*Podarcis muralis*)

As most *Podarcis* species, *P. muralis* shows a cryptic dorsal coloration that probably evolved to avoid predators and enhance thermoregulation, and conspicuous ventral and lateral colour patches that are commonly thought to function as chromatic signals (Pérez i de Lanuza et al. 2014). Many populations of *P. muralis* exhibit a ventral colour polymorphism. Adults of both sexes may show up to five alternative ventral colour morphs: three solid (pure) morphs, i.e. orange (O), white (W) and yellow (Y), and two intermediate mosaics combining orange and white (OW) or yellow and orange (YO) scales (Pérez i de Lanuza et al. 2013, 2019; **Fig. 1.4**). Individuals showing pale yellow ventral coloration, initially considered as pertaining to an additional mixed-morph (i.e. white-yellow), have been identified as subadults not yet expressing the full yellow coloration due to their relatively smaller body sizes and scarcity in natural populations (Calsbeek et al. 2010; Sacchi et al. 2013). Recent research suggests that orange and yellow colour expression are caused by recessive homozygosity at two separate loci involved in the metabolism of pteridines and carotenoids (Andrade et al. 2019; see 1.4.1 and 1.4.2). Interestingly, these colour morphs are shared by geographically distant lineages of the species thought to have diverged up to 2.5 million years ago (Salvi et al. 2013; Andrade et al. 2018; Pérez i de Lanuza et al. 2019). Local morph composition shows considerable geographic variation, although white ventral coloration is typically the most common (>50%), while the orange and especially the yellow morph rarely predominate (Sacchi et al. 2007; Pérez i de Lanuza et al. 2017, 2018*b*; see **Fig 1.4**). Adult ventral colours are fixed at maturity (i.e. one or more years after hatching, depending on the population), and chromatic differences between pure colour morphs are due mainly to variation in reflectance between 400 and 600 nm, suggesting that they can be correctly discriminated by the human eye (Pérez i de Lanuza et al. 2014, 2018*a*). However, no study to date has confirmed this morph classification by quantifying the clustering of colour variation according to the species colour sensitivity, and/or testing the behavioural discrimination of these colours by *P. muralis* lizards (see Box 1 and Box 2). Likewise, newborn ventral coloration (which is perceived as identical to the white morph by human observers; Pérez i de Lanuza et al. 2013) has never been objectively characterized and we ignore how it may be perceived by conspecifics.

Because of their ventral position (which allows the lizards to control their exposure through posture) and conspicuousness to the species' visual system (Pérez i de Lanuza and Font 2015; Pérez i de Lanuza et al. 2018*a*), *P. muralis* colour morphs have been thought to reflect an underlying set of alternative phenotypic optima (e.g. reproductive or life-history strategies), the ventral colours functioning as chromatic signals during social interactions (Calsbeek et al. 2010; Sacchi et al. 2017*a*; Mangiacotti et al. 2019). In particular, the hypothesis that colour morphs may show differential socio-sexual behaviour similar to those described in *Uta stansburiana* has received much attention in the literature (Sinervo et al. 2007; Calsbeek et al. 2010; Scali et al. 2013; Sacchi et al. 2017*b*). Research on *P. muralis* colour morphs has detected inter-morph differences in several (mostly physiological) traits relevant to fitness such as immune response, hormonal profile, and prevalence of infection by parasites, indirectly suggesting the existence of alternative fitness optima (i.e. strategies) in the different morphs (Galeotti et al. 2007, 2010, 2013; Calsbeek et al. 2010; Zajitschek et al. 2012; Sacchi et al. 2017*b*, 2017*a*). Female colour morphs from Italian polymorphic populations have also been suggested to show differential breeding investment strategies (r/k) in a controlled mating experiment in which reproductive output and offspring quality were also found to vary depending on parental morph combination (Galeotti et al. 2013). In addition, field observations suggest an assortative morph bias in pair formation (68% of male-female pairs observed in the wild are homomorphic; Pérez i de Lanuza et al. 2013, 2016) and early microsatellite analyses revealed reduced gene flow between some of the colour morphs (Bellati 2011).

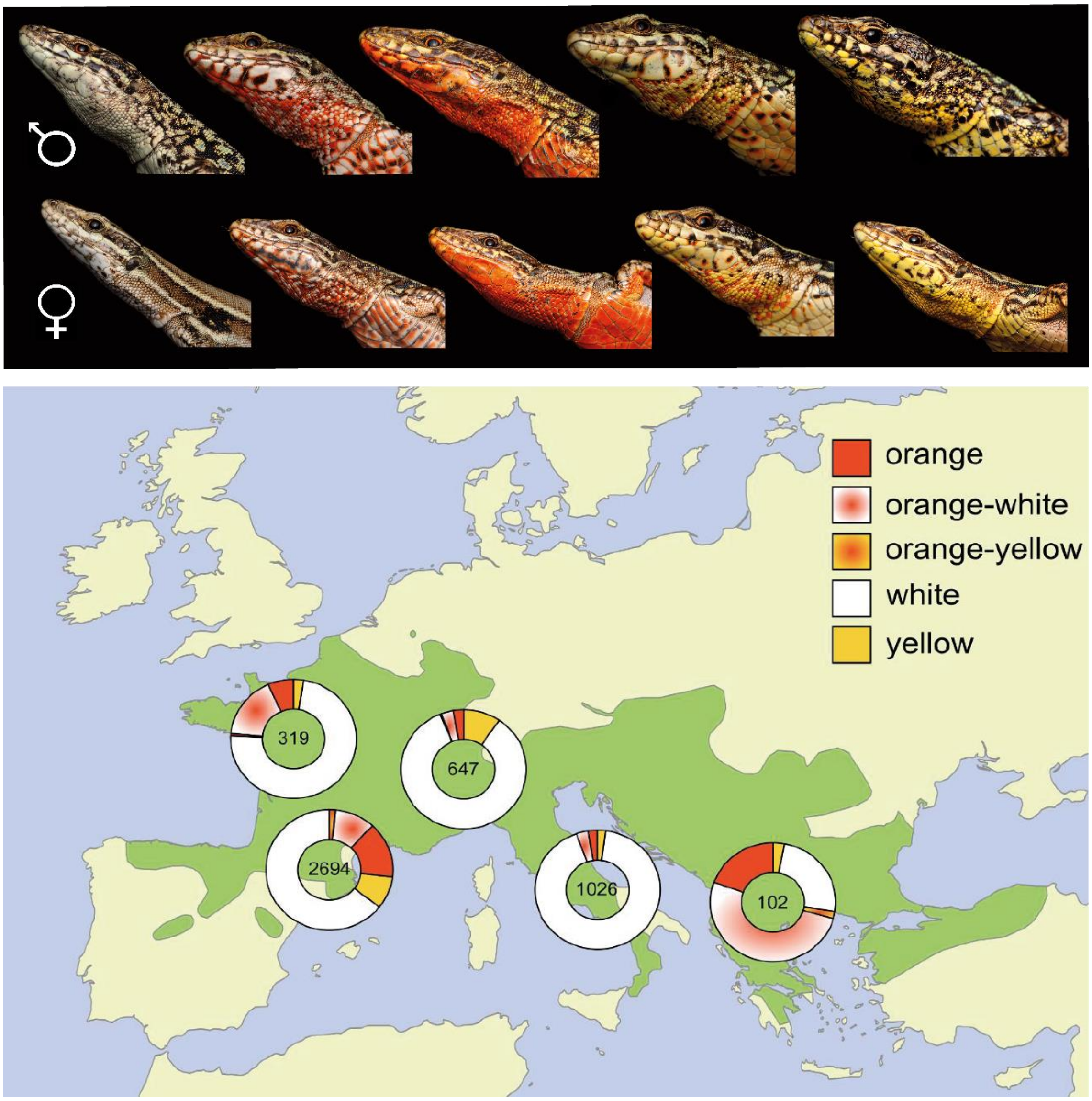


Figure 1.4. Ventral colour polymorphism in the European common wall lizard (*Podarcis muralis*). Up, representative examples of the discrete variation in the ventral surface of adult lizards. Males and females may show up to five different morphs, three pure morphs showing solid orange, white or yellow coloration, and two mixed morphs combining two colours in a mosaic of scales (orange-white and yellow-orange). The coloration extends to the belly in males but is sometimes restricted to the throat in females. Below, native distribution of *Podarcis muralis* (green shading) with pie charts representing the natural morph frequencies pooled by geographically distant sub-lineages. White morph lizards are usually the most common. The number of lizards sampled is indicated inside the pie charts. Figure extracted from Andrade *et al.* (2018) and reproduced here with permission from the authors.

Taken together, these findings suggest that disruptive, correlational selection coupled with assortative mating may have favoured different co-adapted trait complexes in each morph while simultaneously introducing some degree of genetic divergence among them. However, it still unclear how these correlated traits in *P. muralis* morphs may fit within a set of coherent and discrete alternative fitness optima, whether in the context of sexual or natural selection. In fact, as is often the case with studies on polymorphic lizards (see Box 2), the existence of alternative behavioral strategies in *P. muralis* colour morphs has been frequently assumed based on indirect physiological evidence rather than formally tested through quantitative ethological methods (but see Sacchi et al. 2009; Scali et al. 2013, 2019). What is more, the same behavioural strategy has been sometimes attributed to different morphs in different studies: a "high reproductive investment syndrome" (where individuals invest heavily in demanding sexually-selected traits such as body size, hyper-aggressiveness, spatial dominance and exploratory behaviour at the cost of reduced immune function) has been suggested for either orange or yellow males in two separate studies, even though behaviour was not assessed in neither of these studies (Calsbeek et al. 2010; Sacchi et al. 2017*b*). In fact, no previous study has investigated the alignment of polymorphic coloration, socio-spatial behavior, and shared paternity in sufficient detail to draw firm conclusions about the existence of ARS in *P. muralis*. At the same time, insufficient attention has been given to other functional explanations of colour polymorphism maintenance such as the existence of spatio-temporally varying selection or heterosis (see 1.3.2).

1.5.3. Study area

We have investigated colour polymorphism and its correlated traits in the dense *P. muralis* population inhabiting rocky outcrops and human structures found in the Cerdanya plateau (eastern Pyrenees). Lizards in this area might experience more favourable conditions than in other locations in the Pyrenees, as abandoned fields oriented to the south and isolated granite boulders surrounded by sparse vegetation provide abundant suitable habitat, while the dominant East-West orientation of Cerdanya guarantees over 3000 h of sunlight per year (**Fig. 1.5**). The dominant vegetation in our main study site consists of *Fraxinus excelsior, Crataegus monogyna* and *Rosa canina*. The main lizard predator appears to be the diurnal snake *Hierophis viridiflavus*, but other potential predators (i.e. raptors) are common in this area (Font et al. 2012*b*; Pérez i de Lanuza and Font 2015; Pérez i de Lanuza et al. 2016*a*). Wall lizards can be found continuously across the plateau, yet morph richness and ratios have been found to vary greatly among relatively close localities with both biotic (i.e. sex ratio) and abiotic factors (e.g. seasonality and precipitation) apparently playing a role in shaping morph composition (Pérez i de Lanuza et al. 2017, 2018*b*).

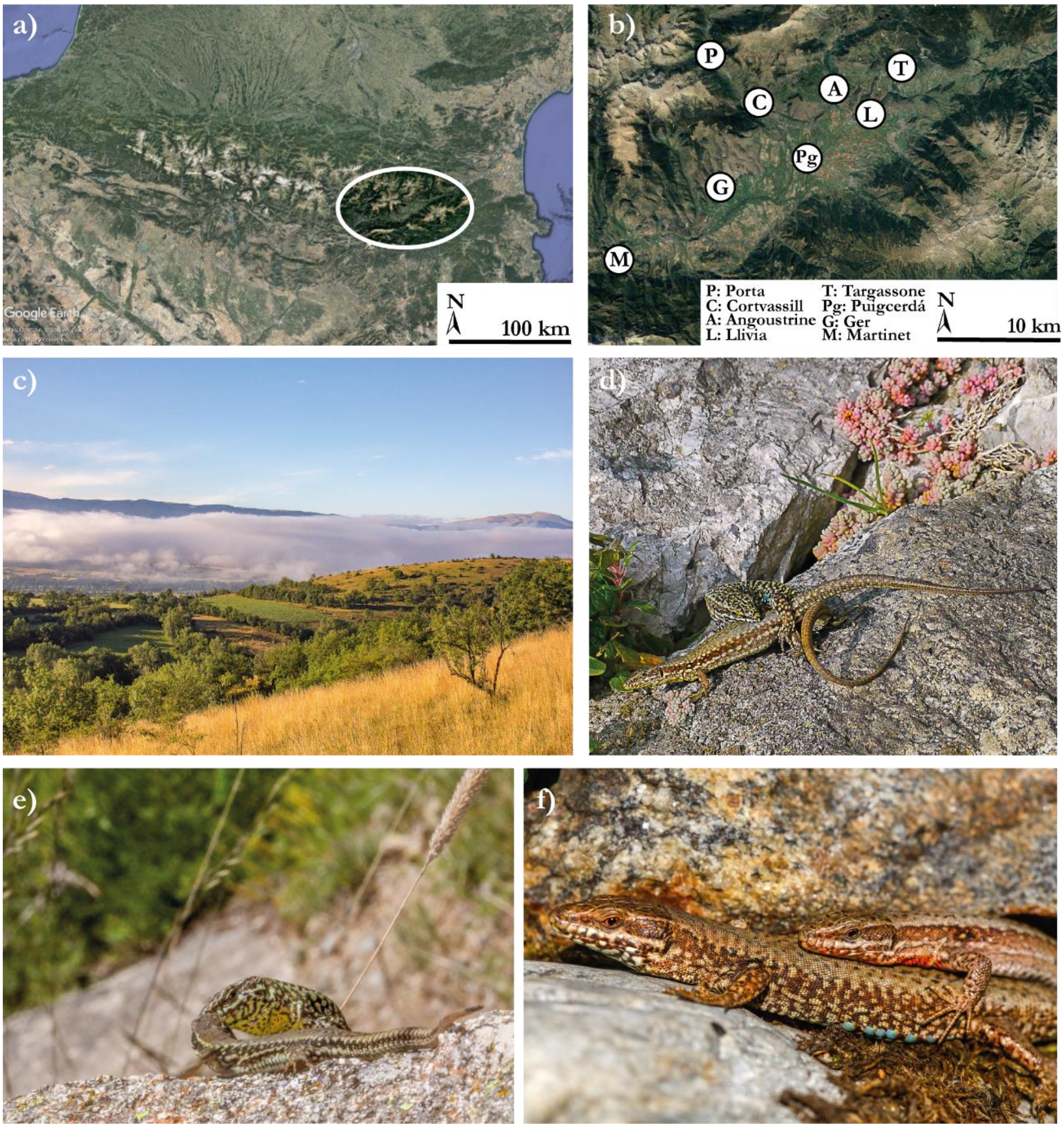


Figure 1.5. Study area and lizard inter-sexual behaviour. a) Satellite image of the Pyrenees, with the location of the Cerdanya plateau encircled. b) Satellite image of the Cerdanya plateu showing a subset of representative *P. muralis* capture localities sampled in this thesis. c) Landscape view from Angoustrine-Villeneuve-des-Escaldes, in the Cerdanya valley (eastern Pyrennees). d) Male and female engaged in the doughnut-shaped copulation posture typical of lacertid lizards. e) early stages of a male-female interaction, when both males and females show sex-specific behaviour (♂: gular extension, back arching, lateral compression; ♀: rapid waving of the anterior limbs in the air, body flattening against substrate, tail vibration). f) pair of lizards lying in physical contact (female on top). These associations become more frequently later in the breeding season and are interpreted as a form of mate-guarding and/or deterrent of male harassment (Censky 1997; in Den Bosch and Zandee 2001; Drummond and Zaldívar-Rae 2007; Heathcote et al. 2016).

Box 1. How do lizards see each other? Studying colour vision from a non-anthropocentric perspective

Colour is not a physical property of natural objects. Rather, colour perception is a psychophysical phenomenon; a fundamentally subjective experience stemming from the interaction between light, a colour-sensitive visual system, and a brain (Kelber et al. 2003; Stevens 2013). First, light needs to impinge on a retina equipped with at least two types of wavelength-dependent photoreceptor cells (i.e. cones). Then, relative comparisons of the input received from the different cones must be performed by the nervous system so as to integrate the information received and allow for wavelength discrimination. Colour is usually defined by three qualities: luminance, chroma, and hue (Endler 1990; Kelber et al. 2003; Osorio and Vorobyev 2008). Luminance is the value in the dark to light scale. Hue is determined by the wavelength in which an objects shows peak reflectance, and is the attribute denoted by terms such as red, green, or purple. Chroma (saturation) describes how reflectance is distributed around its peak: a grey object with a small reddish tint has low saturation, whereas a red object with little white or grey tint is highly saturated. Colour information processing could be carried out in the absence of conscious sensations, yet in humans (and by evolutionary continuity possibly in other animals), it is accompanied by qualia, the subjective experiences that we associate with colour perception (Nagel 1974; Clark 1985; Osorio and Vorobyev 2008). Clearly, colour perception is a far less intuitive phenomenon than commonly believed.

Through history, we have often assumed that other animals perceive their surroundings in a similar way to us. This erroneous assumption is often termed anthropomorphism by omission, as it neglects the fact that animals differ starkly from us in their sensory systems, and many perceive sensory modalities to which humans are entirely insensitive (Rivas and Burghardt 2002). Each species lives in a unique perceptual world (i.e. *Umwelt*) different from that of any other species, which results from the specific set of physiological, behavioural, and environmental constraints that evolution has imposed on its sensory organs and nervous system (von Uexküll 1934; Caves et al. 2019). The risk of misinterpretation associated with an anthropomorphic stance is particularly strong when studying sensory modalities shared by humans and other animals (e.g. vision; Kelber et al. 2003; Osorio and Vorobyev 2008; Stevens 2013; Caro et al. 2017). Probably because we are primarily driven by sight, we have often assumed that vision in most other animals must be poorer, or at best equal to our own.

Sir John Lubbock (1882) seriously challenged this idea when he exposed ants to a beam of light decomposed with a prism and found them particularly anxious to remove their larvae from the apparently dark region (to our sight) well beyond the violet end of the spread-out spectrum. Further research has demonstrated that many organisms, both vertebrate and invertebrate, possess sophisticated visual systems allowing them to perceive light in the near ultraviolet (UV-A) waveband (320-400 nm), which is invisible to us (Cronin and Bok 2016). As stated above, the number of receptor types involved in colour vision (i.e. cones) determines the range of colours an animal could potentially see. Photoreceptor nomenclature is complicated, and potentially confusing. Receptors have been named either according to the part of the spectrum (i.e. colours) to which they are most sensitive, or by their sensitivity relative to other receptors in the eye. As many other catarrhine primates, humans show three types of cones termed SWS (showing relatively higher sensitivity within short wavelengths), MWS (medium wavelengths), and LWS (long wavelengths; Kelber et al. 2003; Osorio and Vorobyev 2005; Wuerger et al. 2005). Along with these, lizards (and many other vertebrates) show a fourth type of photoreceptor cell with its peak sensitivity in the near ultraviolet waveband (i.e. UWS; Cronin and Bok 2016). Colour stimuli that closely match the sensitivity of a single cone are termed primary colours, and for this reason human cones are sometimes referred as blue, green, and red (corresponding to the SWS, MWS and LWS cones, respectively). This nomenclature, though intuitive, should better be avoided since it may lead to the wrong assumption that perception of primary colours depends on a single type of cone. In reality, colour vision depends on the opponent processing of output from the different cones, so that all receptor types are involved in perceiving colour (Kelber et al. 2003; Stevens 2013). Stimuli eliciting a similar response from two or more types of cones may lead to the perception of intermediate colorations (e.g. for example when our SW and MW cones are similarly stimulated and we sense a sort of bluish green) or, alternatively, give rise to an entirely different chromatic experience (i.e. secondary colours; Cuthill et al. 2000; Jones et al. 2001; Ham and Osorio 2007). An example of this latter phenomenon is our yellow, which results from the similar activation of our LW and MW cones (and yet is not perceived as a sort of reddish green). Other eye structures may introduce further complexity by altering the light spectrum arriving to the photoreceptors. In lizards, for instance, there are four types of oil droplets (each associated with a different type of cone and located in front of them in the retina) whose spectral properties are attuned to the type of photoreceptor they accompany. These oil droplets filter the light that arrives at the photoreceptors below certain wavelengths (which reduces the overlap in stimulation between different types of cones) and are thus thought to allow for a more precise discrimination between different wavelengths (i.e. colour discrimination; Loew et al. 1996; Bowmaker and Hunt 2006; Fleishman et al. 2011; Pérez i de Lanuza and Font 2014; Martin et al. 2015).

Note that finding a given number of cones in a species does not imply that the species' colour vision comprises that same number of dimensions (e.g. despite showing 12 different photoreceptors in their retina, mantis shrimps seem to use a unique colour recognition system based on temporal signalling and scanning eye movements rather than a 12-dimensional colour space; Thoen et al. 2014). Ultimately, the dimensionality of colour perception in a species depends on the number of post-receptional neural channels comparing the input of the different types of cones. Humans are trichromats because we present two neural channels involved in colour perception. The first channel compares the input of the red and green cones (i.e. LW-MW), while the second channel obtains the difference between the sum of these two cones and the input from the blue cones (SW-(LW+MW)). Knowledge of the dimensionality of colour vision in other species is scarce, but there is both behavioural and physiological evidence supporting dichromatic and trichromatic vision in some mammals, while many insects, birds, reptiles and fish (which show a fourth type of UV-sensitive photoreceptor cell) are reasonably presumed to be tetrachromats (Bowmaker and Dartnall 1980; Kelber et al. 2003; Osorio and Vorobyev 2008; Jacobs 2009; Stevens 2013; Cronin and Bok 2016; Caro et al. 2017).

Knowledge on other types of perceptual constraints of a species is also crucial if we aim to understand the biological function of its colour traits (Kelber et al. 2003; Osorio and Vorobyev 2005). Visual acuity (i.e. the ability to resolve the spatial details of an object) may be particularly important to consider (Kelber et al. 2003; Osorio and Vorobyev 2005). In general, visual acuity is thought to correlate positively with eye size because (if eye shape is held constant), an increase in eye length produces a longer posterior nodal distance which, in turn, increases the size of the retinal image (Stevens 2013; Veilleux and Kirk 2014). Larger retinal images will generally be sampled by a greater number of independent ganglion cell receptive fields and hence allow for finer spatial detail resolution (i.e. visual acuity). Two further situational factors affect visual acuity: resolving power declines with i) distance to the viewer and ii) when the image is formed on the peripheral retina (low-acuity) instead than on the central foveal region (high acuity) (Stevens 2013; Fleishman et al. 2017). In *Anolis* lizards, considering the limits imposed on acuity by the species' eye Fleishman et al. (2020) revealed that the finer details of the colourful dewlap patterns exhibited by male lizards could only be resolved (for central foveal vision) from a distance of 0.5 m or shorter (Fleishman et al. 2020). In light of these results, the authors infer that while the fine-scale details of *Anolis* dewlap patterns may convey information about individual identity, health status, mate quality or motivational state during close-range social interactions, other long-held hypothesis about the role of *Anolis* dewlaps (such as in signalling species identity through assertion displays broadcasted over longer distances) should be revised. Thus, knowledge on an animal's perceptual world can allow researchers to greatly refine hypotheses about signal function.

The analysis of perceptual processes by studying the effect on a subject's experience or behaviour of systematically varying the physical properties of a stimulus is termed psychophysics (Gescheider et al. 1997). In the absence of easily-implemented methods to investigate neural activity, behavioural experiments offer a suitable way to investigate questions related to colour vision in other animals (Kelber et al. 2003). For instance, Osorio et al. (1999) demonstrated tetrachromatic colour vision in domestic chicks (*Gallus gallus*) by training them to feed from coloured containers designed to be distinguished only by specific combinations of photoreceptors. In addition, modern techniques for colour measurement and analysis have brought significant progress to the field of animal colour vision. Spectrophotometry, for instance, allows researchers to objectively characterize the spectral properties of an animal's colour patches (or surroundings) over a range of wavelengths beyond those perceived by the human visual system (Font et al. 2009; Pérez i de Lanuza 2012; Badiane et al. 2017). Visual modelling can then be used to summarise the chromatic information of a spectrum according to our knowledge of the species' visual system. When studying presumably tetrachromatic animals (e.g. *Podarcis* lizards), we can represent a three-dimensional (i.e. tetrahedral) colour space where each of the four vertices corresponds to maximum stimulation of a different type of cone. The centre of this tetrahedron corresponds to a colour eliciting equal stimulation from every type of cone (i.e. achromatic centre), and spectra varying in their stimulation of one or more types of cones will be represented as a chromatic point displaced from this centre in at least one axis (Vorobyev and Osorio 1998; Kelber et al. 2003; Pérez i de Lanuza et al. 2013*b*). Chromatic distances between two spectra can then be calculated as the Euclidean distance between their chromatic points projected on the tetrahedral colour space, which is often used to investigate the discriminability of two colours (according to a particular species visual system and some theoretical assumptions; Vorobyev and Osorio 1998; Kelber et al. 2003; Pérez i de Lanuza 2012).

In short, if we aim to study questions related with colour and colour vision in a species (e.g. lizards), we should try, as far as possible, to view the world through the eyes of that species. In this thesis, we have used behaviour experiments, spectrophotometry, and visual modelling techniques to investigate colour discrimination (**Chapter II**), and to characterize the spectral properties of lizard colour patches (**Chapters III and V**).

Box 2. Pending questions on the link between behaviour and lizard colour polymorphism

Despite the large volume of studies suggesting a role of behaviour in the maintenance of lizard colour polymorphisms, there remain several unresolved questions. Morph classification within each species often varies across studies, as morph categories have often been defined subjectively (i.e. based on human perception; Thompson and Moore 1991; Carpenter 1995; Calsbeek and Sinervo 2002*c*; Sacchi et al. 2013). Several factors contribute to these inconsistencies: i) quantitative variation in the size or spectral properties of the polymorphic colour patch, ii) mixed-morphs combining two or more colours, and iii) inter-observer variation in colour categorization (Stuart-Fox et al. 2020). For instance, in *Urosaurs ornatus* different researchers have categorized the same male morph as either yellow or green. Moreover, lizards showing yellow or orange throats with a central blue spot have been sometimes conflated in a single morph (i.e. green), and sometimes separated into two different mixed morphs (i.e. orange-blue and yellow-blue; Thompson and Moore 1991; Moore et al. 1998; Lattanzio and Miles 2016; Taylor and Lattanzio 2016). Ultimately, if we aim to study the role of animal coloration, we must obtain an objective, non-anthropomorphic assessment of such coloration informed by our knowledge of the species visual system (see Box 1). In the case of lizard colour polymorphism, overcoming this challenge requires the use of visual modelling and behavioural experiments to examine what morph classification emerges from the clustering of quantified colour variation according to the species colour sensitivity, and which colours are effectively discriminated by the intended receivers (i.e. conspecifics or else).

Two further deficits undermine the alleged link between lizard colour polymorphism and behaviour. Upon finding morph-specific differences in physiological or life-history traits relevant to fitness, many studies suggest the existence of alternative reproductive strategies involving differential behaviour in social interactions (Galeotti et al. 2007; Calsbeek et al. 2010; Sacchi et al. 2017*b*, 2017*a*; Stuart-Fox et al. 2020). Following this reasoning, colour morphs are often thought to function as visual signals conveying information about their owner's strategy (Sinervo et al. 2006*b*; Huyghe et al. 2007, 2009*b*; San-Jose et al. 2014; Yewers et al. 2016; Scali et al. 2019; Mangiacotti et al. 2020; McLean et al. 2020). However, for many species the existence of differential socio-sexual behaviour among colour morphs has rarely been assessed in sufficient detail to draw firm conclusions, and the question of why these alternative reproductive strategies should be associated with different colorations is seldom justified and never formally tested.

Communicative systems are expected to arise when both the sender and the receiver benefit, on average, from signalling and responding to a given signal (Maynard-Smith and Harper 2003; Font and Carazo 2010; Stevens 2013). Honest signals conveying functional information (*sensu* Carazo and Font 2010; Font and Carazo 2010) about the sender's strategy may evolve when animals can form cooperative morph-dependent alliances (e.g. a dominant male may tolerate losing some fertilisations to a subordinate satellite if its presence facilitates territory defence against other dominant males; Waltz 1982; Greenfield and Shelly 2008; Tibbetts et al. 2017). Deceptive signals are expected to evolve when individuals of one morph may benefit from exploiting a behavioural response in the receiver which is adaptive in another context (i.e. female-mimicry complementing a sneaker strategy to bypass male territorial defence; Mason and Crews 1985; Shine et al. 2001; Whiting et al. 2009; Carazo and Font 2013; Stevens 2013; Tibbetts et al. 2017; Font 2019).

How much these questions (i.e. morph classification, behaviour, and signaling theory) have been overlooked may be best exemplified by research on *Uta stansburiana*. In this species, yellow males have been reported to reduce the aggression received from the orange and blue territorial morphs by resembling females both in behaviour (i.e. by performing "female rejection displays" towards approaching males) and coloration (i.e. showing throat colour and dorsal patterning similar to those of females). Unfortunately, these claims seem to be entirely based on early field observations from Sinervo and colleagues, and have never been subject to rigorous scientific scrutiny. Distinctive "female rejection displays" have been characterized in *Sceloporus virgatus* and other Iguanian lizards (Martins 1991, 1993, 1994; Weiss 2002), yet no studies have investigated the existence of such displays in *Uta stansburiana*, nor quantified the frequency with which they are performed by the different male morphs. Likewise, although early work on *Uta stanburiana* claimed that "receptive females have yellow stripes in their throats" (Sinervo and Lively 1996), the number of female morphs has grown in later studies until equating the diversity found in males (Corl et al. 2010), thus obscuring the presumed role of yellow throat coloration in female-mimicry (Sinervo and Lively 1996; Sinervo et al. 2000*a*; Zamudio and Sinervo 2000; Calsbeek and Sinervo 2002*b*). In a similar vein, blue throat coloration has been proposed to function as a phenotypic identifier allowing cooperation between unrelated individuals of a given genotype (i.e. greenbeard effect; Gardner and West 2010; Dawkins 2016). These claims are supported by the observation that blue males tend to settle together and through this achieve higher fitness, in contrast to orange males (whose fitness is negatively affected by other orange neighbours) and yellow males (that are unaffected by the presence of other yellow males (Sinervo and Clobert 2003).

This gain in fitness has been assumed to derive from morph recognition and cooperative territory defence among blue males, although a direct evaluation of these rather complex behaviours is still lacking (Sinervo et al. 2006*b*). Moreover, the alternative hypothesis that the benefits of co-settling in blue males may constitute a transient corollary to settling far from both usurper and sneaker males has not been formally considered (Olsson et al. 2013). These examples from *Uta stansburiana* (mirrored in other polymorphic lizards) suggest the need to devote much more attention to the quantitative examination of behavioural morph differences rather than assuming the existence of alternative reproductive strategies from indirect evidence. This, together with a stronger emphasis on sensory ecology and animal communication theory, should help to bridge the gap between behaviour and colour polymorphism in future studies.

1.6. Thesis aims

In this doctoral thesis, we seek to contribute to our understanding of the mechanisms generating and maintaining phenotypic variability in nature. We will delve into general principles rooted in evolution, sensory ecology, and animal communication theory to investigate the evolutionary causes and consequences of colour polymorphism in the European common wall lizard *(Podarcis muralis*). We will focus on frequently overlooked aspects regarding the link between behaviour, fitness, and colour polymorphism to ascertain whether *P. muralis* colour morphs reflect a set of alternative behavioural or life-history strategies evolved by correlational selection, as is frequently assumed in the literature. Specifically, our thesis aims to i) improve the way we categorize colour morphs by assessing the discriminability of chromatic variation through both visual modelling and behavioural experiments, ii) examine the role of male coloration (including polymorphic throat patches) in the outcome of dyadic male-male contests, iii) investigate whether colour morphs differ in key aspects of their behavioural (i.e. aggressiveness, territoriality, mate-guarding) or life-history traits (i.e. investment in offspring quality vs. quantity) in a way compatible with alternative fitness optima, iv) testing the existence of morph combination effects on reproductive output and offspring viability, and v) contribute to the still limited knowledge on the ontogeny and heritability of colour polymorphism in this species. We will address specific questions in the five following areas.

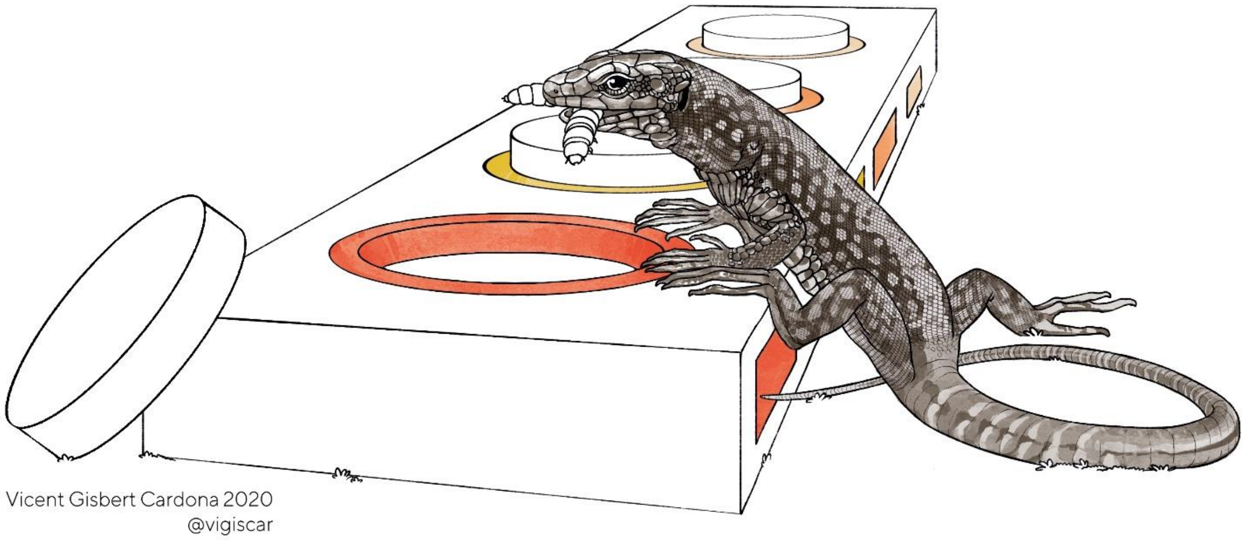
- **a)** Colour morph categorization based on the species visual system and behavioural discrimination by conspecifics (**Chapter II**);
- **b)** Relationship between colour morph, agonistic behaviour, and fighting ability in dyadic confrontations between males (**Chapters II and III**);
- **c)** Search for consistent differences among *P. muralis* colour morphs in key aspects of their behavioural (i.e. aggressiveness, territoriality, mate-guarding) or life-history traits (i.e. investment in offspring quality vs. quantity) compatible with alternative fitness optima (**Chapters III and IV**).
- **d)** Test predictions of correlational selection and heterosis regarding the effect of parental morph combination on reproductive output and offspring viability (**Chapter IV**).
- **e)** Colour morph inheritance and ontogeny (**Chapter V**).

Chapters II, III, and IV have been published in *Journal of Experimental Biology*, *Behaviour*, and *Ecology and Evolution*, respectively. Chapter V is currently undergoing review. Each chapter has been written and formatted as a stand-alone piece for publication, so there is some redundancy among them in methodological details.

Chapter II

"The real thing is that there is no real world but as many worlds as species".

·Jakob von Uexküll (1920)·



This chapter reproduces entirely the published manuscript:

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*Both authors contributed equally to this work

Chapter II:

Through the eye of a lizard: hue discrimination in a lizard with ventral polymorphic coloration

2.1. Abstract

Colour polymorphisms are thought to be maintained by complex evolutionary processes some of which require that the colours of the alternative morphs function as chromatic signals to conspecifics. Unfortunately, a key aspect of this hypothesis has rarely been studied: whether the study species perceives its own colour variation as discrete rather than continuous. The European common wall lizard (*Podarcis muralis*) presents a striking colour polymorphism: the ventral surface of adults of both sexes may be coloured orange, white, yellow, or with a mosaic of scales combining two colours (orange-white, orange-yellow). Here we use a discrimination learning paradigm to test if *P. muralis* is capable of discriminating colour stimuli designed to match the ventral colours of conspecifics. We trained 20 lizards to eat from colour-coded wells bored in wooden blocks. Blocks had four colour-coded wells (orange, white, yellow, and an achromatic control), but only one contained food (mealworm larvae). After six trials, the lizards performed significantly better than expected by chance, showing a decrease in both the number of wells explored and the latency to finding the food. Using visual modelling techniques we found that, based on their spectral properties and the lizards' cone sensitivities, the ventral colours of *P. muralis* correspond to discrete rather than continuous colour categories, and that colour discriminability (i.e. distance in perceptual space) varies depending on the morphs compared, which may have implications for signal detection and discrimination. These results suggest that *P. muralis* can discriminate hue differences matching their own ventral colour variation.

Keywords: colour discrimination, colour polymorphism, learning experiment, lizards, visual modelling

2.2. Introduction

Understanding the processes responsible for the evolution of population polymorphisms is one of the most exciting challenges facing evolutionary biology. Colour polymorphic species such as the peppered moth, *Biston betularia*, have been extensively used as models to test important evolutionary hypotheses about the origins and maintenance of phenotypic variation (Majerus 1998; Gray and McKinnon 2007; Oliveira et al. 2008; Svensson et al. 2009; Mckinnon and Pierotti 2010; Wellenreuther et al. 2014; Svensson 2017). However, assessing colour variants and their functional significance in colour polymorphic species is not straightforward. Colour variation is often described from the perspective of a human viewer but should instead be judged from the perspective of the appropriate receivers (Bennett et al. 1994; Eaton 2005), which requires the use of modern instrumentation and methods for objective colour characterization (e.g. reflectance spectrophotometry and visual modelling). Also, the widely-held assumption that the colours of the different morphs act as chromatic signals and that conspecifics use colour variation to identify alternative phenotypes (e.g. related to age, sex, individual quality, or reproductive strategies) and adjust their behaviour accordingly has rarely been tested.

Recent work with pollinating insects and birds and with cichlid fish underscores the importance of considering perceptual mechanisms in the study of colour polymorphisms (Chittka and Raine 2006; Muchhala et al. 2014; Thairu and Brunet 2015). Cichlids show striking and hypervariable inter- and intrapopulation colour polymorphisms, and abundant evidence has demonstrated that colour discrimination, visual ecology, and sensory drive play a critical role in the evolution of this interesting polymorphic clade (e.g. Seehausen et al. 2008). However, there is no information on morph discrimination for most colour polymorphic species, including lizards (but see Teasdale et al. 2013; Merkling et al. 2016).

The European common wall lizard *Podarcis muralis* (family Lacertidae) is attracting much interest in studies of colour polymorphisms (e.g. Calsbeek et al. 2010; Galeotti et al. 2013; Pérez i de Lanuza et al. 2013*a*, 2017). To the human eye, this species may show up to five discrete ventral colour morphs that are fixed at sexual maturity: white, yellow and orange pure colour morphs, as well as white-orange and yellow-orange mixed phenotypes that display a mosaic of scales of two different colours (sensu Pérez i de Lanuza et al. 2013*a*; Pérez i de Lanuza and Font 2015). These colours extend over the throat and the belly in males but, at least in some populations, are restricted to the throat in females (females in these populations have a white belly). Much effort has been devoted to trying to identify consistent behavioural, morphological, physiological or ecological correlates of the colour variation, but the results are so far inconclusive (Galeotti et al. 2007, 2013; Calsbeek et al. 2010; Pérez i de Lanuza et al. 2014).

While it is often assumed that the ventral colours in *P. muralis* function as social signals, the evidence in this regard is very scant. It is possible that the ventral coloration, while correlated with other phenotypic traits, has no effect on receiver behaviour and is therefore not a chromatic signal to conspecifics. Rather, a link between polymorphic coloration and alternative phenotypes could result from pleiotropic effects of whatever genes are responsible for the polymorphism. However, there are some indications that lizards assess each other's ventral colours and adjust their behaviour based on their own colour relative to that of others with which they interact. For example, although morphs are not spatially segregated, males and females pair assortatively by ventral colour (Pérez i de Lanuza et al. 2013*a*, 2016*b*). Also, male ventral colour seems to be important in the resolution of lab-staged fights (Abalos et al. 2016). Further, it has been suggested that females may adjust their breeding strategy according to their own and their mate's colour morph (Galeotti et al. 2013).

A necessary condition for the colour of alternative morphs to function as social signals is that the animals themselves can perceive them as different stimuli, which ultimately depends on their visual perception, not ours (Teasdale et al. 2013; Pérez i de Lanuza and Font 2014). Research on colour polymorphic *P. muralis* rests on the reasonable assumption that lizards perceive their own chromatic variation as categorically distinct phenotypes (i.e. morphs), much as humans do. However, nobody has formally tested this assumption. Given the known differences between the visual systems of lizards and humans, establishing the existence of discrete colour morphs from the lizards' perspective is essential for many current hypotheses about the genetic underpinnings of the polymorphism and the evolutionary processes generating and maintaining it (e.g. Cote et al. 2008; Paterson and Blouin-Demers 2017).

Although the human visual system has little trouble identifying discrete colour morphs in *P. muralis*, lizards could perceive their own colour variation in a different way. *Podarcis muralis* has, in common with other diurnal lizards, a sophisticated colour vision system with four types of single cones that are sensitive to light in the wavelength range between 320 and 700 nm (Pérez i de Lanuza and Font 2014; Martin et al. 2015*a*). Their retinas also contain large numbers of long-wavelength-sensitive double cones that are thought to be responsible for luminance (i.e. brightness) perception (Loew et al. 2002; Olsson et al. 2013). As the ventral colours differ both in spectral shape and in luminance (Pérez i de Lanuza et al. 2013*a*; Pérez i de Lanuza and Font 2015), discrimination of the alternative morphs could be based on either of these variables.

Discrimination experiments are a useful tool to confirm animal colour vision and the perception of colour differences (Kelber et al. 2003; Kelber and Osorio 2010). There is no shortage of papers testing the ability of lizards to visually discriminate between stimuli of different size, shape, pattern, luminance, or colour (i.e. hue). In his comprehensive review of learning processes in reptiles, Burghardt (1977), listed 12 such studies, of which half involved some type of hue discrimination, and more have been published in the ensuing decades. These studies have shown that lizards can discriminate between stimuli differing only in hue (e.g. Wagner 1933; Swiezawska 1949; Rensch and Adrian-Hinsberg 1963; Benes 1969; Dücker and Rensch 1973) or in luminance (e.g. Vance et al. 1965; Garzanit and Richardson 1974; Peterson 1976; Hodgkinson and Still 1980). Unfortunately, few studies have used standard colour stimuli with known reflectance properties (e.g. Ostwald, Munsell) and many do not include luminance controls (i.e. greys). Luminance controls may be of little relevance if the focus of the study is learning per se rather than colour discrimination (e.g. Leal and Powell 2012). But if the goal is to establish that colour vision is present, luminance controls are essential to ensure that the animals respond differentially to hue independent stimuli (Kelber et al. 2003). Also, most studies using colours as discriminanda make no attempt to use colours that resemble natural stimuli that the animals might encounter in the field, such as colours of prey or conspecifics (but see Hews and Dickhaut 1989).

Here we use a behavioural experiment adapted from previously used experimental designs (Leal and Powell 2012; see also Clark et al. 2014) to test if *P. muralis* can discriminate the ventral colour variation shown by this species. Additionally, we reanalyse spectral data collected in previous studies (Pérez i de Lanuza et al. 2013*a*, 2014; Pérez i de Lanuza and Font 2015) and use visual modelling techniques based on the receptor noise model (Vorobyev and Osorio 1998) to assess the colour variation and quantify the degree of discriminability among colour morphs from a lizard's visual perspective.

2.3. Methods

We captured 20 lizards (10 males and 10 females) by noosing (i.e. using a pole with a slipknot that tightens around the neck of the lizard) on July 8, 2015 in Angostrina (Eastern Pyrenees, France). The lizards were individually held in cloth bags and transferred by car to the Ethology lab at the University of Valencia (470 km) on the day following their capture. In the laboratory, lizards were housed in individual terraria (20 x 40 and 26 cm high) provided with water, a shelter, and a brick over which an incandescent reflector lamp (40 W; Radium ©, Parabolica RP50) was suspended. Terraria were housed in an animal room with temperature and light cycle mimicking average field conditions at the capture site (thermal gradient of 24-40°C inside the terraria during the day; 12.5L: 11.5D). In addition, ultraviolet (UV)-rich fluorescent tubes (Reptistar 5.0: Sylvania, Danvers, MA, USA; colour temperature 6500K) suspended above the terraria were switched on for 1.5 h (12.00–13.30 h) three times weekly. During the colour discrimination experiments (July 11 to August 27), lizards had access to food only during the experimental trials, but individuals failing to eat in five consecutive trials were discarded from the experiment and fed 3-4 times weekly. After the experiments were completed, all lizards were released back at their capture location on August 31. Lizards were captured under research permit number 2013095-0001 from the Préfecture des Pyrénées-Orientales (France). This research complied with the ASAB/ABS Guidelines for the Use of Animals in Research and all applicable local, national and European legislation.

2.3.1. Colour discrimination experiment

For the colour discrimination experiment, we trained lizards to eat mealworm larvae (*Tenebrio molitor*; ca. 2 cm long and 150 mg) dusted with vitamins (Exo Terra, Montreal, QC, Canada) from a well in a wooden block. The block had four evenly spaced circular wells (2 cm diameter, 1.5 cm deep) and each well was associated with a different colour by means of two coloured paper stickers: a ring-shaped sticker surrounding the well's entrance and a rectangular sticker marking its position on the lateral side of the block (**Fig. 2.1**). In total, we used 10 wooden blocks, each of which was used by only two lizards. Each block had three wells fitted with orange, white and yellow stickers resembling the ventral colours of *P. muralis*, and a fourth grey-coloured sticker having the same luminance as the training colour (see stimuli design below). To prevent the lizards from locating prey using chemical cues we placed two live mealworm larvae inside all wells for two weeks before the start of the experiment, and two nights per week during the experiment (Monday and Thursday; 19:30 - 9:30).

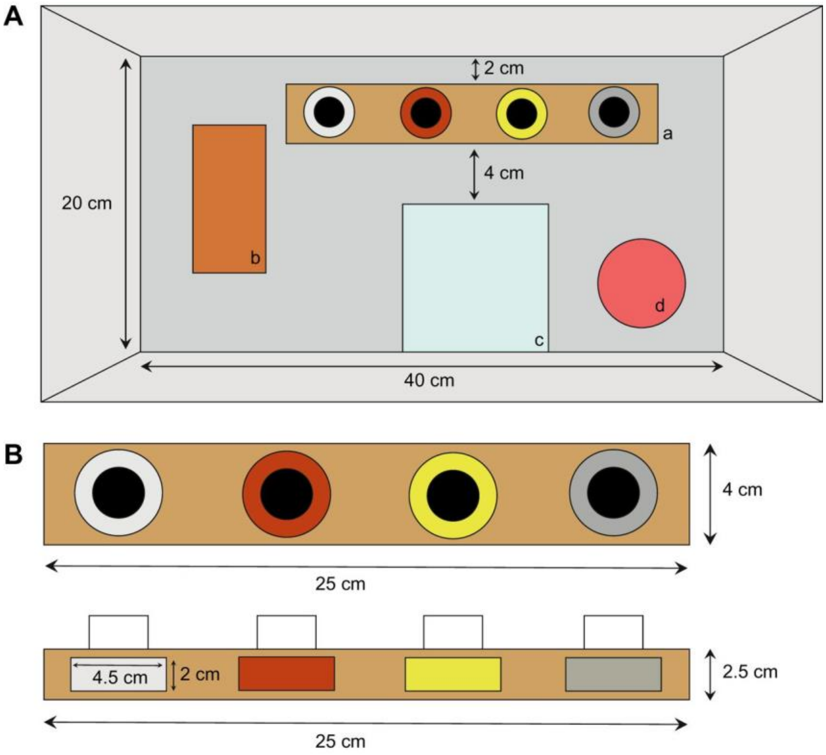

Figure 2.1. Experimental terraria. A) Disposition of elements within the individual terraria: a) wooden block (only inside the terraria during experiments); b) basking brick; c) shelter; d) water dish. B) Schematic view of the experimental wooden block as seen from above (lids removed) and from the side facing the shelter (below, lids on wells).

We trained half the animals (five males and five females) to eat from the orange well, and the other half to eat from the yellow well (**Fig. 2.S1**). To begin a trial, we introduced two larvae in the orange or yellow well, and positioned the wooden block inside the terrarium of the animal to be tested (4 cm from the shelter's entrance; see **Fig. 2.1**). A trial ended when the lizard located and ate the prey or after 25 min had elapsed. We tested each lizard on alternate days to ensure motivation toward the food reward (one trial/day). Prior to each trial the position of the colour stickers was determined using a random number generator, discarding combinations in which the larvae would remain in the same position as in the previous trial. During trials, both the UV-rich fluorescent tube and the incandescent lamp were on, providing a continuous light spectrum across the entire visual range of *P. muralis* (see irradiance spectrum in **Fig. 2.S2**). We conducted trials during the lizards' natural daily period of activity (10.00-13.30 and 16.00-19.00 local time).

The experiment comprised a training phase and a testing phase. Training consisted of six trials (12 days) during which the mealworm larvae were in the orange or yellow well and we gradually reduced their visibility by partially covering the well with a white plastic lid weighing 8.5 g (i.e. covering 50% of the opening in trials 1 and 2, 75% in trials 3 and 4, 95% in trials 5 and 6). During the testing phase (18 trials, 36 days), we presented the wooden block with all four wells completely covered and videotaped every trial using a photographic camera (Canon © EOS 60D, Tokyo, Japan) mounted on a tripod. We played back each filmed trial and one researcher (J.A.) recorded whether or not the animal found and consumed the prey, the number of incorrect lids lifted (errors), and the time elapsed since it left the refuge until it lifted the correct lid (latency).

2.3.2. Experimental stimuli design

Experimental stimuli were designed to resemble the natural colour variation found in *P. muralis* ventral coloration. We prepared a palette of whites, yellows and oranges in Adobe Illustrator and printed them on five types of paper differing in whiteness, brightness and shade (resulting in 790 stimuli). These stimuli were measured with a portable spectrometer (see details below) and differences with natural lizard reflectance spectra (i.e. averaged over at least 164 spectra per morph; **Fig. 2.2**) were explored graphically, comparing colour variables (i.e. hue, chroma, and brightness), and calculating chromatic and achromatic distances between any two colours using visual modelling (see details below). We chose the three chromatic stimuli that best matched natural colours (orange: CMYK = 0%, 99%, 91%, 0%, Couché mate 130 g/m^2; white: CMYK = 6%, 10%, 21%, 2%, Color copy 250 g/m^2; yellow: CMYK = 0%, 23%, 86%, Couché mate 130 g/m^2; **Fig. 2.2** and **S3**).

By presenting an achromatic control with the same luminance as the training colour, we controlled the possibility that lizards base their discrimination on luminance differences among the colour stimuli. We designed two achromatic controls, isoluminant with the chromatic yellow and orange experimental stimuli. Unfortunately, no evidence regarding how lizards judge luminance differences is available. Therefore, we conservatively designed the achromatic stimuli to show an absolute luminance (i.e. the integral of the spectral curve) similar to the chromatic stimuli (orange-achromatic: CMYK = 0%, 0%, 0%, 82%, Couché mate 130 g/m^2; yellow-achromatic: CMYK = 0%, 0%, 0%, 67%, Couché mate 130 g/m^2, **Fig. 2.2** and **2.S3**). However, as it has been hypothesised that luminance is perceived by a sensory channel involving the long-wavelength sensitive cones (as single cones: Fleishman et al. 1997; Fleishman and Persons 2001; or as the main component of double cones: Osorio and Vorobyev 2005), we also compared luminance between the chromatic stimuli and their corresponding achromatic stimuli using visual models assuming that luminance is processed by the long-wavelength sensitive cones (see methodological details below).

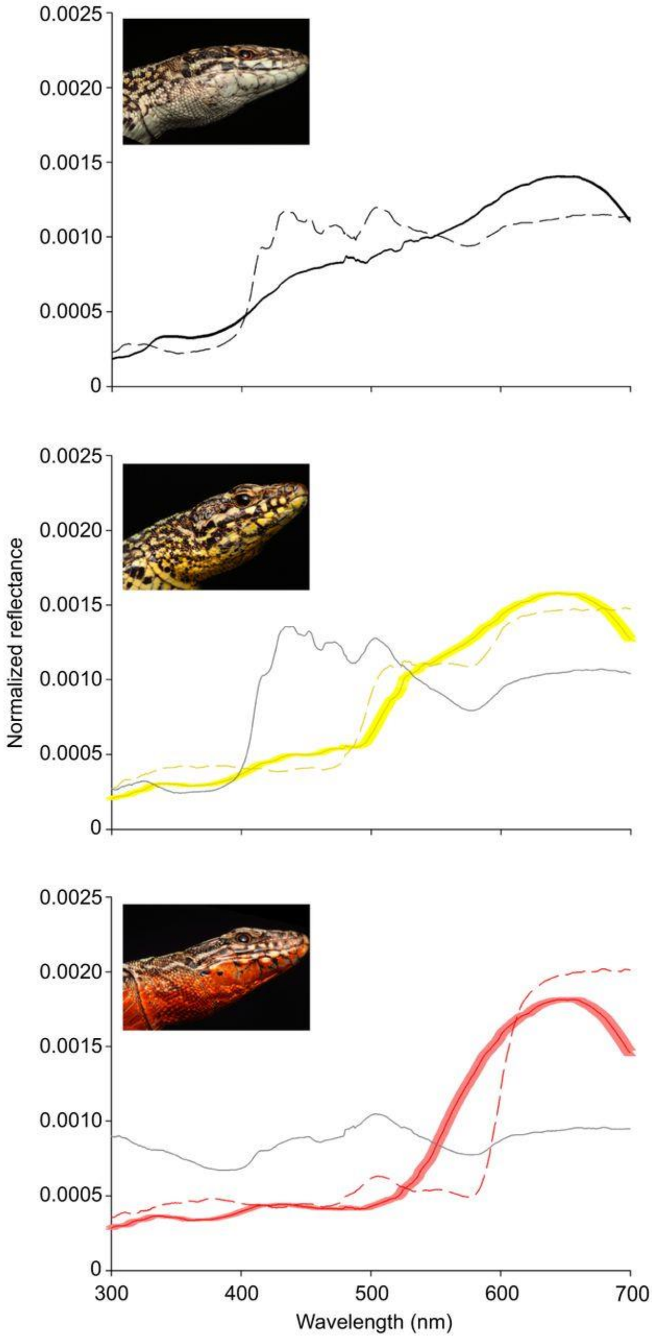

Figure 2.2. Reflectance spectra. Lines represent spectra from natural *Podarcis muralis* throats (solid lines and surrounding area = mean ± 1 SEM) and the corresponding artificial stimuli (long-dashed lines represent the chromatic stimuli; grey lines for yellow and orange morphs represent the corresponding achromatic stimuli). For clarity, the spectra have been normalized dividing the reflectance at each wavelength by the entire reflectance under the curve (i.e. luminance). See sample sizes for natural spectra in the text.

2.3.4. Statistical analyses

In order to account for inter- and intra-individual variability within trials we grouped experimental trials in blocks of three, hence defining six blocks where number of errors and mean latency were calculated. We then compared the mean number of errors per individual in each block with the average expected by chance (Baldwin 1979; Margules and Gallistel 1988; Brannon and Terrace 1998). With four options to choose, the maximum number of errors is three. If choosing randomly, lizards are equally as likely to make zero, one, two or three errors. By multiplying each number of errors by its probability (1/4) we obtain the mean number of errors expected by chance ($\bar{X}_{\text{errors}} = 0 \text{ x } 1/4 + 1 \text{ x } 1/4 + 2 \text{ x } 1/4 + 3 \text{ x } 1/4 = 6/4 = 1.5$). We established better-than-chance performance in a block of trials as the criterion for successful learning.

To check for a decrease in both the mean number of errors and the mean latency with time, we ran two generalized linear mixed models (GLMMs) with errors (Poisson distribution) and latency (Gaussian distribution) as dependent variables, block and sex as fixed factors, and animal identity, training colour and (only in the model with number of errors) whether or not the animal located the prey as random factors. We checked graphically that both the number of errors and latency followed non-normal distributions (qqplots in R; R Core Team 2016). We power-transformed latency to follow a normal distribution by calculating the fourth root of each value (Shapiro-Wilk test for normality: $W = 0.99$, $p = 0.14$). We did not transform the number of errors as transforming count data is not recommended (O'Hara and Kotze 2010), and adjusted a GLMM following a Poisson distribution after checking the mean and the variance of this variable had similar values ($\bar{X} = 1.12$, $S^2 = 0.98$).

In addition, as a more conservative test of discrimination learning, we coded the performance of each individual lizard in each trial as either 1 (if the lizard's first choice was correct) or 0 (if the lizard made any number of errors). Then, we fitted a logistic mixed model with the lizard's performance as dependent variable, trial and sex as fixed effects, and animal identity, training colour and whether or not the animal located the prey as random factors. We plotted the fitted model with confidence intervals against time (i.e. trial) to check for a significant increase in the probability of correct first choice from chance levels (0.25).

Model fitting and model selection were conducted using backward single term deletions ($p \leq 0.05$) of the saturated model followed by model comparisons via likelihood ratio tests (lme4 package in R: Bates *et al.,* 2015; R Core Team 2016). We did not find a problem of overdispersion in the Poisson model ($\varphi = 1.002$). We explored graphically that residuals from both models conformed to normality and homoscedasticity assumptions by plotting them against the logarithm of the fitted values.

2.3.5. Visual modelling

To determine quantitatively the discriminability between pairs of ventral colours (i.e. white against yellow, white against orange, and yellow against orange) we used Vorobyev and Osorio's (1998) receptor noise limited model. Calculations were performed in R 3.3.2 (R Core Team 2016) using the package PAVO (Maia et al. 2013). We assumed a cone abundance ratio of 1:1:1:4 (corresponding to the UV-, short-, middle- and long-wavelength-sensitive cones; J.F. Le Galliard, personal communication based on results from Martin et al. 2015*a*), and a Weber fraction of 0.05 for the long-wavelength sensitive cone (Siddiqi et al., 2004; previously used in other studies with lacertids: Marshall and Stevens, 2014; Martin et al., 2015*a*). Cone absorbance spectra of *P. muralis* were obtained from the literature (Martin et al., 2015*a*). As illuminant we used the irradiance spectrum inside the experimental terraria resulting from the combination of the light provided by the incandescent lamp and the UV-rich fluorescent tube (**Fig. 2.S2**). The discriminability between morphs was expressed as just noticeable differences (JND). A value of 1 JND is traditionally assumed as the threshold of discrimination between two colour patches, i.e. pairs of colours giving values <1 JND are not discriminable, values between 1 and 3 JND represent colours that are discriminable under good illumination conditions, and values above 3 JND represent easily discriminable colours (Siddiqi et al. 2004; Marshall and Stevens, 2014). However, as behavioural data to confirm this assumption in *P. muralis* are completely lacking, these thresholds have to be interpreted with caution. Therefore, we conservatively adopted a threshold of 3 JND to declare that two colours were discriminable to the lizards.

To determine quantitatively whether our experimental stimuli resembled the lizards' ventral coloration, we also used the Vorobyev and Osorio's receptor noise model to calculate chromatic and achromatic distances between natural spectra and the spectra obtained from the artificial stimuli. Based on these analyses, we chose for behavioural experiments those artificial chromatic stimuli that minimize the chromatic distances when compared with the natural spectra (see below), and those artificial achromatic stimuli that minimize the achromatic distances when compared with the artificial chromatic stimuli.

For visual modelling we used reflectance spectra of throat coloration from the same Pyrenean population of *P. muralis* where experimental animals were obtained (Angostrina, Eastern Pyrenees, France). Spectra were compiled from previously published studies (Pérez i de Lanuza et al. 2013*a*, 2014; Pérez i de Lanuza and Font 2015), resulting in a dataset of 643 adult lizards showing pure morphs (199 white males, 88 white females, 135 yellow males, 57 yellow females, 131 orange males, and 33 orange females). Reflectance spectra were obtained with a USB-2000 portable spectrometer and a PX-2 xenon strobe light source (Ocean Optics Inc.; Dunedin, FL, USA), calibrated with a Spectralon white diffuse reflectance standard (Labsphere) (see Font *et al.*, 2009; Pérez i de Lanuza et al., 2013*a*, 2014; Badiane et al. 2017 for more details). Irradiance inside the experimental terraria was measured with a second USB-2000 spectrometer calibrated by means of a LS1-CAL calibration light source (Ocean Optics), using a cosine-corrected irradiance probe (Ocean Optics CC-3-UV). To assess differences in discriminability (i.e. chromatic distances) between the different pairs of ventral colours, we used a generalized linear model including the paired colour combinations (i.e. white-yellow, white-orange, and yellow-orange) and sex as factors, as well as their interaction.

As parameters used to run visual models (i.e. cone proportion: 1:1:1:4; Weber fraction = 0.05) are not supported by any empirical data in *P. muralis*, and because small variations in visual parameters can affect the results of visual modelling (Lind and Kelber 2009; Bitton et al. 2017), we repeated the analyses to assess whether our results are robust to variations in these parameters. Therefore, we also run models with cone proportions of 1:1:1:1, 1:1:1:8, and 2:3:3:11 (i.e. the original count presented by Martin et al., 2015*a*), and values of Weber fraction of 0.03 and 0.07.

2.4. Results

2.4.1. Colour discrimination experiment

Twelve lizards (seven males: three trained to eat from the orange well and four from the yellow one; and five females: three trained to orange, two to yellow) completed the experiment. The remaining eight individuals failed to uncover a well in five consecutive trials and were discarded from the experiment.

Table 2.1. Mean number of errors and confidence interval (CI) in each of six consecutive blocks of trials (three trials per block) comprising the training phase the experiment, and p-values for one-way Wilcoxon tests ($\mu < 1.5$). N = Trials per block (out of 36) in which all 12 trained lizards uncovered at least one well.

Block	**N**	**Mean**	**CI**	***p***
1	32	1.34	0.35	0.158
2	34	1.29	0.41	0.153
3	32	1.19	0.36	0.049
4	36	1.08	0.36	0.013
5	35	1.09	0.30	0.008
6	33	0.73	0.27	$1.92\ e^{-5}$

The mean number of errors per block was significantly smaller than expected by chance (1.5) in all but the first two blocks of trials (**Table 2.1**; **Fig. 2.3**). Both the number of errors (**Fig. 2.3**) and latency (**Fig. 2.S4**, **Table 2.2**) showed a significant reduction with time (Errors ~ Stage, Z = -2.40, Std. Coeff. ± SE = -0.30 ± 0.13, P = 0.016, see **Table 2.S2**; Latency ~ Stage, t = -3.49, Std. Coeff. ± SE = -0.55 ± 0.16, P < 0.0001, see **Table S1**). Also, we found an effect of sex on the number of errors (Errors ~ Sex, $Z = 2.58$, Std. Coeff. ± SE = 0.34 ± 0.13, $P = 0.001$) due to females committing less errors than males on average across blocks (μ ± SEM, males = 1.24 ± 0.09, females = 0.94 ± 0.10; **Fig. 2.2**). After finding violations of normality in the model with the number of errors, we run a bootstrap analysis based on 10,000 simulations and compared the mean coefficients, their standard errors and p-values with those obtained before. This analysis confirmed our GLMM results showing similar coefficients but smaller standard errors and p-values (see details in **Table S1**).

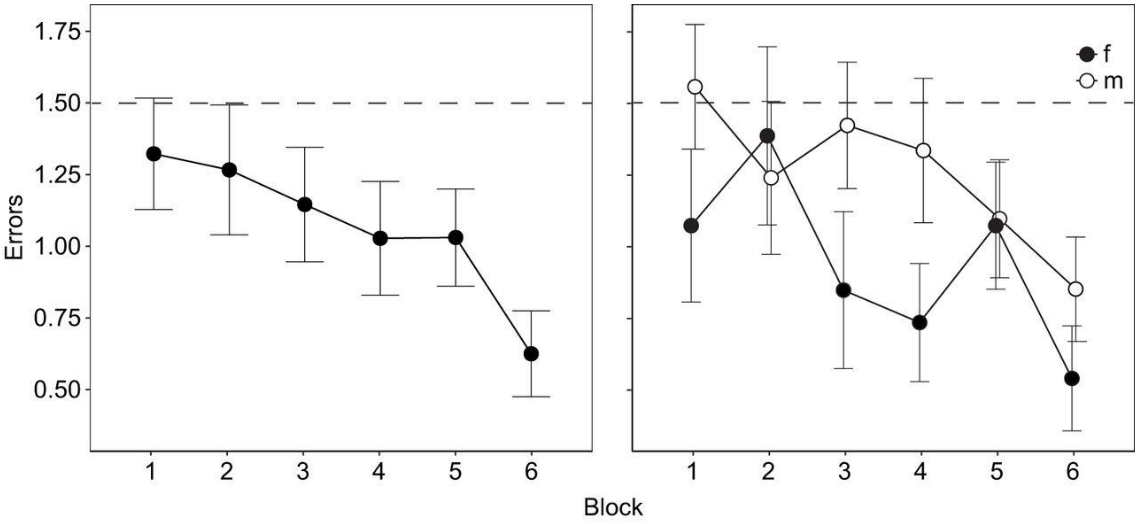

Figure 2.3. Mean number of errors per block of trials. Left, mean number of errors pooling males and females (12 lizards, three trials per block). Right, mean number of errors per block of trials, grouped by sex (f = females, m = males). Each well of the wrong colour uncovered by a lizard in the allotted time (25 min) was considered an error (maximum of three errors). Error bars represent the standard error of the mean. After two blocks of trials, the lizards responded to training by showing a significantly smaller number of errors than expected by chance (dashed line at 1.5).

Plotting the logistic mixed model fitted with confidence intervals against time shows that the probability of the lizards' first choice being correct nearly doubled during the experiment, from chance levels in the first trial ($p \pm CI = 0.23 \pm 0.08$) to even odds in the last trial ($p \pm CI = 0.41 \pm 0.04$; **Fig. 2.4**).

Throughout the experiment no colour was overrepresented in the total record of errors (trained to orange, $\chi^2 = 3.15$, $df = 2$, $p = 0.207$; trained to yellow, $\chi^2 = 0.703$, $df = 2$, $p = 0.704$; **Fig. 2.5**). However, wrong first choices were biased toward yellow in lizards trained to orange more frequently than to white or to the achromatic grey control ($\chi^2 = 8.41$, $df = 2$, $p = 0.015$), while lizards trained to yellow showed a marginally non-significant bias toward orange ($\chi^2 = 5$, $df = 2$, $p = 0.08$; **Fig. 2.5**).

2.4.2. Visual modelling

The natural spectra from the three morphs are relatively segregated in colour space (**Fig. 2.6**). Assuming a discrimination threshold of 3 JND and the proposed cone proportions (i.e. 1:1:1:4) and Weber fraction (i.e. 0.05), the three colours are chromatically discriminable considering all paired combinations (**Fig. 2.7**). Similar results were found using models with alternative values of cone proportions and/or Weber fraction (see Materials and Methods). The effect of considering other visual parameters is graphically illustrated in **Fig. 2.S6**.

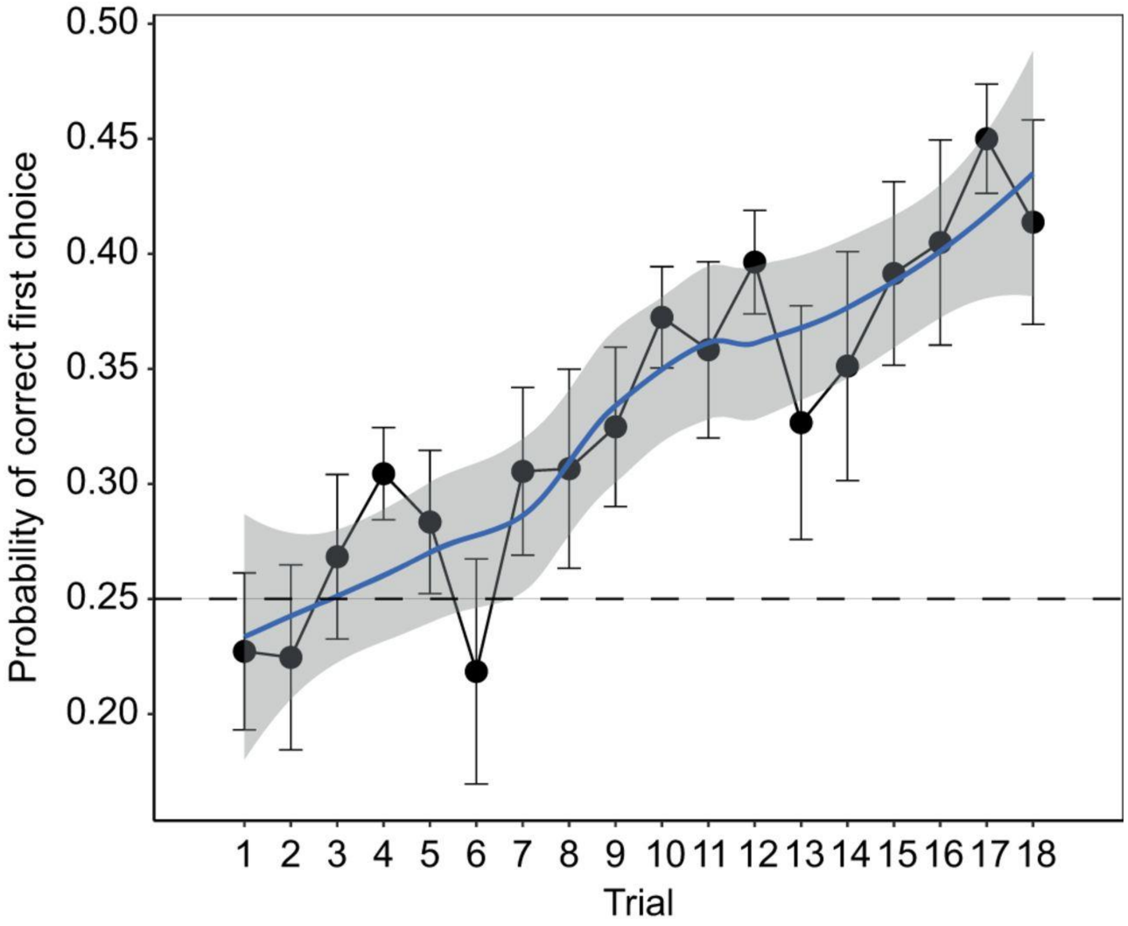

Figure 2.4. Fitted logistic mixed model of the probability of correct first choice against time (i.e. trial). Circles and error bars represent mean probability with 95% confidence intervals in each trial. Blue line and shaded area show the smoothed tendency line with its confidence interval. The learning curve shows that the probability of correct first choice nearly doubled during the experiment.

Discriminability varies with morph combination (t = -5.66, Std. Coeff. ± SE = -0.13 ± 0.02, P < 0.00001), the white-orange combination being more discriminable than the white-yellow and the yellow-orange combinations (P < 0.00001), and the white-yellow combination is more discriminable than the orange-yellow combination (P < 0.00001). Male morphs are more discriminable than female morphs (t = -4.27, Std. Coeff. ± SE = -0.07 ± 0.02 P = 0.00002), and the interaction between morph combination and sex is significant (*t* = 4.10, Std. Coeff. ± SE = 0.05 ± 0.01, *P* = 0.00005). Results remain significant using visual models with alternative visual parameters (**Table 2.S3**).

Table 2.2. Mean latency in seconds, standard error (SEM), and confidence interval (CI) in each of six consecutive blocks of trials (three trials per block) comprising the training phase of the experiment. N = Trials per block (out of 36) in which the trained lizards found the prey.

Block	**N**	**Mean**	**CI**	**SEM**
1	27	284.67	119.75	58.26
2	30	325.60	116.91	57.16
3	29	310.07	127.80	62.39
4	35	243.09	99.48	48.95
5	30	217.03	125.91	61.56
6	31	152.13	31.65	64.64

2.5. Discussion

Our results show that adult *P. muralis* learn to perform a procedural task (lifting the one lid among four giving access to prey in a colour-coded well), as shown by a significant reduction in both the number of errors and latency throughout the experiment. The fraction of lizards that successfully learned to perform the discrimination task (12 out of 20) is similar to that observed in experiments with other lizard species (e.g. Leal and Powell 2012). The number of wrong choices was smaller than expected by chance after six trials (a week of testing) and the probability of correct first choice after 18 trials nearly doubled from initial chance levels. These results demonstrate that *P. muralis* is capable of discriminating experimental colour stimuli designed to match natural colours on the ventral surface of this species. Although the achromatic controls did not match perfectly the luminance of chromatic stimuli, as lizards did not choose the achromatic (grey) control incorrectly more often than the other available incorrect stimuli, we can reasonably assume that the discrimination was based on wavelength (i.e. hue) rather than luminance differences among stimuli. This evidence provides support for the idea that *P. muralis* is capable of discriminating its own ventral colour variation based on hue. Results of reflectance spectrophotometry and visual modelling reinforce this conclusion, showing that ventral colours of *P. muralis* correspond to discrete rather than continuous colour categories based on their spectral properties (independent of the human visual system) and lizard cone sensitivities. Interestingly, lizards were able to discriminate the two artificial stimuli showing the smallest perceptual distance (i.e. yellow vs orange), which is in the lower range of perceptual distances generated by the natural yellow and orange morphs (**Fig. 2.7**).

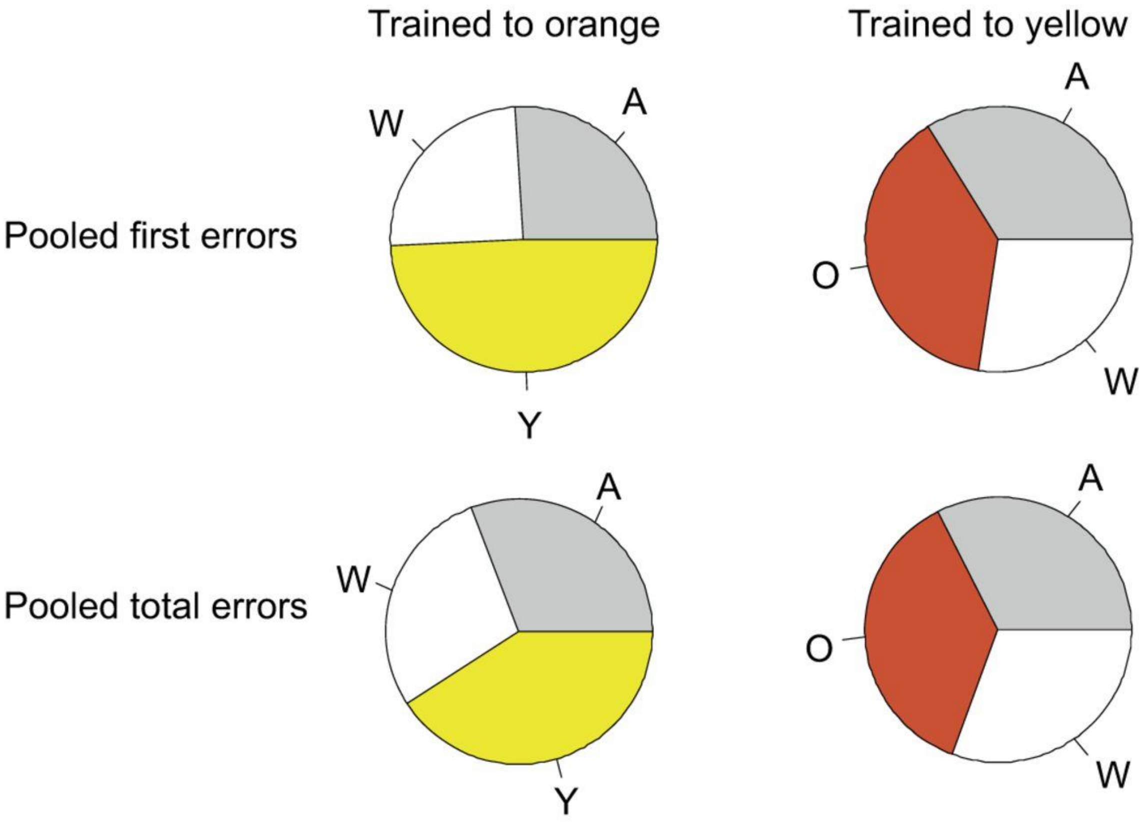

Figure 2.5. Pie charts representing the relative frequency of each colour among the pooled errors performed by the lizards. Morphs: O = orange; W = white; Y = yellow; A = achromatic stimulus matching the luminance of either orange or yellow.

Our results bear out the assumption that the ventral polymorphism of *P. muralis* is discrete, encompassing several chromatically distinct morphs. On a practical note, our results also provide support for the use of a categorical classification of ventral colours in *P. muralis*, although perhaps not in other lacertid species. For example, in *Zootoca vivipara* the assumption that the polymorphism is represented by categorically distinct colour morphs unleashed a heated controversy (Vercken et al. 2007, 2008; Cote et al. 2008). Unfortunately, although mate choice is involved in the maintenance of colour polymorphism in *Z. vivipara* (Sinervo et al. 2007; Fitze et al. 2014; San-Jose et al. 2014), colour discrimination among morphs was not tested and thus there is no conclusive evidence that lizards discriminate morphs visually, or that ventral colours in this species act as social signals.

Previous studies of the visual system of *P. muralis* demonstrated that ventral colours differ in conspicuousness when viewed against other body patches or against natural backgrounds (i.e. rocks, vegetation), which raises interesting questions regarding their potential role as social signals (Pérez i de Lanuza and Font, 2015). The results presented here indicate that colour discriminability varies according to the morphs being compared, white and orange being more discriminable than white and yellow, and yellow and orange ventral colours, and white and yellow more discriminable than yellow and orange combinations. Although behavioural results supporting this conclusion are lacking, chromatic distances obtained with visual modelling techniques may be good predictors of colour discriminability even when distant colours are compared (Fleishman et al. 2016). Therefore, discriminability differences among pairs of ventral colours of *P. muralis* may have implications for signal detection and discrimination. This may be especially relevant for intra- and intersexual interactions that can be modulated by ventral colour, such as mate choice or male-male contests (Pérez i de Lanuza et al. 2013*a*, 2016*b*; Abalos et al. 2016).

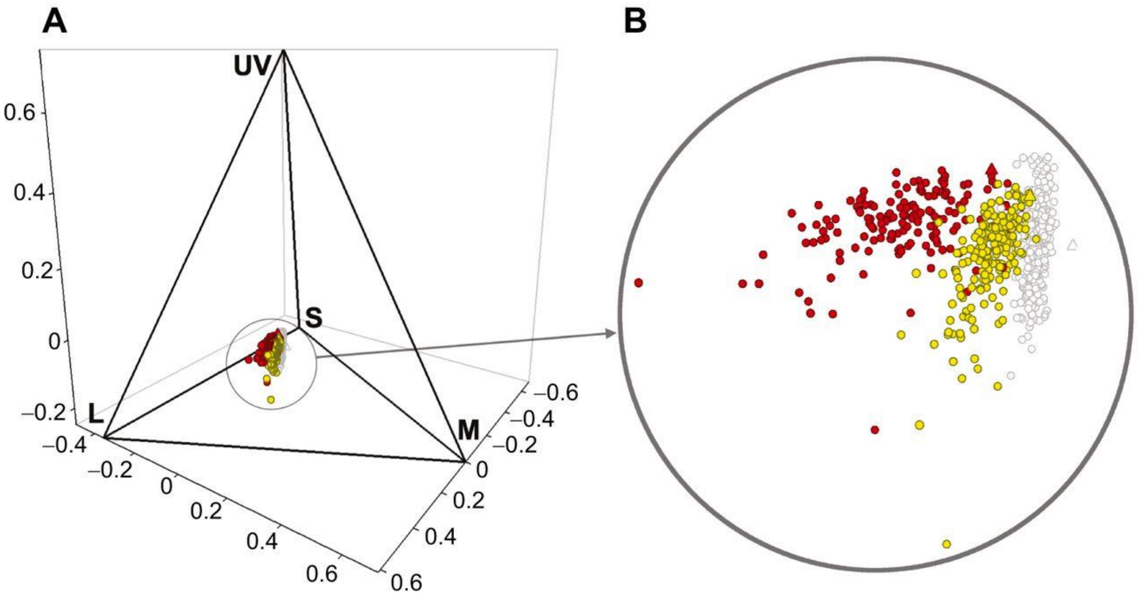

Figure 2.6. Chromaticity diagram showing the location of chromatic points. Panel A shows the entire receptor space and panel B a detail of the volume occupied by the chromatic points. Circles correspond to chromatic points from the natural ventral colours (pooling males and females). Triangles correspond to the artificial stimuli. The colours of symbols indicate the morph. See sample sizes in the main text. The overlap between the white and the yellow volumes amounts to 27.8% of the white volume and 4.9 of the yellow volume; the overlap between the white and the orange volumes represents 5.1 % of the white volume and 0.7 % of the orange volume; the overlap between the yellow and the orange volumes is 12.2% of the yellow volume and 9.1 of the orange volume. The white artificial stimulus is located outside (but close to) the cloud of white natural colours. The other two artificial stimuli fall within their respective cloud of natural colours, but are located at the periphery. However, note that the perceptual distance between the artificial stimuli is smaller than that of many chromatic points of different morphs.

We found that sexes differ in colour morph discriminability, a finding that could have biological relevance (Zhou et al. 2015). However, this result may be a consequence of sexual dichromatism (i.e. slight differences in spectra) resulting in small (but significant) differences in colour distances between males and females (see, for example, the chromaticity diagram in **Fig. 2.6**). We also found that males and females differ in the number of errors, but this difference may be caused by the small sample size used in the experiment, which allows for extremely good performers to bias our results.

Although colour polymorphisms provide invaluable models to study the evolution and maintenance of polymorphisms generally (e.g. Roulin 2004; Sinervo and Calsbeek 2006; Chunco et al. 2007; Pryke and Griffith 2009*b*; Mckinnon and Pierotti 2010; McLean and Stuart-Fox 2014; Wellenreuther et al. 2014; Svensson 2017), insufficient attention has been paid to colour (morph) discrimination and its implications for understanding the significance of polymorphic coloration. It is often assumed that colour polymorphic animals perceive their own colour variation as different morphs, and that colour variants represent qualitatively different signals. However, this assumption has an unstable foundation if no evidence of colour discrimination is provided. We encourage other researchers to obtain evidence that their study animals are capable of chromatically discriminate their colour variation as independent colour morphs, and show behavioural evidence of doing so.

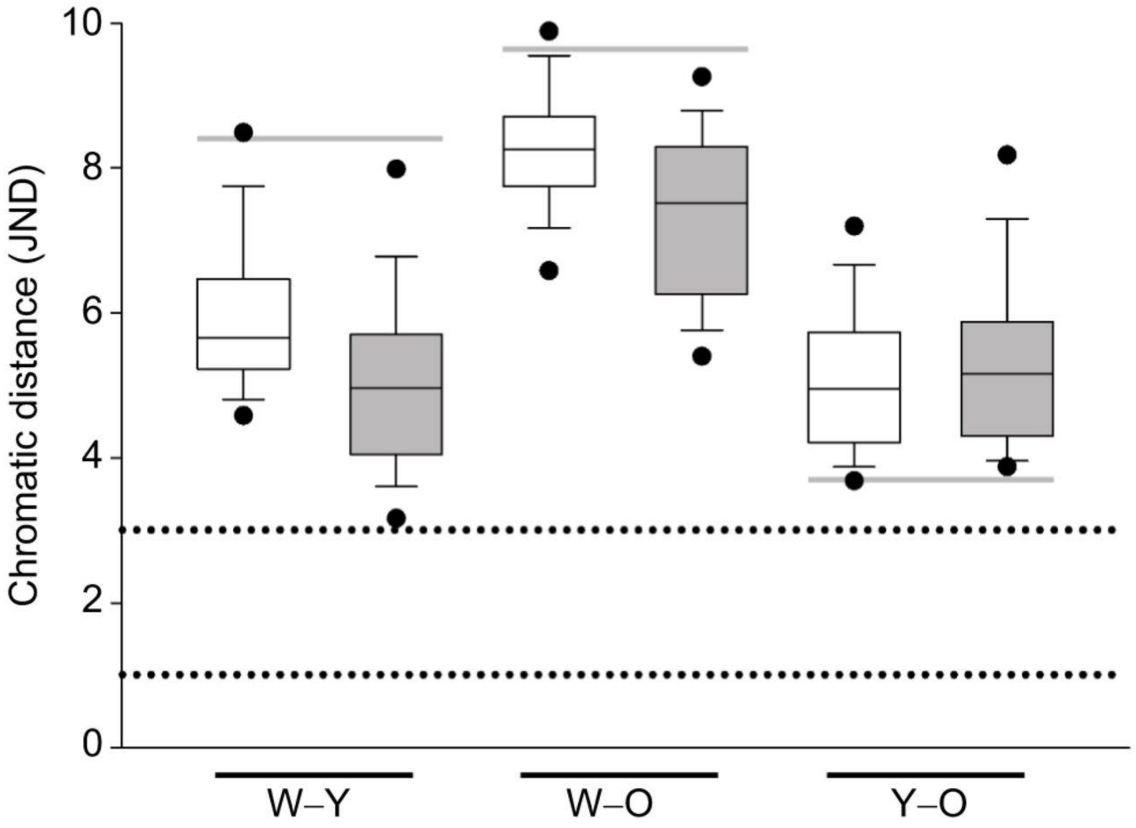

Figure 2.7. Box-plots showing chromatic distances generated by pairs of colour morphs. Morphs: W = white, Y = yellow, O = orange. White box-plots correspond to males and grey box-plots correspond to females. In each case, horizontal lines, boxes, error bars and points indicate, respectively, the median, the 25-75% range, the 10th and 90th percentiles, and the 5th and 95th percentiles. Horizontal dotted lines indicate the discriminability thresholds of 1 JND (values above 1 JND indicate pairs of colours that are discriminable under good illumination conditions) and 3 JND (values above 3 JND indicate easily discriminable pairs of colours). Results considering other cone proportions and other values of Weber fraction are detailed in **Fig. 2.S5**. The impact of these variations is graphically reported in **Fig. 2.S6**. Horizontal grey lines indicate chromatic distances of the experimental stimuli for each pair of colour morphs.

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Competing interests: No competing interests declared.

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2.6. Supplementary Material

Tables

Table 2.S1. Results of mixed effects model used to explore the reduction in latency (seconds elapsed since the lizard left its refuge until it consumed the prey) over sequential blocks of trials (Block).

Term	*b*	SEM	SD	*t*	*p*
Fixed					
Intercept	4.11	0.23		17.79	< 0.0001
Block	-0.16	0.46		-3.49	0.0006
Random					
ID			0.49		
Train			$>10^{-4}$		
Residual			1.02		
Model: Latency ~ Block + (1|ID) + (1|Train)					

Table 2.S2. Results of mixed model exploring the decline in the number of wrong lids uncovered by the lizards before finding the prey (Errors) in sequential blocks of trials (Block). Coefficients, standard errors and p-values are compared with those resulting from a bootstrapping analysis (10.000 simulations). Our results are strong against the violation of normality in the residuals of the model.

	Model					Bootstrapping (10,000 simulations)				
Term	*b*	SE	SD	*Z*	*p*	*Mean β*	SE	SD	*Z*	*p*
Fixed										
Intercept	0.30	0.24		1.25	0.211	0.29	0.25		1.16	0.245
Block	-0.30	0.14		-2.23	0.026	-0.30	0.13		-2.40	0.016
Sex	0.34	0.14		2.40	0.017	0.34	0.13		2.58	0.010
Random										
ID			$<10^{-5}$					$<10^{-5}$		
Train			$<10^{-5}$					$<10^{-5}$		
Eat			0.31					0.31		

Model: Errors ~ Block + Sex + (1|Eat) + (1|Train) + (1|ID)

Table 2.S3. Results from GLM analyses comparing chromatic distances for each pair of morphs (i.e. Morph pair), including Sex as a second factor and the interaction between Morph pair and Sex, for each visual model generated by the combination of the four alternative cone proportions and the three alternative values of the Weber fraction.

			Weber fraction											
			0.05				**0.03**				**0.07**			
			b	**SE**	***t***	***p***	***b***	**SE**	***t***	***p***	***b***	**SE**	***t***	***p***
cone proportion	**1:1:1:4**	**Intercept**	0.98	0.03	29.79	< 0.00001	1.20	0.03	36.55	< 0.00001	0.83	0.03	25.34	< 0.00001
		Morph pair	-0.13	0.02	-5.66	< 0.00001	-0.13	0.02	-5.66	< 0.00001	-0.13	0.02	-5.66	< 0.00001
		Sex	-0.07	0.02	-4.27	0.00002	-0.07	0.02	-4.27	0.00002	-0.07	0.02	-4.27	0.00002
		interaction	0.05	0.01	4.10	0.00005	0.05	0.01	4.10	0.00005	0.05	0.01	4.10	0.00005
	1:1:1:1	**Intercept**	0.99	0.03	29.70	< 0.00001	1.21	0.03	36.36	< 0.00001	0.84	0.03	25.31	< 0.00001
		Morph pair	-0.15	0.02	-6.24	< 0.00001	-0.15	0.02	-6.24	< 0.00001	-0.15	0.02	-6.24	< 0.00001
		Sex	-0.08	0.02	-4.70	< 0.00001	-0.08	0.02	-4.70	< 0.00001	-0.08	0.02	-4.70	< 0.00001
		interaction	0.05	0.01	4.61	< 0.00001	0.05	0.01	4.61	< 0.00001	0.05	0.01	4.61	< 0.00001
	1:1:1:8	**Intercept**	1.16	0.03	33.67	< 0.00001	1.39	0.03	40.09	< 0.00001	1.02	0.03	29.44	< 0.00001
		Morph pair	-0.13	0.02	-5.23	< 0.00001	-0.13	0.02	-5.23	< 0.00001	-0.13	0.02	-5.23	< 0.00001
		Sex	-0.05	0.02	-2.70	0.007	-0.05	0.02	-2.70	0.007	-0.05	0.02	-2.70	0.007
		interaction	0.04	0.01	3.28	0.001	0.04	0.01	3.28	0.001	0.04	0.01	3.28	0.001
	2:3:3:11	**Intercept**	0.87	0.03	26.20	0.00001	1.09	0.03	32.91	< 0.00001	0.72	0.03	21.77	< 0.00001
		Morph pair	-0.13	0.02	-5.64	0.00001	-0.13	0.02	-5.64	< 0.00001	-0.13	0.02	-5.64	< 0.00001
		Sex	-0.08	0.02	-4.63	0.00001	-0.08	0.02	-4.63	< 0.00001	-0.07	0.02	-4.63	< 0.00001
		interaction	0.05	0.01	4.22	0.00003	0.05	0.01	4.22	0.00003	0.05	0.01	4.22	0.00003

Model: chromatic distances ~ Morph pair*Sex

Figures

Figure 2.S1. Frame of a video recording in which two trained lizards in adjacent terraria feed on *Tenebrio molitor* larvae after uncovering the trained colour-identified well.

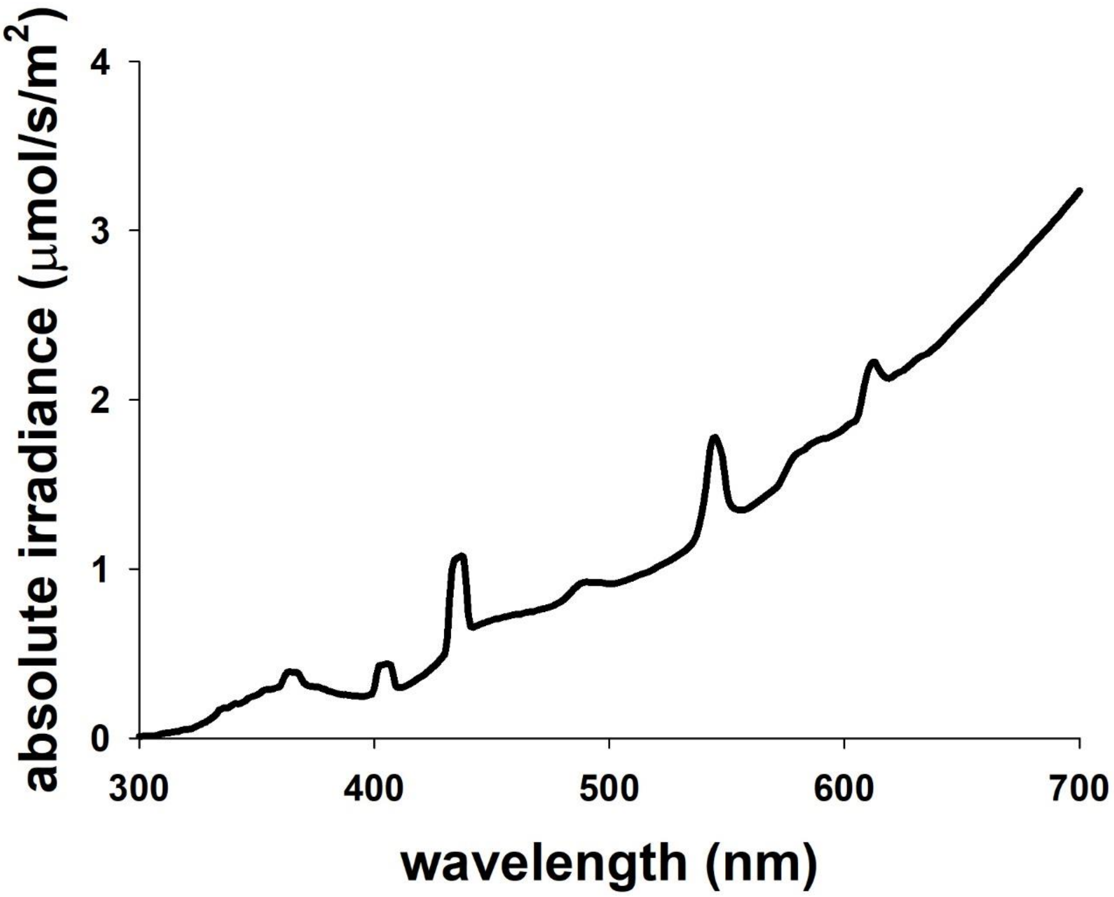

Figure 2.S2. A representative measurement of the absolute irradiance in the experimental terraria resulting from the combination of the ultraviolet-rich fluorescent tube and the incandescent lamp.

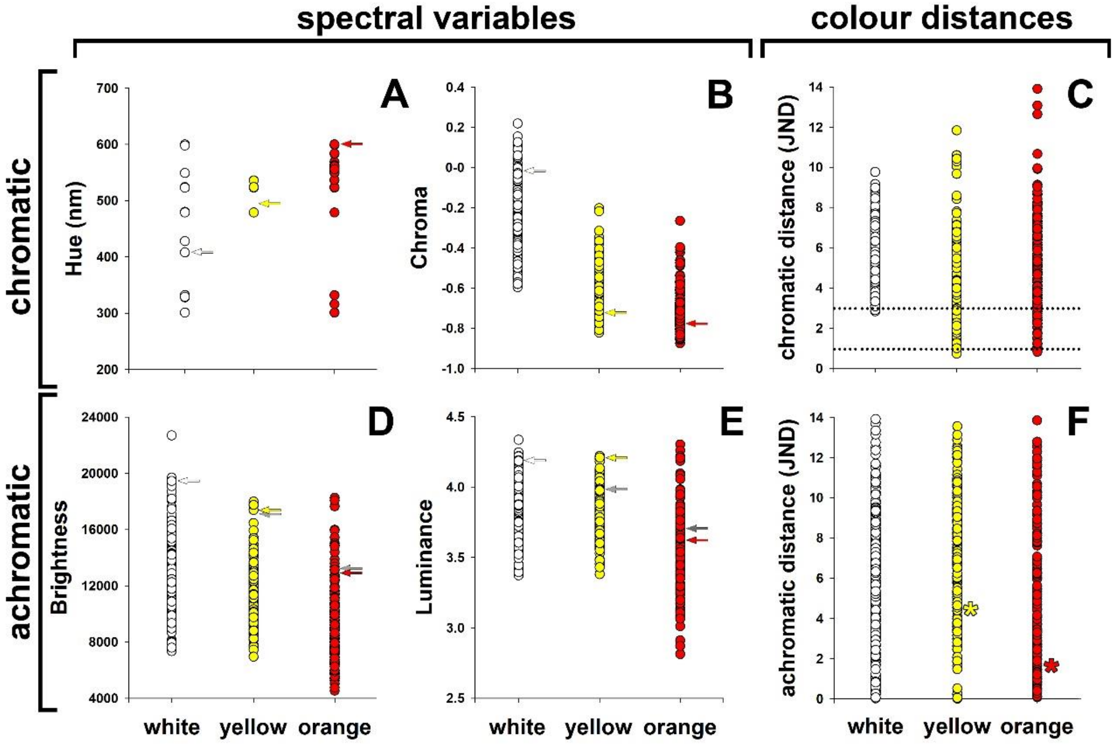

Figure 2.S3. Spectral variables (A, B, D, E) and chromatic (C) and achromatic (F) distances calculated with visual models from male and female throats (circles). Hue was calculated as the wavelength of maximum slope of the curve. Chroma was calculated as (R450 - R700)/R700. Brightness was calculated as the sum of the relative reflectance over the entire spectral range. Luminance was calculated considering the response of the long-wavelength cone type for visual modelling. In A, B, D, and E panels, the arrows indicate the values of the artificial stimuli used in the experiment (in D and E, the grey arrows indicate the values of the achromatic stimuli). In C and F panels, circles indicate the chromatic and achromatic distances between each natural stimulus of each morph against the corresponding artificial chromatic stimuli used in the experiment. Horizontal dotted lines in C indicate the assumed discriminability thresholds of 1 and 3 JND (see details in the main text). The yellow and the orange stars in F indicate the achromatic distance calculated between the yellow and orange artificial stimuli against the corresponding achromatic stimuli.

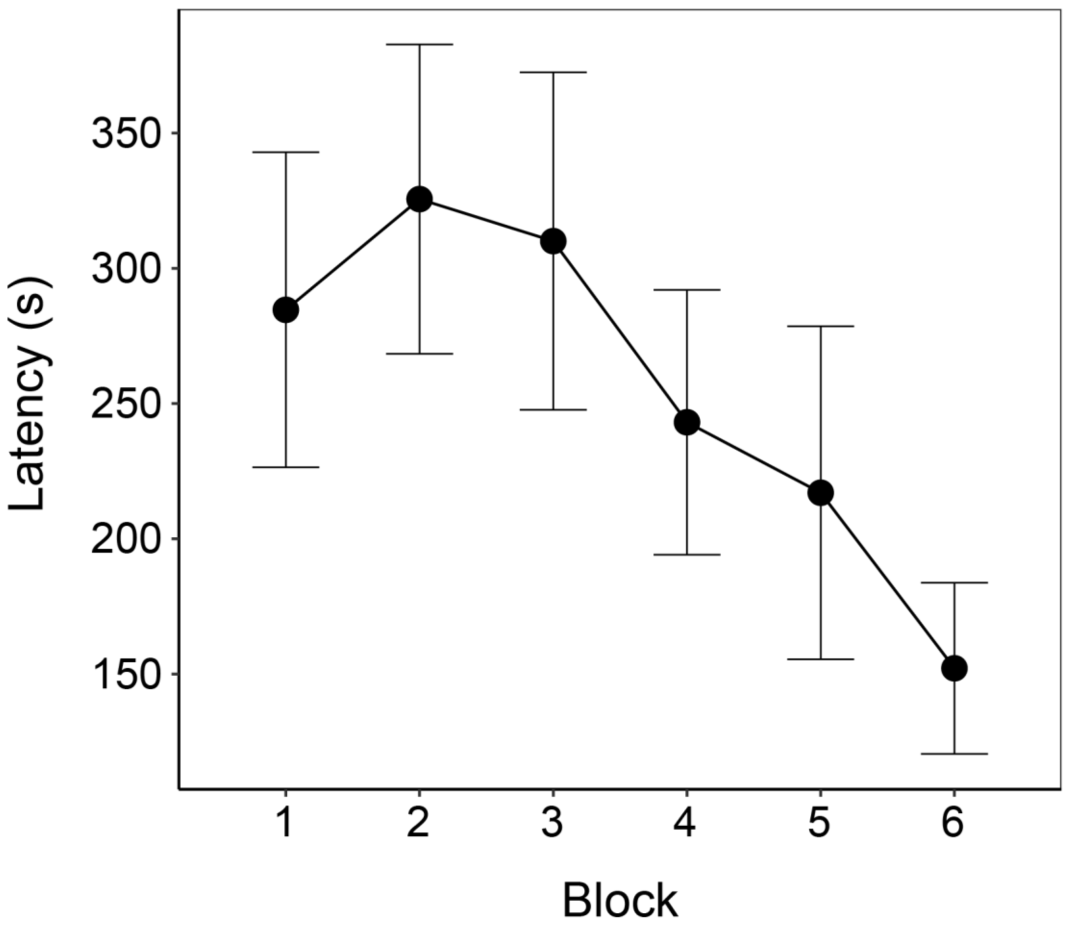

Figure 2.S4. Mean latency per block of trials (12 lizards, three trials per block). Latency was defined as the time (s) elapsed since the lizard left the refuge until it lifted the right lid. Error bars represent the standard error of the mean.

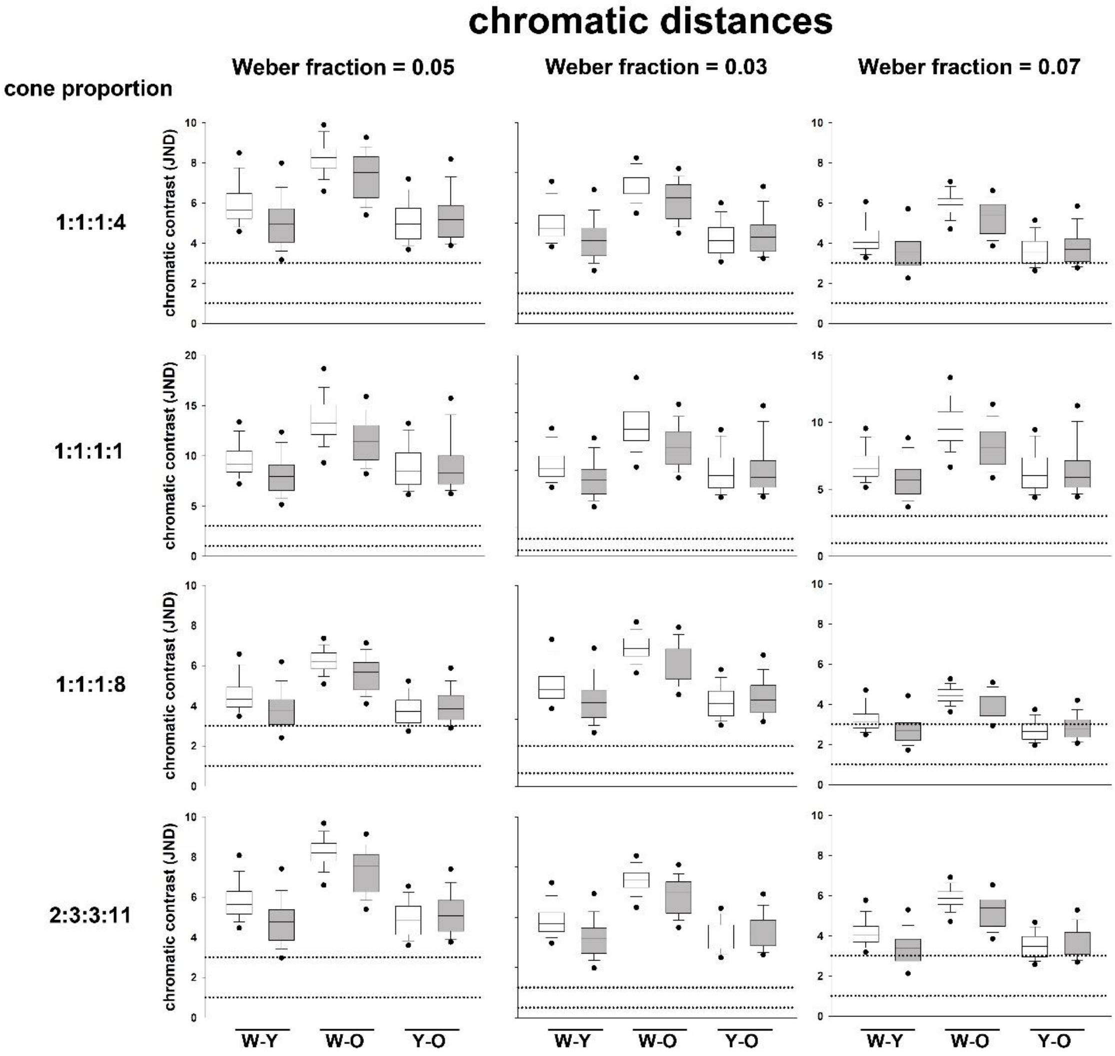

Figure 2.S5. Box-plots showing chromatic distances generated by pairs of colour morphs (W = white, Y = yellow, O = orange) generated by the alternative visual models used in the analyses considering four different cone proportions and three values of the Weber fraction. White box-plots correspond to males and grey box-plots correspond to females. In each case, horizontal lines, boxes, error bars and black dots indicate, respectively, the median, the 25-75% range, the 10th and 90th percentiles, and the 5th and 95th percentiles. Horizontal dotted lines indicate the discriminability thresholds of 1 JND (values above 1 JND indicate pairs of colours that are discriminable under good illumination conditions) and 3 JND (values above 3 JND indicate easily discriminable pairs of colours). Results from the corresponding analyses in **Table 2.S3**. Note that the scale of the Y axis varies depending on the panel.

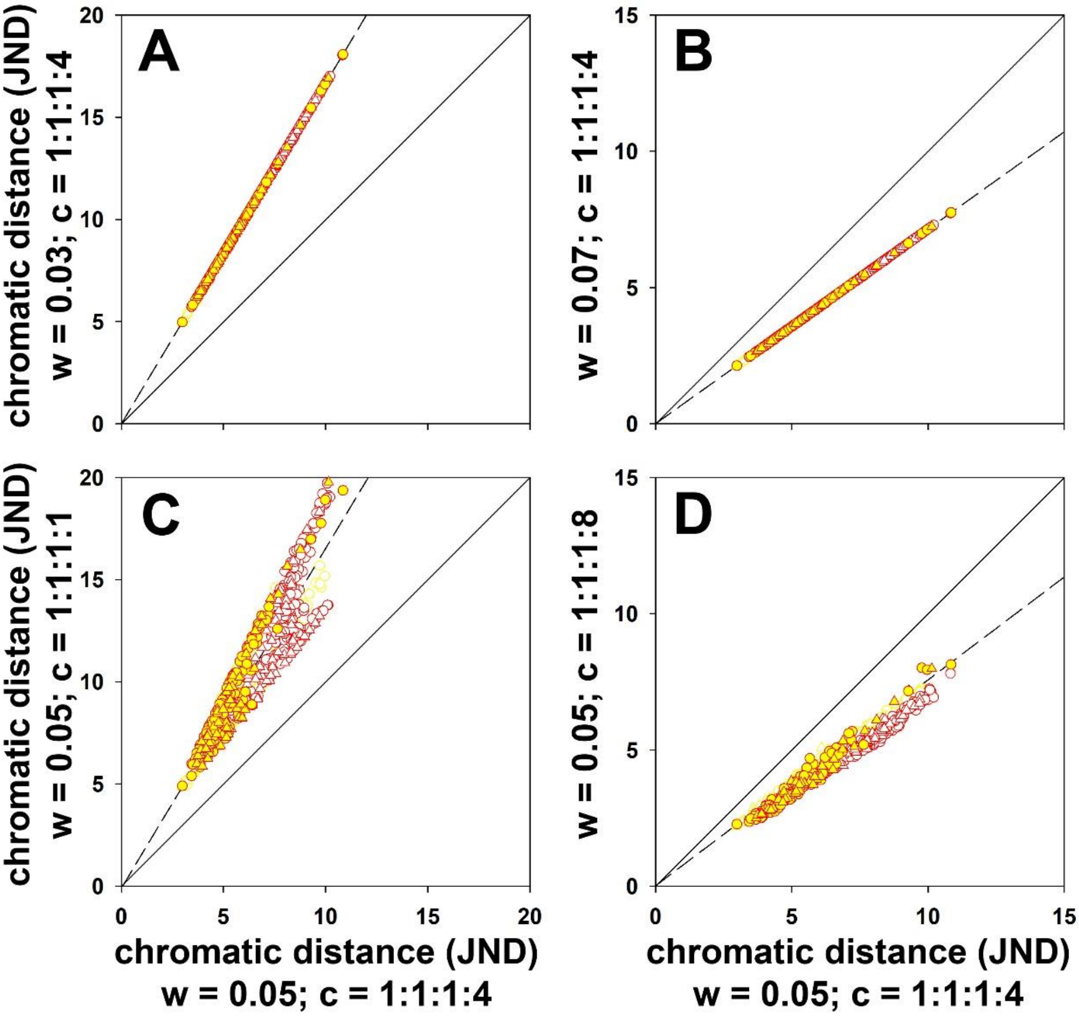

Figure 2.S6. Effect of varying the Weber fraction (w) and cone proportions (c) on the chromatic distances between morphs (measured in JND). Comparison of our main model assuming a cone proportion of 1:1:1:4 (ultraviolet-:short-:medium-:long-wavelength sensitive cones) and a Weber fraction of 0.05 with models with values of the Weber fraction of 0.03 (A) and 0.07 (B), and with models with cone proportions of 1:1:1:1 (C) and 1:1:1:8 (D). Circles indicate males and triangles indicate females. The two colours of each triangle or circle (outline and fill) indicate the two morphs generating this chromatic distance, i.e. white and yellow circles and triangles represent chromatic distances between white and yellow morphs, white and red circles and triangles represent chromatic distances between white and orange morphs, etc. The dashed lines indicate the regression. The continuous lines represent the 1:1 reference line.

Chapter III

"The Universe is a dark forest. In this forest, hell is other people. An eternal threat that any life that exposes its own existence will be swiftly wiped out. This is the picture of cosmic civilization. It's the explanation for the Fermi Paradox".

·Cixin Liu, The Dark Forest·

"I come in peace… take me to your lizard".

·Douglas Adams, The Hitchhiker's Guide to the Galaxy·

This chapter reproduces entirely the published manuscript:

Abalos, J., Pérez i de Lanuza, G., Carazo, P., and E. Font. (2018). The role of male coloration in the outcome of staged contests in the European common wall lizard (*Podarcis muralis*). *Behaviour* 153(5): 607-631. doi: 10.1163/1568539X-00003366

Chapter III:

The role of male coloration in the outcome of staged contests in the European common wall lizard (*Podarcis muralis*)

3.1. Abstract

Colour signals play a key role in regulating the intensity and outcome of animal contests. Males of the common wall lizard (*Podarcis muralis*) show conspicuous ventrolateral ultraviolet (UV)-blue and black patches. In addition, some populations express a striking ventral colour polymorphism (i.e. discrete orange, white and yellow morphs). In this study, we set out to evaluate the potential signalling function of these colour patches by staging pairwise combats between 60 size-matched adult lizards (20 per morph). Combats were held in a neutral arena, with each lizard facing rivals from the three morphs in a tournament with a balanced design. We then calculated a fighting ability ranking using the Bradley Terry model, and used it to explore whether ventral colour morph, the size of UV-blue and black patches or the spectral characteristics of UV-blue patches (i.e. brightness, hue, chroma) are good predictors of fighting ability. We did not find an effect of the UV-blue patches on contest outcome, but the size of black patches emerged as a good predictor of fighting ability. We also found that winners were more aggressive when facing rivals with black patches of similar size, suggesting that black patches play a role in rival assessment and fighting rules. Finally, we found that orange males lost fights against heteromorphic males more often than yellow or white males. In light of these results, we discuss the potential signalling function of ventrolateral and ventral colour patches in mediating agonistic encounters in this species.

Keywords: fighting ability, intra-sexual competition, colour polymorphism, UV signals, melanin, *Podarcis* muralis

3.2. Introduction

In a world of limited resources, males of many vertebrate systems often engage in contests with members of their own species, either over access to females or resources that are necessary to attract potential mates. Chromatic signals mediate the intensity of these confrontations by allowing animals to assess rivals prior to physically interacting, thereby reducing the costs of aggressive conflicts (Briffa 2014). For example, in male widow birds (*Euplectes ardens*) the size and spectral properties of the red collar predict territorial behaviour and the intensity of aggressive responses against simulated intruders (Pryke et al. 2001). Similar colour ornaments acting as agonistic signals (i.e. armaments) have been described in numerous vertebrates and invertebrates (e.g. Pryke and Griffith 2006*a*; Miyai et al. 2011; Todd et al. 2011). In lizards, several colour signals have been shown to convey information about dominance and male fighting ability in intrasexual confrontations (Stapley and Whiting 2006; Baird 2013; Olsson et al. 2013). For instance, in the Australian frillneck lizard (*Chlamydosaurus kingii*), the carotenoid-based colour of the frill acts as a reliable signal of fighting ability, and males exhibiting more colourful frills are dominant in size-matched dyadic contests (Hamilton et al. 2013). Similarly, research on *Anolis* has demonstrated that the display of brightly coloured dewlaps plays an important communicative role in several contexts, including male-male contests (Jenssen et al. 2000; Tokarz et al. 2003; Simon 2011), its role in determining contest outcome being dependent on the degree of territoriality of the species (Lailvaux and Irschick 2007).

Lacertid lizards comprise 42 different genera and more than 300 species inhabiting a wide variety of habitats across the Old World (Uetz and Hošek 2015). Although their mating systems are insufficiently documented, they seem to be characterized by strong male-male competition over resources (e.g. territoriality in the genus *Podarcis*; (Edsman 1990; Font et al. 2012*a*) or females (e.g. mate-guarding in *Lacerta agilis*; Olsson 1994*a*; and *Lacerta schreiberi*; Marco and Pérez-Mellado 1999). The role of colour signals in this group has been relatively neglected, possibly because lacertids have been traditionally considered to be mainly chemosensory (Mason and Parker 2010). Despite this historical bias, lacertids have a colour vision system as sophisticated as that of other lizards that rely heavily on vision for many aspects of their biology (Pérez i de Lanuza and Font 2014; Martin et al. 2015*a*), and very often exhibit complex colour patterns that at least in some cases seem to have evolved under strong intrasexual competition in males (Bajer et al. 2011; Olsson et al. 2011; Pérez i de Lanuza et al. 2013*b*).

The common wall lizard (*Podarcis* muralis) is a suitable lacertid model in which to test ideas about the role of colour signals in animal contests. Males of *P. muralis* exhibit a complex ventrolateral pattern on their outer ventral scales (OVS) combining black melanin-based patches (Bowker et al. 1987) and conspicuous ultraviolet (UV)-blue structurally based patches (**Fig. 3.1**, **3.2b**; Pérez i de Lanuza and Font 2015). Chromatic variables of male UV-blue patches have been found to correlate with bite force and body condition (Pérez i de Lanuza et al. 2014), while a recent study showed that the overall area covered by black patches correlates positively with male dominance status (While et al. 2015). In addition to ventrolateral colour patches, some populations of *P. muralis* express a pigment-based ventral colour polymorphism with discrete white (w), orange (o) or yellow (y) pure morphs, as well as intermediate orange-yellow and white-orange morphs (**Figure 3.1**; Pérez i de Lanuza et al. 2013*a*). Although recent studies suggest that different phenotypic optima and breeding strategies may be favoured in each morph, the evolutionary origins and functional significance underlying colour polymorphism in *P. muralis* are still far from resolved (Calsbeek et al. 2010; Galeotti et al. 2013; Sacchi et al. 2015). As in many *Podarcis* species, *P. muralis* shows a resource-based polygynous mating system in which males set out territories that overlap with the home ranges of several females, engaging in frequent contests with intruding males (Edsman, 1990). During these male-male interactions, lizards compress their body laterally (Kitzler 1941; Olsson 1992, 1994*a*) in a way that exposes the ventrolateral colour patches, but also the ventral coloration, which could hence also be involved in signalling during agonistic encounters (see Huyghe et al. 2012). These territorial contests are pivotal for the reproductive success of males, since females seem to be attracted to good quality territories rather than to males of certain phenotypic characteristics (Font et al. 2012*a*; Olsson et al. 2013), and frequently remain within the same territory even when the owner is removed and another male takes his place (Edsman 2001).

In this study we explored the effect of ventral and ventrolateral colour patches during pairwise agonistic interactions between size-matched males with no previous experience that confronted each other in a neutral arena (i.e. no residency effect). We designed our dyadic encounters as a tournament in order to calculate an overall fighting ability for each individual that we could relate to chromatic variables of ventral and ventrolateral colour patches and to levels of winner/loser aggression. We calculated individual fighting ability using a Bradley-Terry model (B-T; Bradley and Terry 1952; Firth and Turner 2012), which allows for simultaneous analysis of the relative influence of multiple individual traits on fighting ability, can accommodate an incomplete matrix of confrontations, and takes proper account of dependency among contests involving the same individual (Stuart-Fox et al. 2006).

Figure 3.1. Left: male common wall lizard *Podarcis muralis*. This species has a relatively cryptic dorsum and conspicuous ventrolateral coloration. The ventrolateral area usually presents a complex pattern with black and UV-blue patches. Right: ventral view of both sexes to illustrate the colour polymorphism. Individuals from the three main morphs present orange, yellow or white ventral coloration. In our study population, the ventral colour is restricted to the throat in females, but extends to the belly in males.

3.3. Materials and methods

3.3.1 Study species

Animals participating in this study came from a colour polymorphic population of *P. muralis* from the Cerdanya valley in the eastern Pyrenees (France). To minimize potential previous fighting experience between opponents, lizards were captured in eight different areas at least 300 m apart, or separated by a geographic barrier (e.g. river). Between May 17-19 2014, we captured 20 adult males of each pure morph (i.e. white -w-, yellow -y- and orange -o-) by noosing, and transported them to the laboratory at the University of Valencia (Valencia, Spain). We only captured lizards with a snout-to-vent length (SVL) larger than 65 mm to restrict the sample to large adult males, ensuring that all individuals participating in the tournament had fully developed colour patches (Pérez i de Lanuza et al. 2013*a*, 2014) and were large enough to exhibit full-blown agonistic behaviour (Edsman 1990), while minimising the range of possible size differences between potential contestants (Sacchi et al.

2009). For each lizard, we used SVL (± 1 mm) and body mass (± 0.1 g) measures at the time of capture to calculate a body condition index (BCI) as the residual from a least-squares linear regression of log(body mass) against log(SVL) (Green 2001).

3.3.2. Animal housing and maintenance

In the laboratory, we housed lizards individually in glass terraria (20 x 40 and 25 cm high) with an artificial grass substrate, a water dish, a shelter, and a small brick for basking over which we suspended a 40 W incandescent lamp. Shelters consisted of two tiles (one serving as base and one serving as a removable lid) separated with felt so as to leave a 90 x 90 x 12 mm cavity for the lizard to take refuge. We covered all but the front wall of every terrarium with brown cardboard paper to prevent visual contact between animals from adjoining terraria. We set the temperature and light cycle to mimic average field conditions during the reproductive season at the capture site (15º C at night, 26º C during the day; 12.5L:11.5D), and additionally provided all lizards with full spectrum light (Reptistar 5.0, Sylvania, Danvers, U.S.A.) for 1 h three times weekly (12.00-13.00 h) to ensure an effective calcium metabolism, preventing avitaminosis and metabolic bone disease (Adkins et al. 2003). We fed lizards *Tenebrio molitor* larvae dusted with vitamins (Exo Terra, Montreal, Canada) three times weekly and allowed lizards to acclimate to laboratory conditions for 14 days before the experiments. Overall, animals stayed in the laboratory less than two months (May 19-July 13) before being released back to their capture location in the field.

3.3.3. Experimental design

In order to examine the effect of colour patches on male-male contest outcome, we used a tournament design in which every male faced three different rivals (one of each morph) with a minimum of three days between consecutive contests. The order of contests was randomized for each morph combination to control for a potential order effect. At the conclusion of the 90 initially planned contests, we staged nine additional heteromorphic fights between the three more successful individuals of each morph in order to increase the nestedness of the final tournament network and give additional resolving power to the B-T model (Stuart-Fox *et al.,* 2006); note these fights were pooled with the rest in our analyses. To reduce the potential noise introduced by size asymmetry and prior experience (Baird, 2013), we allowed a maximum size difference between contestants of 10% in SVL, and only confronted males from different capture areas (see above). Finally, to eliminate the effect of residency, we staged the encounters in a neutral experimental arena consisting of a glass terrarium (70 x 30 and 40 cm high) divided into two equal compartments by an opaque partition. Each compartment was illuminated by a 40 W lamp and a full spectrum lamp (Reptistar 5.0, Sylvania, Danvers, U.S.A.) equipped with a high frequency ballast (Quicktronic, OSRAM, Munich, Germany) (Evans et al. 2006). We performed five combats daily between the June 4 and the July 1 2014 at the natural peak activity hours for this population (10.00-13.30 and 16.00-19.00, local time). Immediately before each trial, we randomly determined the initial side of the arena for each opponent, chased the lizards into their shelter, and transferred them into the experimental arena along with their own basking stone. We then disassembled the shelter leaving the base tile in the arena and allowed a 30 min acclimation period before withdrawing the opaque partition and allowing the opponents to interact. All contests were filmed with a digital video camera (XM2, Canon®, Tokyo, Japan). The experimental arena was thoroughly cleaned with water, then alcohol, and again with water after every combat.

In each contest, we designated a winner only if one of the males ceased agonistic behaviours (**Table 3.1**) and repeatedly fled if approached by its opponent. In addition to this categorical measure, we also calculated a quantitative variable based on the level of aggression shown by each lizard in each staged contest. In order to do this, we played back filmed contests and recorded each time a lizard performed any of the behaviours described in **Table 3.1.** We assigned a score to each of these behaviours based on Carazo et al. (2008), which allowed us to calculate an individual aggression score as the sum of every agonistic behaviour performed by each lizard in each contest (i.e. one aggression score per lizard and contest). We also calculated a measure of aggression ratio for each contest as the quotient between the winner aggression score and the sum of both winner and loser aggression scores (i.e. one aggression ratio per contest). We interrupted combats if they escalated to the point of risking injuries (bite-holds in head or limbs lasting more than 1 min), which was only necessary in one combat. None of the contests resulted in observable injuries.

Table 3.1. Behaviours used to calculate the individual Aggression Score (AS) of lizards participating in the tournament (see text for details).

Behaviour	**Description**	**Score**
No response	-	0
Stare	Looks toward rival	1
Approach	Reduces distance with rival	2
Chase	Quickly follows fleeing rival.	3
Display	Throat extension, trunk compression and back arching.	4
Mouth gaping	Opens mouth	5
Lunge	Hits rival with closed mouth	6
Bite	Holds rival for < 2 s.	7
Bite-hold	Holds rival for > 2 s.	8

3.3.4. Colour measurements

We determined morph visually at the time of capture in the field (Pérez i de Lanuza et al. 2013*a*). We recorded spectrophotometric measurements in a single session at the conclusion of all contests to minimize the stress induced by manipulation prior to the experiments. We recorded reflectance spectra of the UV-blue patches with a portable USB-2000 spectrometer equipped with a PX-2 Xenon strobe light (Ocean Optics Inc., Dunedin, Fl. U.S.A.; for further details see Font et al. 2009). Measurements encompassed the 300-700 nm range to cover the entire visual spectrum of *P. muralis* (Pérez i de Lanuza and Font 2014; Martin et al. 2015*a*). We recorded spectra from the second, third and fourth UV-blue patches in both sides and averaged them to provide an individual mean spectrum for each lizard. We then extracted data for the standard variables describing colour: brightness (spectral intensity), chroma, and hue (Endler 1990; Bradbury and Vehrencamp 1998). We calculated brightness of the UV-blue patches (OVS-Qt) as the total area under the 300-700 nm reflectance curve, hue (OVS-hue) as the wavelength where peak reflectance is located , and UV-chroma (OVS-C_{UV}) as the area under the 300-400 nm reflectance curve divided by the area under the entire spectral curve (i.e. 300-700 nm) (Endler 1990; Molina-Borja et al. 2006; Pérez i de Lanuza et al. 2014).

In addition, we measured the relative ventrolateral area covered by black and blue patches in each lizard. For this purpose, we obtained an image of both sides of every lizard using a portable digital scanner (Lide 700F, Canon®, Tokyo, Japan), and then calculated the proportion of black and blue coloured area out of the total ventrolateral surface (**Fig. 3.2b**) using ImageTool Ver. 3.0 (Wilcox et al. 2002). We defined total ventrolateral surface as the area covered by the first two rows of OVS, between the insertions of the fore and hind limbs. We determined the proportion of coloured area by dividing the blue or black area by total ventrolateral surface; the same researcher (J.A.) measured all areas following a blind protocol. We confirmed the reliability of these measurements by calculating repeatability (r) as defined by Lessells and Boag (1987) in a subsample of lizards ($N = 15$; $r_{blue} = 0.97$; $r_{black} = 0.97$). Due to an endoparasitic infection unnoticed at the time of capture, one lizard died in the laboratory before its colour patches could be measured, reducing the final sample for colour variables to 59 individuals.

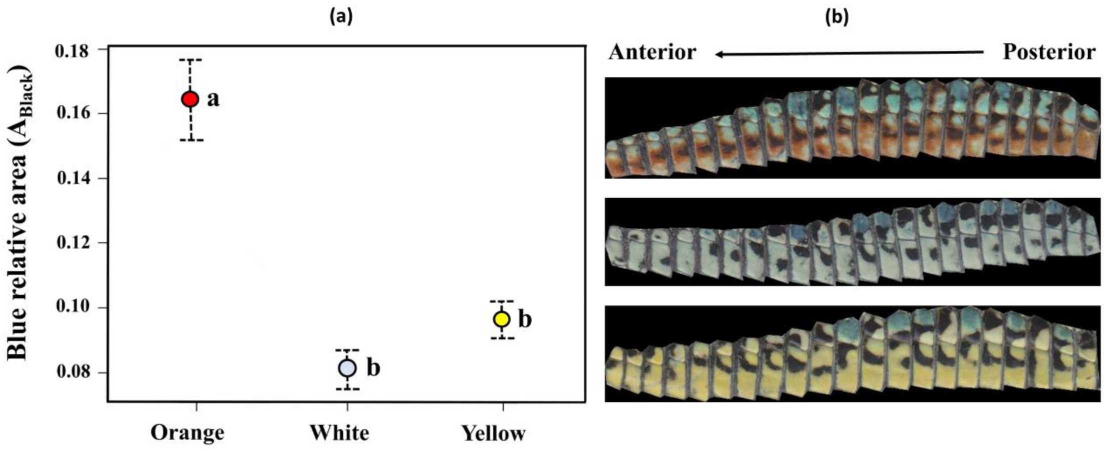

Fig. 3.2. a) Means of the relative blue area (A_{Blue}) in the ventrolateral patches, for each morph. Error bars represent the standard error of the mean. b) Representative pictures of the ventrolateral pattern in males of the three pure morphs. Blue coloration sometimes extends to the second row of ventral scales in orange males, while this is rare in white or yellow morph males.

3.3.5. Statistical analyses

We used the Bradley-Terry model (B-T model) for planned comparisons to extract a global index of fighting ability for each contestant (Bradley and Terry 1952). Staged contests can be considered as a subset of all the possible interactions between rivals from which relative fighting ability can be estimated. The B-T model is a type of generalized linear model that takes proper account of dependencies within and between contests while accommodating an incomplete matrix of all possible interactions (Firth 2005). This model assumes every contestant has a positive value of fighting ability, such that the most likely contest outcome is given by the ratio of the abilities of both contestants (Whiting et al. 2006), and estimates a hierarchy based on transitivity (Stuart-Fox et al. 2006). Using the R package BradleyTerry2 (Firth and Turner 2012) in R 2.1.5.1 (R Core Team, 2014) we fitted B-T models to 99 fights involving 60 lizards, obtaining a ranking based on individual fighting ability estimates. The model was calculated with "bias reduction", as is recommended when players face each other only once, and coding ties as half a win for each rival (Firth & Turner, 2012).

We used a generalized linear model (GLM) to explore the relationship between ventral colour morph (considered as a fixed factor), the three spectral variables of the UV-blue patches (i.e. OVS-Qt, OVS-Hue, OVS-C_{UV}), the relative area of blue (A_{Blue}) and black (A_{Black}) colour patches, body size (SVL), and fighting ability estimates as the dependent variable. We checked that all variables conformed to heteroscedasticity and normality assumptions, and assumed a Gaussian distribution for fitting the GLM. We started with the following full model representing our main hypothesis: *Fighting ability ~ Morph + SVL + OVS-Qt+ OVS-*C_{UV} *+ OVS-Hue +* A_{Blue} + A_{Black}. Model fitting was done manually in R 2.1.5.1 (R Core Team, 2014) and model selection was conducted using backward single term deletions ($P \leq 0.05$) of the saturated model followed by model comparisons via likelihood ratio tests.

We run linear mixed effects models to explore the relationship between individual aggression score and colour variables. We also explored potential receptor-dependent costs of coloration (i.e. variation in aggression score caused by rival coloration) by running linear mixed models with SVL, OVS-Hue, OVS-C_{UV}, OVS-Qt, Morph, A_{Blue}, A_{Black} and rival aggressive score as fixed factors, and intercepts for winner and loser identity as random effects. Finally, in order to assess the relationship between aggression ratio, body size, morph, and the size of black colour patches, we run the following post hoc linear mixed effects model: $Aggression\ ratio \sim Winner\ A_{Black} * Loser\ A_{Black} + Winner\ Morph * Loser\ Morph + Winner\ SVL * Loser\ SVL + (1 \mid Winner) + (1 \mid Loser)$. P-values were obtained via likelihood ratio tests of the full model after single term deletions.

3.4. Results

We could identify a winner in 76 out of 99 contests (**Fig. 3.S1**). Overall, the mean aggression score of each individual male across contests was significantly correlated with the estimate of fighting ability calculated with the B-T model from the global tournament network (*Mean aggression score ~ Fighting ability*, R = 0.59, $t_{56} = 5.515$, $P < 0.0001$, **Fig. 3.S2**).

3.4.1. Inter-morph differences

Yellow males won 66% of heteromorphic combats ($N = 36$), while white males won 58% ($N = 39$) and orange males only 24% ($N = 37$). Yellow and white males defeated orange males in the majority of contests (W > O: 76%, $N = 17$; Y > O: 75%, $N = 20$), while results for contests between white and yellow males were more balanced (Y > W: 58%, $N = 19$). However, males with different ventral coloration did not differ in their mean aggression score across contests (*Mean AS*, $F_{2,58} = 1.73$, $P = 0.187$). We found no significant differences in body size (*SVL*, $F_{2,60} = 1.84$, $P = 0.168$) or body condition (*BCI*, $F_{2,60} = 2.858$, $P = 0.0658$) among morphs, despite a trend for yellow males in our sample to show lower BCI than males of the other two morphs (**Fig. 3.S3**). UV-blue spectral variables did not differ between morphs (*OVS-Qt*, $F_{2,59} = 2.807$, $P = 0.0691$; *OVS-Hue*, $F_{2,59} = 0.088$, $P = 0.916$; *OVS-*C_{UV}, $F_{2,59} = 1.349$, $P = 0.268$). A_{Black} showed marginally non-significant differences between morphs (A_{Black}, $F_{2,58} = 3.038$, $P = 0.0561$), likely due to males of the white morph having slightly larger A_{Black} than males from the orange morph (Tukey's post hoc, W-O ± SEM = 0.058 ± 0.024, $t_{2,58} = 2.39$, $P = 0.052$; Y-O ± SEM = 0.042 ± 0.025, $T_{2,58} = 1.69$, $P = 0.218$; Y-W ± SEM = -0.016 ± 0.025, $t_{2,58} = -0.64$, $P = 0.801$). Orange morph males had higher A_{Blue} than white and yellow males (**Fig. 3.2**; A_{Blue}, $F_{2,58} = 25.49$, $P < 0.0001$; Tukey's post hoc, W-O ± SEM = -0.083 ± 0.012, $t_{2,58} = -6.75$, $P < 0.0001$; Y-O ± SEM = -0.067 ± 0.013, $t_{2,58} = -5.33$, $P < 0.0001$), while the white and yellow morphs did not differ (Tukey's post hoc, Y-W ± SEM = 0.016 ± 0.013, $t_{2,\,58} = 1.24$, $P = 0.434$).

3.4.2. Predictors of fighting ability

In accordance with the observed differences in contest outcome, we detected a marginally significant difference in the fighting ability of different morphs (one way-ANOVA: *Fighting ability ~ Morph*, $T_{2,59} = 3.337$, $P = 0.043$; **Fig. 3.3**). Orange males seem to have slightly lower fighting ability than yellow males (Tukey's post hoc, W-O ± SEM = 0.007 ± 0.003, $t_{2,\,58} = 2.08$, $P = 0.104$; Y-O ± SEM = 0.008 ± 0.003, $t_{2,\,58} = 2.37$, $P = 0.054$; Y-W ± SEM = -0.001 ± 0.003, $t_{2,\,58} = 0.35$, $P = 0.93$).

However, after model selection, only $\mathrm{A}_{\mathrm{Black}}$ (Mean± SEM = 0.249 ± 0.128) remained as a significant predictor of individual fighting ability (*Fighting ability ~* A_{Black}, $t_{56} = 2.453$, *Std. Coef.* = 0.31, $P = 0.017$, **Fig. 3.4**). $\mathrm{A}_{\mathrm{Black}}$ was not related to mean aggression score (Spearman correlation: $\mathrm{S}_{56} = 26808$, $\varrho = 0.175$, $P = 0.188$), BCI ($T_{56} = 0.032$, $P = 0.974$) or $\mathrm{A}_{\mathrm{Blue}}$ ($t_{56} = -1.23$, $P = 0.223$). We found no relationship between the spectral variables of UV-blue patches and fighting ability ($R < 0.1$, $P > 0.2$ in all cases).

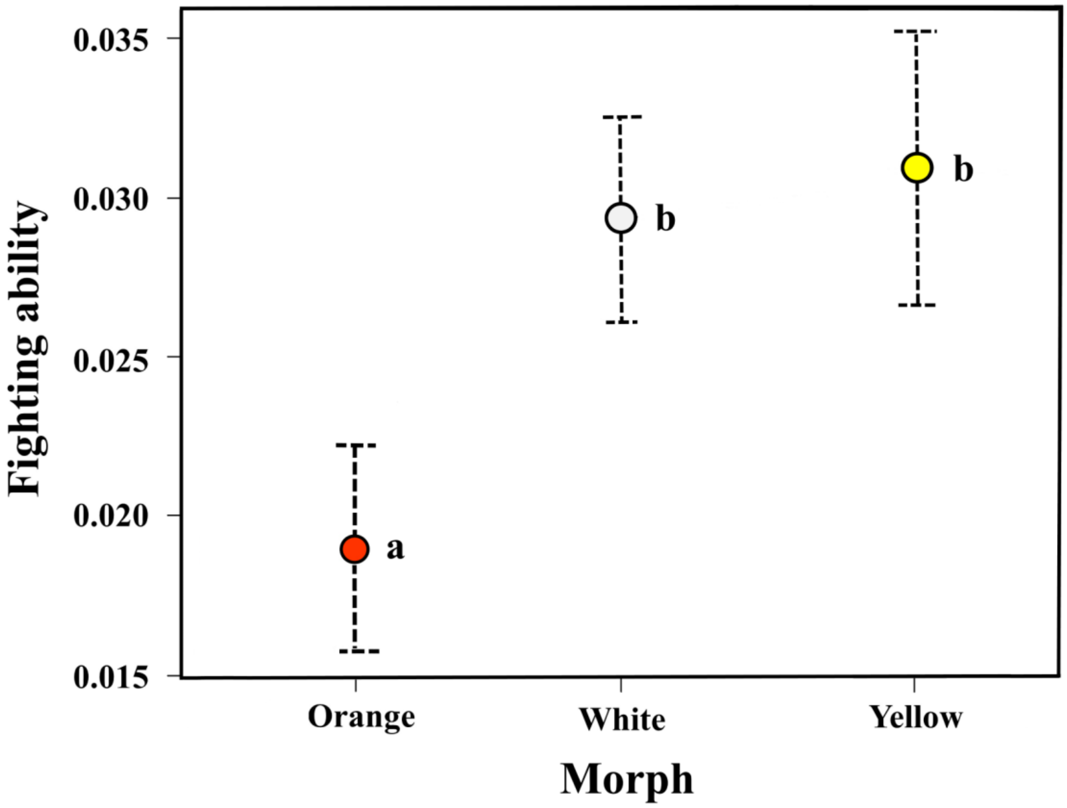

Figure 3.3. Within-morph means of fighting ability estimates obtained with the Bradley-Terry model. Error bars represent the standard error of the mean.

3.4.3. Predictors of aggression score and aggression ratio

Winner or loser aggression scores were unrelated to colour variables from any of the contestants. In every mixed model, the aggression score of one rival was best predicted only by the aggression score of its opponent (*Winner aggression score ~ Loser aggression score*, $t_{5,\ 76} = 5.6$, $P < 0.0001$). In contrast, aggression ratio showed a significant relationship with the A_{Black} of both contestants. Specifically, combats between males with similar A_{Black} resulted in higher aggression ratios. After model selection, aggression ratio was found to be significantly related to the interaction between the A_{Black} of each contestant (*Winner* A_{Black}**Loser* A_{Black}; $\chi^2 = 4.74$, df = 1, $P = 0.029$) (**Table 3.2**), but not with their morph (*Winner Morph*Loser Morph*; $\chi^2 = 6.63$, df = 1, $P = 0.16$), nor their SVL (*Winner SVL*Loser SVL*; $\chi^2 = 0.0003$, df = 1, $P = 0.99$). To examine the significance of this interaction, we used a 3D plot, which suggests that aggression ratio increases as the asymmetry between winner and loser A_{Black} decreases (**Fig. 3.5**).

Table 3.2. Mixed effects linear model used to explore the relationship between body size, morph, black coloration, and aggression ratio in staged contests between *Podarcis muralis* male lizards.

	Term	*Coefficient*	*Standard error*	X^2	*Df*	*P-value*
Fixed factors	Winner A_{Black}* Loser A_{Black}	0.19	0.09	4.74	1	0.029
	Winner A_{Black}	-0.09	0.12	0.53	1	0.47
	Loser A_{Black}	-0.02	0.13	0.03	1	0.87
		Variance	*Standard deviation*			
Random factors	Loser identity	0.41	0.64			
	Winner identity	0.33	0.57			

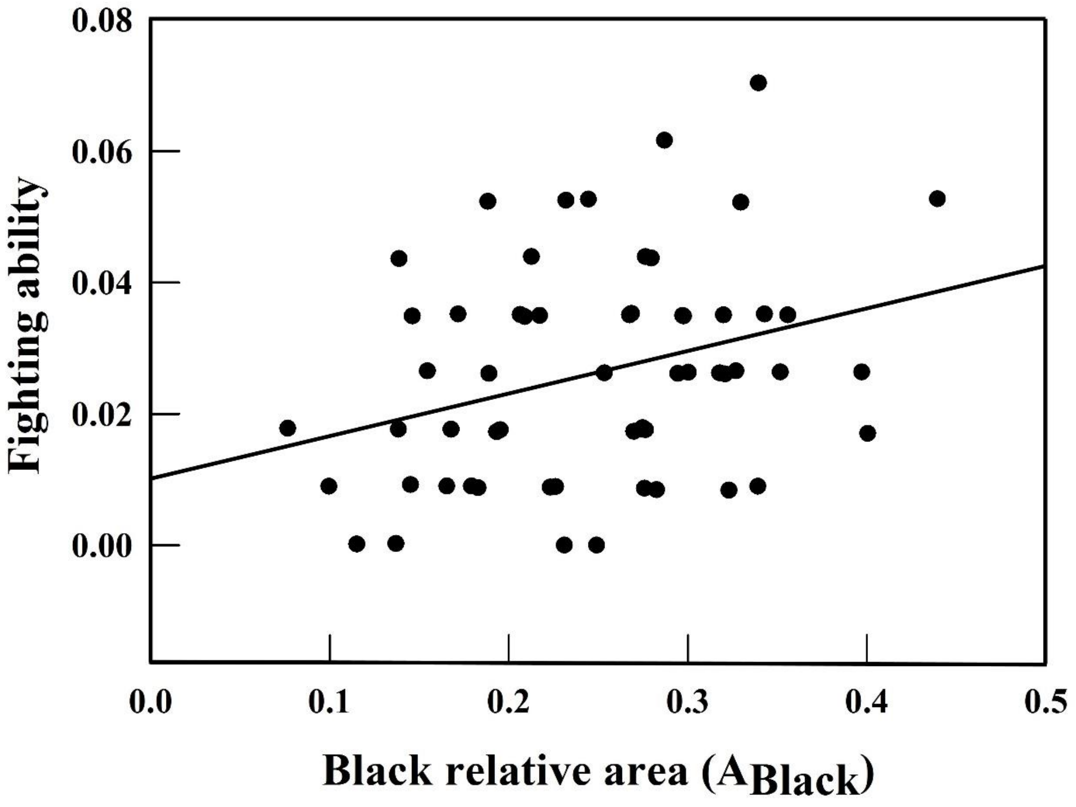

Figure 3.4. Scatterplot showing the relationship between fighting ability and relative black area in the ventrolateral scales (A_{Black}) for each individual participating in the tournament.

3.5. Discussion

In this study, we set out to assess the potential role of *P. muralis* ventral (i.e. morph; orange, yellow or white) and ventrolateral (i.e. UV-blue and black) colour patches in determining contest outcome in male-male conflicts. Neither the size nor the spectral variables of UV-blue patches affected contest outcome, but both A_{Black} and ventral colour morph did. A_{black} was a particularly good predictor of fighting ability that also affected the difference in aggression score between winners and losers, in a way that suggests it may be functional as an agonistic signal (see below).

3.5.1. Black ventrolateral patches as chromatic signals of fighting ability

As stated, A_{Black} was the best predictor of fighting ability in our models, and we also found that the interaction between winner and loser A_{Black} was a significant predictor of the aggression ratio of a contest. Namely, winners behaved relatively more aggressively towards losers as the difference in winner-loser A_{Black} decreased, and less so as it increased. This effect was strong and persisted despite controlling for the interaction between both rivals' SVL in our post hoc model. Hence black coloration does not appear to act as a simple surrogate of size (Gosá 1987) but rather as an agonistic signal used by males to assess their rivals' fighting ability, and adjust their investment in the fight accordingly. This is in agreement with a recent study reporting that the amount of ventral black coloration strongly correlates with dominance status in two different lineages of *P. muralis* (i.e. Tuscany and Western Europe lineages; While et al. 2015). Furthermore, results from this study strongly suggest that the introgression of the Italian lineage (with exaggerated black coloration) onto Western France is likely driven by male-male competition favouring this character (While *et al.,* 2015).

Several studies across different taxa have also documented the influence of black (melanic) coloration in the formation of hierarchies, dominance status, and in the resolution of contests (insects: Tibbetts et al. 2010; fish: Horth 2003; Johnson and Fuller 2014; birds: Gonzalez et al. 2002; Chaine et al. 2011; reptiles: Lebas and Marshall 2001; Osborne 2005; Mafli et al. 2011; Qi et al. 2011). Interestingly, and in agreement with predictions from the sequential assessment game model (Enquist and Leimar 1983), we found that the most aggressive contests were those involving opponents with similarly-sized melanic patches, which has also been reported in organisms as phylogenetically distant as paper wasps (genus *Polistes*; Sheehan and Tibbetts 2010; Tibbetts et al. 2010), or the bluefin killifish (*Lucania goodie*; Johnson and Fuller 2014). Thus, melanin-based signals seem to convey valuable information about opponents' fighting abilities in a broad diversity of taxa.

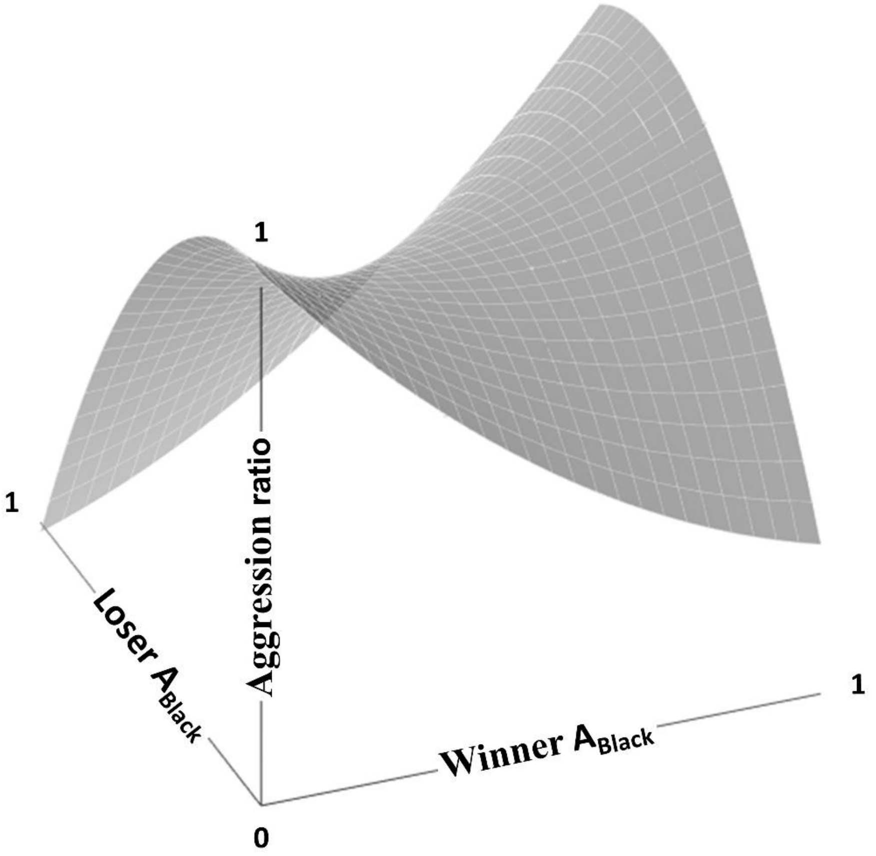

Figure 3.5. 3D plot exploring the relationship between aggression ratio and the interaction between the black relative area (A_{Black}) of both opponents.

Melanin-based signals are usually considered to have low production costs in vertebrates (Hill and Brawner 1998; Badyaev and Young 2004; Stoehr 2006; but see Roulin 2016), but there is evidence in different species suggesting they frequently act as badges of status with socially-enforced costs (Møller 1987; Senar and Camerino 1998; Tibbetts and Dale 2004; Westneat and Diep 2013; Roulin 2016). In addition, activity in the melanocortin system -which is responsible for the production of melanin-based coloration- covaries with several behavioural and physiological traits in vertebrates, such that darker individuals are often more aggressive, sexually more active and more resistant to various sources of stress than lighter animals (Ducrest et al. 2008). We suggest future studies should examine the mechanisms underlying honest signalling by means of melanin-based colour patches in *P. muralis*.

3.5.2. Size and reflectance of UV-blue patches are not related to contest outcome

We did not find a relationship between fighting ability or aggression score and the size or any of the spectral characteristics of UV-blue patches. Taken at face value, these results seem to argue against the possibility that conspicuous UV-blue patches convey information about male competitive skills. However, there is now compelling evidence suggesting that UV-blue patches may play an important role in intra-sexual competition in several *Podarcis* species (Marshall and Stevens 2014; Pérez i de Lanuza et al. 2014; Martin et al. 2015*c*), including *Podarcis muralis* (MacGregor et al. 2017*b*). It is possible that UV-blue patches play a significant role in more natural contexts than the short-range encounters artificially enforced by our experimental design (see also Martin et al. 2015*c*). It is also possible that, due to potential within-season changes in coloration, our spectrophotometric measurements, taken after the experiment was completed and towards the end of the reproductive season in this population, failed to reflect the true characteristics of UV-blue patches during combats (Martin et al. 2015*c*). Finally, our negative results may be due to UV-blue patches playing an important role only in the early stages of contests (when assessment takes place), but failing to predict contest outcome if the conflict escalates into physical aggression (Baird et al., 2013). We suggest future studies should aim to experimentally manipulate the area and spectral variables of the UV-blue patches, and set up contests in larger enclosures.

3.5.3. Morph-specific bias in fighting ability

Our finding that orange males have a propensity to lose heteromorphic confrontations seems to contradict a previous study that did not report any inter-morph differences in fighting ability in Italian polychromatic populations of *P. muralis* (Sacchi et al. 2009). Although this discrepancy might arise from geographical/phylogenetic differences between the Italian and French lineages, it could also have originated from crucial differences in the experimental design and data analysis. Sacchi et al. (2009) found no differences among morphs in the aggression exhibited during contests, and no effect of ventral coloration over contest score (calculated as the difference in aggression score between contestants). In agreement with these results, in the present study we found no differences in levels of mean aggression score among morphs, nor of an effect of the opponents' ventral coloration on aggression ratio. However, the analysis of fighting ability estimates revealed significant inter-morph differences in the ability to win intrasexual confrontations. Estimating fighting ability directly from contest outcome in a nested tournament experimental design (rather than extrapolating it from aggressive scores obtained from independent subsets of contests; Sacchi et al., 2009), may have allowed for a more powerful analysis of the role played by ventral coloration in the agonistic context. Additionally, as Sacchi et al. (2009) performed combats in a resident-intruder scenario, residency -a strong determinant of contest outcome in lizard encounters (Whiting 1999; Olsson and Shine 2000)- may have easily masked the relatively weaker effect of ventral coloration (Stuart-Fox and Johnston 2005). In *Lacerta agilis*, for example, resident males defeated intruder males in all staged combats regardless of their coloration (Olsson 1993), while males with larger colour patches were more likely to win fights when confrontations took place in a neutral arena (Olsson 1994*a*). Other previous studies with lizards have also linked colour polymorphisms to differences in fighting ability using experimental designs that control for a residency effect. For example, in the agamid *Ctenophorus decresii* orange males consistently showed the highest levels of aggression when confronted to the other morphs (Yewers et al. 2016), while in *Ctenophorus pictus* red-headed males outcompeted yellow-headed males in dyadic contests (Healey et al. 2007). In the phrynosomatid *Urosaurus ornatus* blue-green throated males were more likely to defeat orange throated males (Carpenter 1995*b*). In contrast to our results, in populations of *Podarcis melisellensis* with the same type of orange-yellow-white ventral polymorphism as *P. muralis*, orange males show greater fighting ability than yellow or white males (Huyghe et al., 2012).

Sexual selection often plays a role in the origin and maintenance of population polymorphisms as strong intra-sexual competition might promote the evolution and maintenance of alternative mating strategies (Taborsky et al. 2008), which frequently correlate with discrete phenotypic traits (e.g. colour morphs; Wellenreuther et al., 2014**)**. For example, in *Uta stansburiana* periodic oscillations in the relative frequencies of three discrete morphs reflect a cyclical "rock-paper-scissors" game that is driven by frequency-dependent selection on three alternative reproductive strategies (Sinervo and Lively, 1996). Following these results, much attention has been paid to the possibility that other lizard colour polymorphisms might similarly reflect the existence of alternative reproductive tactics maintained by "rock-paper-scissors" dynamics of selection. For instance, San-José *et al.* (2014) found that the lacertid *Zootoca vivipara* experiences similar fluctuations in the relative frequencies of morphs that appear to be consistent with frequency-dependent cycles of cumulative selection. However, the reason why alternative mating strategies should be associated with different colorations remains an intriguing question far from being resolved (Pérez i de Lanuza et al. 2013*a*; but see Sinervo et al. 2006*b*), and future experimental studies in polymorphic systems should test the presumed signalling role of the alternative ventral colours.

Until now, research on colour polymorphism in *P. muralis* has revealed several between-morph differences in morphological and physiological traits (Galeotti et al. 2007, 2010, 2013; Sacchi et al. 2007; Calsbeek et al. 2010) that might suggest that different phenotypic optima are being favoured in each morph (i.e. increased body size and susceptibility to infection in the orange morph; Calsbeek et al., 2010), but it is still unclear whether these differences correlate with morph-specific behavioural syndromes and/or mating strategies (but see Sacchi et al., 2009, Pérez i de Lanuza et al., 2013a; Sacchi et al., 2015). The lower fighting ability of orange males in our experiments could be explained by a number of causes, such as by inter-morph behavioural differences in territory acquisition and defence or in inherent fighting ability. The relative impact of colour signals on contest outcome has been found to vary in species of *Anolis* with different levels of territoriality (Lailvaux and Irschick, 2007). It would be interesting to investigate whether the differences we observed in the fighting ability and black coloration of orange males might be explained by differences in territorial behaviour, and hence in the relative importance of black coloration, across morphs. We suggest future studies should take a closer look at inter-morph differences in fighting ability and other behavioural and physiological aspects relevant to male-male competition (e.g. territoriality and sperm competition).

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3.6. Supplementary material

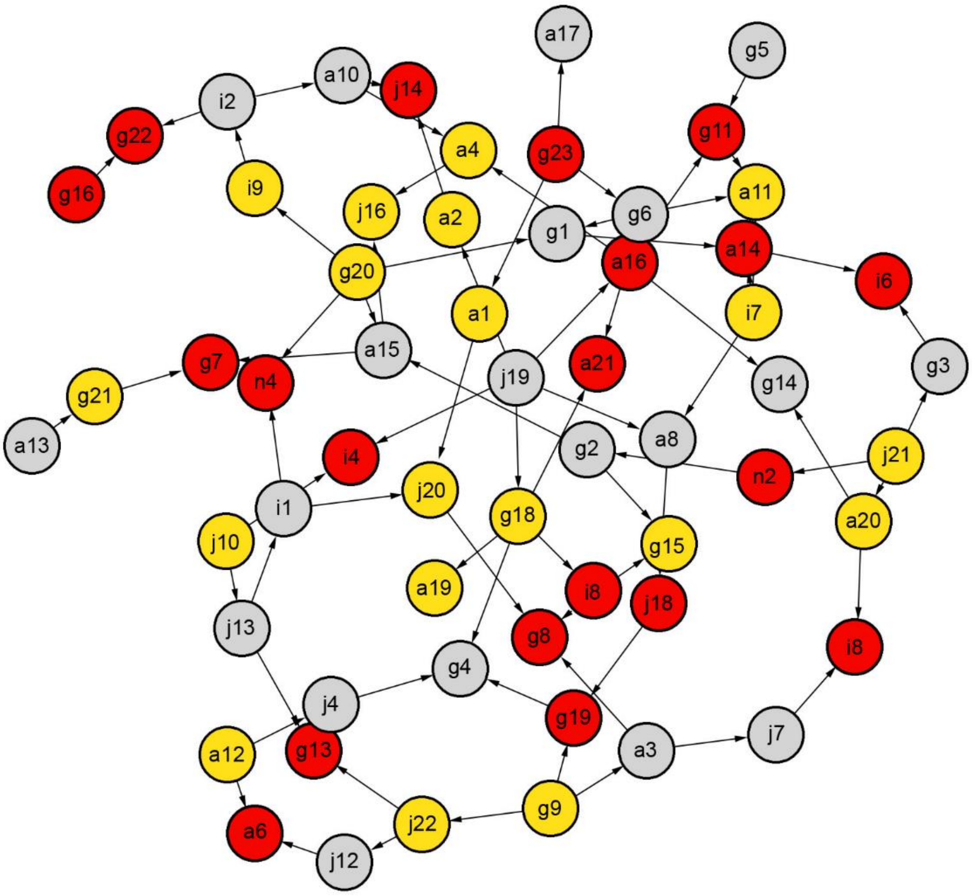


Figure 3.S1. Tournament network including all the 76 contests (out of 99 staged) in which a winner could be determined. Numbers inside circles denote individuals, and the colour represents their morph (red = o, grey = w, yellow = y). Arrows connect opponents that were confronted, pointing toward the winner. The Bradley-Terry model calculates individual fighting ability estimates from nested tournament networks such as this one.

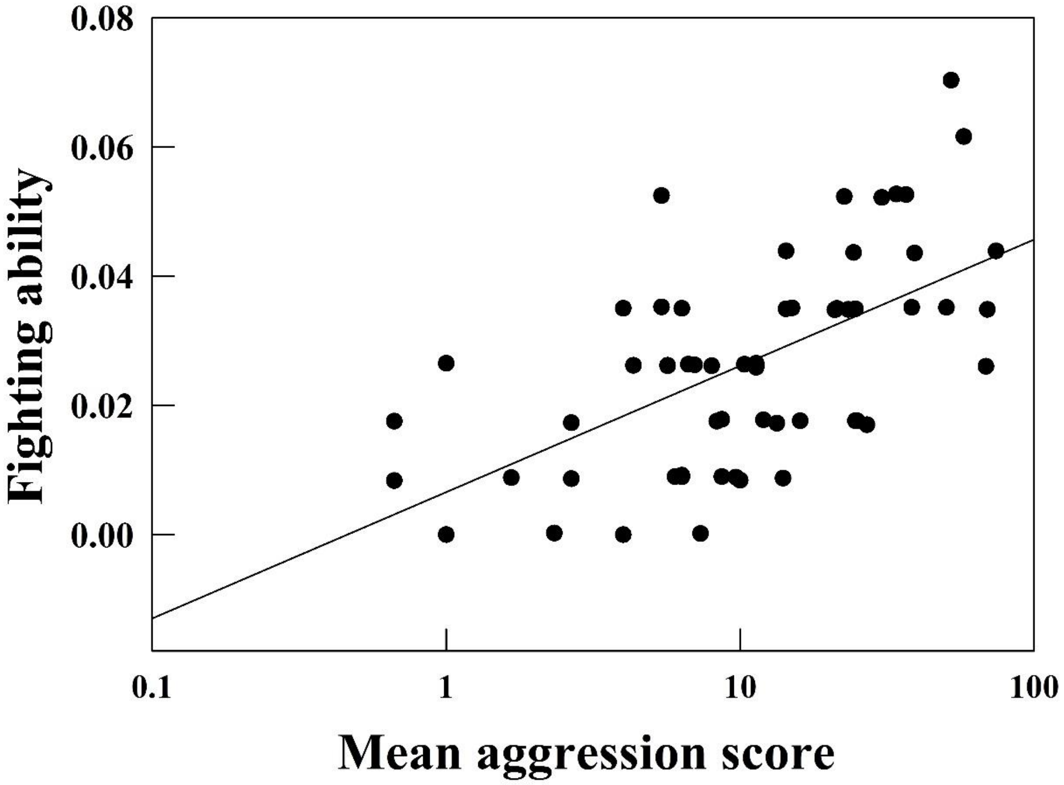


Figure 3.S2. Scatterplot showing the relationship between mean aggression score (AS) and fighting ability for each individual participating in the tournament.

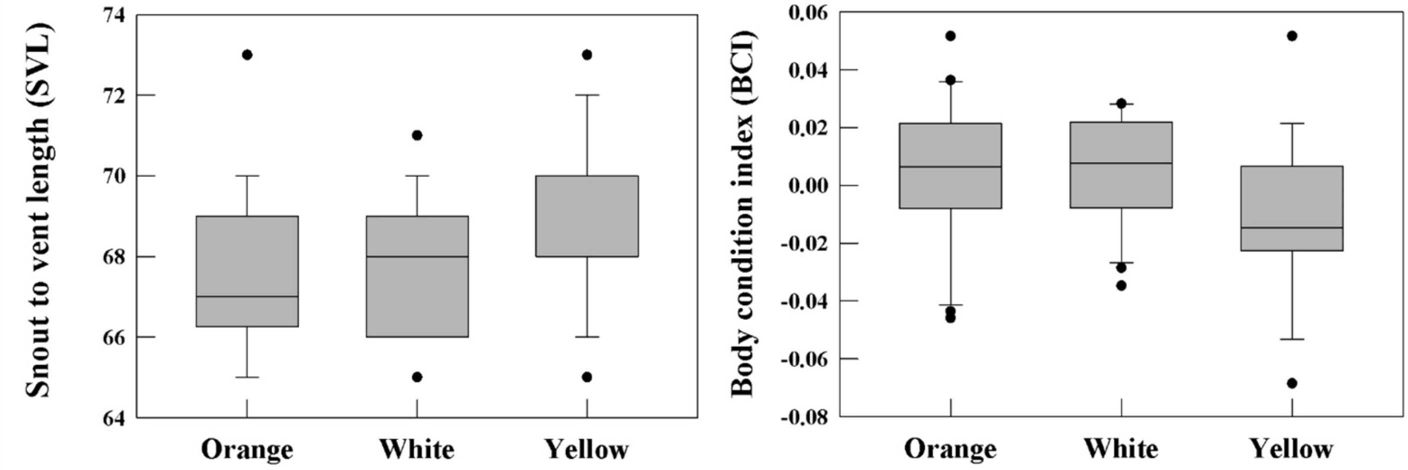
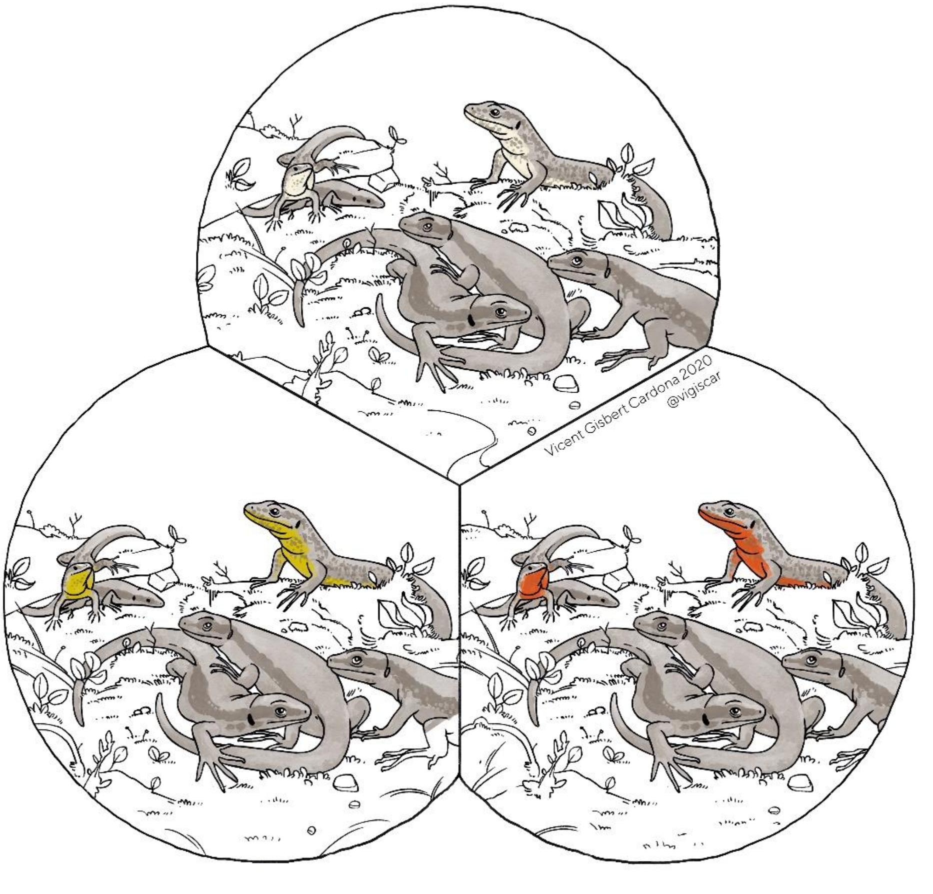


Figure 3.S3. Box plots showing the values of BCI and SVL separated by colour morph.

Chapter IV

"Everyone knows that dragons don't exist. But while this simplistic formulation may satisfy the layman, it does not suffice for the scientific mind. The brilliant Cerebron, attacking the problem analytically, discovered three distinct kinds of dragons: the mythical, the chimerical, and the purely hypothetical. They were all, one might say, non-existent, but each non-existed in an entirely different way".

·Stanislaw Lem, The Cyberiad·



This chapter reproduces entirely the published manuscript:

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Chapter IV:

No evidence for differential socio-sexual behaviour and space use in the colour morphs of the European common wall lizard (*Podarcis muralis*)

4.1. Abstract

Explaining the evolutionary origin and maintenance of colour polymorphisms is a major challenge in evolutionary biology. Such polymorphisms are commonly thought to reflect the existence of alternative behavioural or life-history strategies under negative frequency-dependent selection. The European common wall lizard *Podarcis muralis* exhibits a striking ventral colour polymorphism that has been intensely studied and is often assumed to reflect alternative reproductive strategies, similar to the iconic “rock-paper-scissors” system described in the North American lizard *Uta stansburiana*. However, available studies so far have ignored central aspects in the behavioural ecology of this species that are crucial to assess the existence of alternative reproductive strategies. Here we try to fill this gap by studying the social behaviour, space use, and reproductive performance of lizards showing different colour morphs, both in a free-ranging population from the eastern Pyrenees and in ten experimental mesocosm enclosures. In the natural population, we found no differences between morphs in site-fidelity, space use or male-female spatial overlap. Likewise, colour morph was irrelevant to socio-sexual behaviour, space use, and reproductive success within experimental enclosures. Our results contradict the commonly held hypothesis that *P. muralis* morphs reflect alternative behavioural strategies, and suggest that we should instead turn our attention to alternative functional explanations.

Keywords: alternative strategies - colour polymorphism - mesocosm - free-ranging population - *Podarcis muralis* - social behaviour

4.2. Introduction

Explaining the maintenance of phenotypic variability over time remains a central question in evolutionary biology. Population polymorphisms are a particularly widespread form of phenotypic variability (Galeotti et al. 2003; Roulin 2004; Gray and McKinnon 2007; Mckinnon and Pierotti 2010; Svensson 2017). In polymorphic populations, individuals of the same sex and age may exhibit different phenotypes (e.g. colour morphs) that are heritable, fixed in adults, and not condition-dependent (Galeotti et al. 2003; Roulin 2004; Mckinnon and Pierotti 2010). Selectively neutral polymorphisms are expected to be lost eventually due to stochastic processes (i.e. genetic drift; Roulin 2004), and the long-term maintenance of polymorphisms within a population requires some form of balancing selection, for example via non-random mating, source-sink dynamics, overdominance, or rare morph advantage (Galeotti et al. 2003; Roulin 2004; Roulin and Bize 2007; Wellenreuther et al. 2014; Svensson 2017).

Sexual selection often plays a major role in the maintenance of colour polymorphisms (Roulin and Bize 2007; Wellenreuther et al. 2014). Discrete variation among conspecifics in behaviour or life-histories associated with reproduction (termed alternative reproductive strategies; ARS) is frequently coupled with alternative colour morphs (Zamudio and Sinervo 2000; Shuster and Wade 2003; Roulin 2004; Roulin and Bize 2007; Ducrest et al. 2008; Wellenreuther et al. 2014; Willink et al. 2019). ARS are particularly frequent in males of polygynous (or polygynandrous) species, which experience a high variance in mating success and, thus, stronger sexual selection. In these species, the uneven distribution of fertilizations among males playing the conventional strategy allows the evolution of behavioural ARS (e.g. monogynist, satellite, sneaker, etc.) adapted to exploit distinct mating niches (Waltz 1982; Shuster and Wade 2003; Greenfield and Shelly 2008; Shuster 2008; Taborsky et al. 2008; Shuster et al. 2013). Genetically fixed strategies are favoured whenever males tend to experience only one selective regime during their lifetime, so that specialising in alternative resources has higher fitness than being a generalist (Roulin, 2004; Brockmann, 2002; Zamudio & Sinervo, 2003). For instance, certain characteristic of the environment (e.g. heterogeneous distribution of resources, short breeding season) can interact with aspects of the species' ecology (e.g. short lifespan, adaptive site-fidelity) producing resource-defence mating systems (i.e. territoriality) in which subordinate males are unlikely to disperse. Males of such species tend to experience a single social environment during their lifetime, promoting the evolution of fixed, rather than conditional, behavioural strategies (Shuster and Wade 2003; Zamudio and Sinervo 2003). Balancing selection can maintain these alternative strategies, even if genetically fixed, whenever they obtain equal average fitness across contexts. This can happen in a wide array of scenarios, such as marked seasonality or spatial environmental heterogeneity (Brockmann, 2001; Taborsky & Brockmann, 2010). In sympatry, ARS can obtain equal fitness through frequency-dependent selection (Gross, 1996; Shuster & Wade, 2003; Taborsky *et al.*, 2008). Occasionally, two or more strategies can cycle in frequency over time if presenting a lower frequency confers a fitness advantage (negative frequency-dependent selection (NFDS; Brockmann, 2001; Roulin, 2004; Taborsky et al., 2008; Takahashi et al., 2010; Willink et al., 2019). Colour polymorphism may participate of this evolutionary process and be maintained under two different conditions. Distinct colour morphs may be directly selected for because of an adaptive advantage they confer in the context of ARS (e.g. sexual mimicry in damselflies; Willink et al. 2019; Svensson et al. 2020). Alternatively, colour morphs may be an indirect by-product of selection on other attributes related to the ARS (i.e. when genes involved in morphology, physiology or behaviour have pleiotropic effects on colour production; Galeotti et al. 2003; Roulin 2004, 2016; Roulin and Bize 2007; Wellenreuther et al. 2014).

One of the best studied cases of colour polymorphic ARS is the side-blotched lizard, *Uta stansburiana*. Adult males of this species present one of three alternative throat colours (blue, orange, yellow), each of which is associated with different socio-spatial behaviours. Orange-throated males establish large territories overlapping with several females by outcompeting blue-throated males in territorial disputes. These vast territories make orange males vulnerable to losing fertilizations in favour of the non-territorial yellow morph, which uses female-mimicry to sneak copulations opportunistically. In turn, blue-throated males compensate their competitive disadvantage by guarding females directly and hence securing more fertilizations against the yellow sneaker males (Sinervo & Lively, 1996; Zamudio & Sinervo, 2000; Sinervo & Zamudio, 2001; Alonzo & Sinervo, 2001a; Calsbeek & Sinervo, 2002a; Sinervo *et al.*, 2006, 2007). This dynamic gives rise to periodic oscillations in the relative frequencies of *U. stansburiana* male colour morphs, in a cyclical "rock-paper-scissors" (RPS) game whereby each colour morph, when predominant, is vulnerable to invasion by another colour morph (Sinervo and Lively 1996; Sinervo and Calsbeek 2006). These results sparked a proliferation of studies aimed at detecting similar differences in reproductive behaviour among the numerous species of lizards with colour polymorphism (Huyghe et al. 2007, 2009*a*; Olsson et al. 2007*a*, 2013; Bastiaans et al. 2013; San-Jose et al. 2014; Yewers et al. 2016, 2018; Fernández et al. 2018). For a number of reasons, morph-specific ARS, morph fluctuations, and rock-paper-scissors dynamics similar to those described in *Uta stansburiana* have been predicted to occur in Eurasian lacertids, particularly in wall lizards (genus *Podarcis*, family Lacertidae; Sinervo et al. 2007; Calsbeek et al. 2010; Mangiacotti et al. 2019). First, ventral colour polymorphisms involving three alternative colours (i.e. orange, white, and yellow) have been documented in adult individuals of at least 11 out of the 24 species currently recognized within the *Podarcis* genus, and is thus thought to have an ancestral origin (Arnold et al. 2002; Huyghe et al. 2007; Sacchi et al. 2007; Runemark et al. 2010; Andrade et al. 2019; Pérez i de Lanuza et al. 2019; Jamie and Meier 2020). Second, many of these species show high site-fidelity, low inter-annual survival, and occupy habitats where resources relevant to reproduction (e.g. stone-walls) are unevenly distributed (Strijbosch et al. 1980; Barbault and Mou 1988; Edsman 1990, 2001; Carretero 2007; Sinervo et al. 2007; Calsbeek et al. 2010; Font et al. 2012*a*). Third, males of many wall lizards experience strong intra-sexual competition, mainly in the contexts of territorial disputes and sperm competition. Females seem to be attracted to high quality and/or familiar patches of habitat rather than to males with certain phenotypic characteristics (Edsman 1990, 2001; Font et al. 2012*a*). Moreover, behavioural observations and genetic analyses have confirmed that receptive females often mate with more than one male before oviposition, which results in a high incidence of multiple paternity (Oppliger et al. 2007; Uller and Olsson 2008; Heathcote et al. 2016). Consequently, adult males try to secure fertilizations by investing significant time and energy in the defence of territories offering resources valuable to females (such as basking spots, shelters, optimal egg-laying sites, etc.) against other males (Edsman 1990; Font et al. 2012*a*; Baird 2013). The outcome of this territorial disputes is crucial to male reproductive success, and patterns of shared paternity have often been found to reflect spatial and social dominance among males (Oppliger et al. 2007; Uller and Olsson 2008; While et al. 2015; MacGregor et al. 2017*b*). For these reasons, alternative colour morphs in many wall lizards are often believed to represent the visible mark of heritable ARS involving differential socio-spatial behaviours in males (Huyghe et al. 2007; Sinervo et al. 2007; Calsbeek et al. 2010; Pérez i de Lanuza et al. 2017; Andrade et al. 2019).

The European common wall lizard (*Podarcis muralis*) shows the widest distribution within the genus *Podarcis* and many populations exhibit a striking colour polymorphism (Arnold et al. 2002). Adults of both sexes may show up to five alternative ventral colour morphs: three uniform (pure) morphs, i.e. orange (O), white (W) and yellow (Y), and two intermediate mosaics combining orange and white (OW) or yellow and orange (YO) (Pérez i de Lanuza et al., 2013, 2019; **Fig. 4.1**). These colour morphs are fixed at maturity (Pérez i de Lanuza et al., 2013), and recent research suggest that orange and yellow colour expression is caused by recessive homozygosity at two separate loci in the regulatory regions of two genes associated with pterin (SPR) and carotenoid (BCO2) metabolism, respectively (Andrade et al. 2019). Interestingly, each of these morphs is found in geographically distant sub-lineages of the species thought to have diverged up to 2.5 million years ago (Salvi et al. 2013; Andrade et al. 2018; **Fig 4.S1**). Local morph composition shows considerable geographic variation, although white ventral coloration is typically the most common (>50%), while the orange and especially the yellow morph rarely predominate. The yellow and yellow-orange morphs are often the most infrequent and in Pyrenean populations they seem to be geographically restricted to a subset of localities (< 50%) characterized by male-biased sex ratios and marked climatic seasonality (Pérez i de Lanuza et al. 2017, 2018*b*).

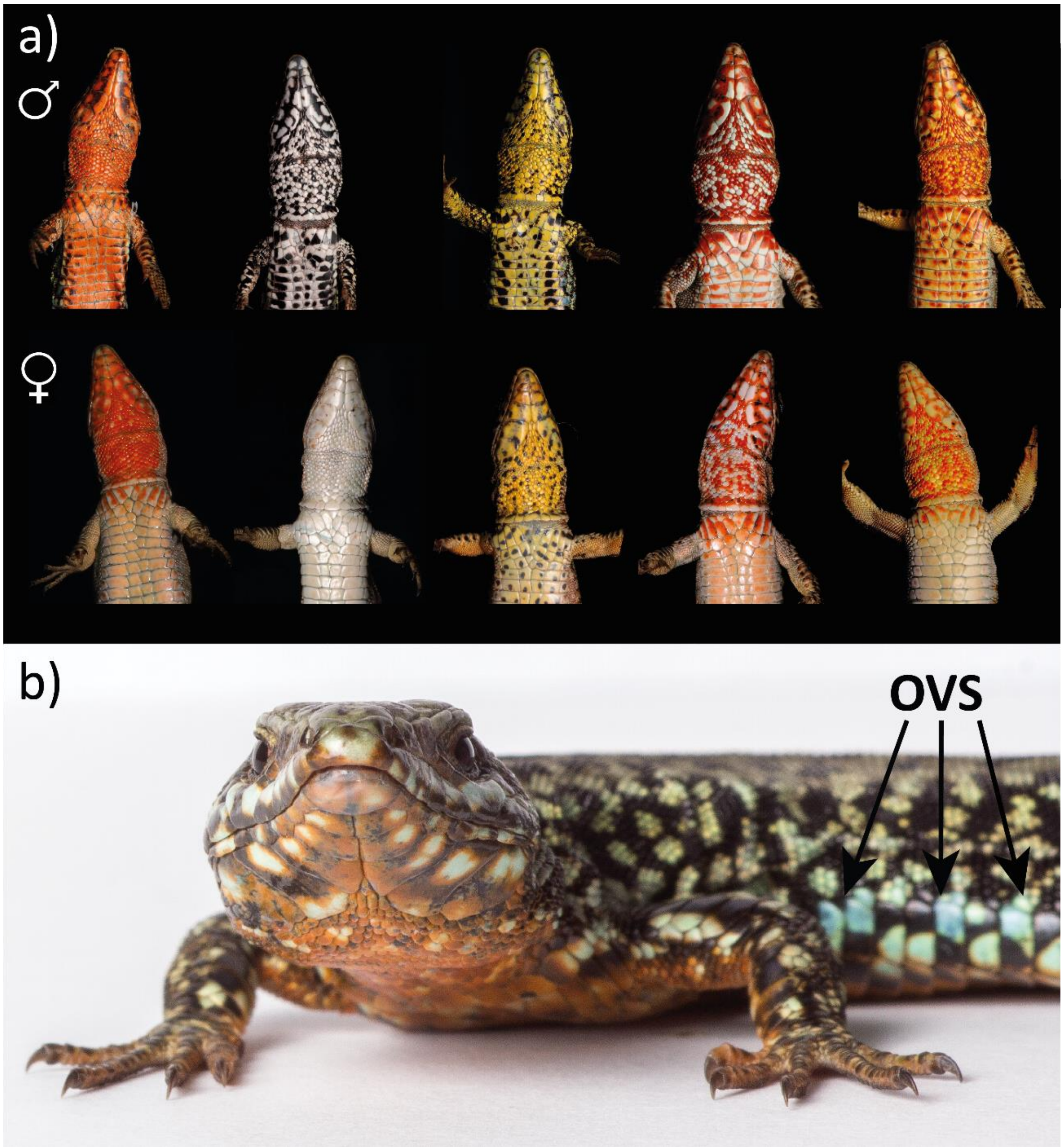


Figure 4.1. a) Colour variation in the ventral surface of adult *Podarcis muralis* lizards. b) Close-up of an orange morph male showing UV-blue and black spots in its outer ventral scales (OVS).

At least the pure morphs in *P. muralis* are often assumed to reflect alternative behavioural or life-history strategies (e.g. Calsbeek et al., 2010; Galeotti et al., 2010; Zajitschek et al., 2012; Scali et al., 2013). The colours are indeed well-suited to function as colour signals. They are highly conspicuous to the species visual system, heritable, and their ventral position allows the lizards to control their exposure through posture (Pérez i de Lanuza and Font 2015, 2016; Pérez i de Lanuza et al. 2016*a*; Andrade et al. 2019). Moreover, the alternative colours show discrete variation and are chromatically discriminated as categorically distinct by conspecifics (Pérez i de Lanuza et al. 2013, 2018*a*;), which makes them particularly suited to convey information about strategy (Tibbetts et al., 2017). Research on *P. muralis* has revealed several differences in morphological, physiological and behavioural traits across colour morphs (e.g. Calsbeek *et al.*, 2010; Zajitschek *et al*., 2012; Scali *et al.*, 2013; Galeotti *et al.,* 2013; Sacchi *et al.,* 2017a; Pérez i de Lanuza *et al.,* 2018). However, there is no clear evidence that these correlated traits reflect morph-specific strategies, whether in the context of sexual or natural selection. Furthermore, available studies have focused on morphology and physiology (Galeotti et al. 2007, 2010, 2013; Sacchi et al. 2007, 2017*a*; Calsbeek et al. 2010; Pellitteri-Rosa 2010), while central aspects in the behavioural ecology of this species have received little attention (Sacchi et al. 2009, 2015; Abalos et al. 2016; Pellitteri-Rosa et al. 2017). In particular, the interaction between socio-spatial behaviour, reproductive success, and shared paternity is key to ascertain whether *P. muralis* colour morphs obtain their fitness using alternative behavioural strategies during the breeding season. If behavioural ARS underlie colour polymrphism in *P. muralis*, the alternative colour morphs may show equal reproductive success but differential investment in social dominance, territoriality, space use, and/or post-copulatory sexual behaviour (e.g. mate-guarding), which often translate into morph-biased patterns of co-siring and clutch monopolisation (Sinervo and Lively 1996; Sinervo et al. 2000*a*; Zamudio and Sinervo 2000; Formica et al. 2004). However, no previous study has investigated the alignment of polymorphic coloration, social behaviour, and reproductive performance in sufficient detail to draw firm conclusions about the existence of behavioural ARS in *P. muralis*. To fill this gap, we monitored morph differences in spatial behaviour in a free-ranging polymorphic population from the eastern Pyrenees across a period of five years. We complemented this with a a mesocosm experiment using ten experimental populations with balanced sex ratio and morph frequencies to study the spatial and socio-sexual behaviour of *P. muralis* pure colour morphs in a controlled environment. Our experimental design was aimed to detect behavioural differences in space use or social behaviour among the colour morphs, as well as morph differences in shared paternity, rather than frequency-dependent effects on morph fitness. For this reason, we introduced the morphs in equal frequencies to optimise our sample size of individual lizards representing each morph within the enclosures. Incidentally, as the balanced morph ratios employed are highly unlikely to occur in natural populations, this design also allows us to test whether the higher prevalence of white morph lizards observed across the species distribution range results from some form of frequency-dependent fitness effect.

4.3. Materials and methods

4.3.1. Spatial behaviour in a free-ranging population of *P. muralis*

During the spring seasons of 2006-2010 we collected data on the activity and spatial behaviour of a population of wall lizards in Angoustrine (42°28'43'' N, 1°57'12'' E), eastern Pyrenees. The study site (ca 140 x 500 m = 7 ha; **Fig 4.2**) consists of a series of abandoned terraced fields characterized by granite outcrops and old dry-stone walls partially covered in vegetation (see Font *et al.*, 2012). Lizards were mostly sighted perching on the stone walls, usually remaining within the boundaries of a single wall for the whole breeding season. In any particular year, lizards showing at least six re-sightings on the same wall were considered resident, while lizards showing five or fewer re-sightings and/or sighted at walls located more than 100 m apart were considered non-resident transients (Edsman, 1990). We only considered lizards measuring at least 56 mm from snout to vent (SVL), which ensures they had developed full-blown adult ventral coloration (**Fig. S2**; Pérez i de Lanuza, Font, & Carazo, 2013).

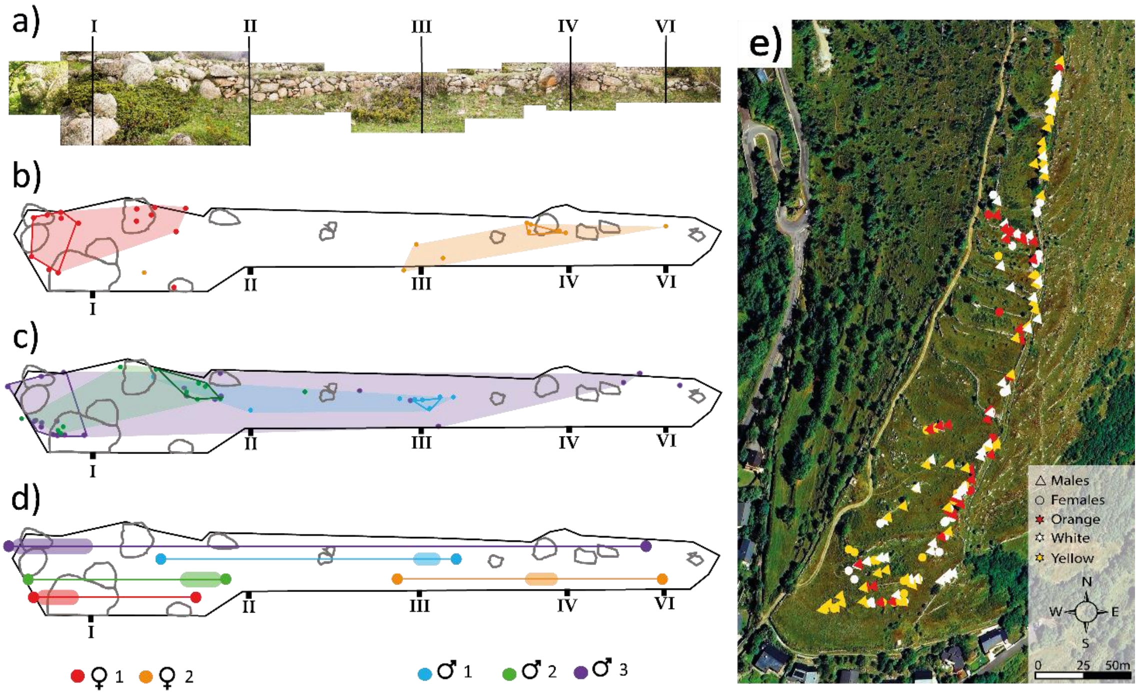


Figure 4.2. Space use in a free-ranging population of *P. muralis*. a) Photographic composition of a stone wall in Angoustrine. Roman numbers mark reference points for precision. b & c) Schematic representations of the wall vertical surface used as home- (colour shades, 95% MCP) and core-ranges (solid-line polygons, 50% MCP) by two females (b) and three males (c) during the breeding season of 2010. D) Diagram of the linear home and core-range lengths estimated for each lizard as the width of the corresponding MCP (solid-lines = home-range, colour shades = core-range). e) Google Earth satellite image of the study site in Angoustrine (Map data: Institut Cartogràfic de Catalunya), with arithmetic centre of each pure morph lizard core-range during the period examined (367 lizards, 125 females, 242 males).

To examine potential inter-morph differences in activity levels, for each lizard we counted the total number of sightings, the mean days elapsed between consecutive sightings, and the mean distance between consecutive sightings. As frequently done when a species' habitat is physically constrained (e.g. river fauna; Kramer, 1995; Ahlers et al., 2010; Kornilev et al., 2010), we calculated a one-dimensional measure of home-range size for each lizard inhabiting a particular stone wall. We operationally defined the width of the 95% minimum-convex-polygon (MCP) encompassing the lizard's cluster of re-sightings on a stone wall as the lizard's linear home-range size, and the width of the 50% MCP as the linear core-range in which the animals were most frequently observed (**Fig.4.2**; Powell, 2000; Heupel et al., 2004; Grassman et al., 2005). To determine what fraction of male core-ranges constitutes a territory (Maher and Lott 1995), we defined the exclusive-range of each male as the part of its core-range that did not show overlap with the core-range of any other male (i.e. territory; Kerr & Bull, 2006). Then, for each male with a reliable linear home-range estimate (≥ 17 sightings, see **Appendix S1**) we measured spatial overlap by counting the number of resident females whose core-ranges overlapped at least partially with either the home-range, or the exclusive-range of the focal male. To account for the vertical dimension of the lizards' home ranges we also calculated the mean perching height of each resident lizard sighted.

4.3.2. Mesocosm experiment

4.3.2.1. Lizard capture and housing

We captured 190 lizards (100 females and 90 males) by noosing from 12 polymorphic localities spread across the Cerdanya valley (Eastern Pyrenees). In each of these localities, we captured 2-8 lizards (SVL ≥ 56 mm) showing each of the pure colour morphs (O, W, Y) so as to avoid a geographical bias in our sample. No lizards were captured from populations lacking any of the pure colour morphs. To ensure captured females were not gravid, we captured females at the end of the previous breeding season (September 2017), and transferred them to the Statión d'Ecologie Théorique et Expérimentale (SETE, Moulis, France). There, we housed females in groups of 3-5 coming from the same locality in outdoor circular plastic tanks (170 cm diameter, 60 cm high), where they were kept under natural conditions for 130 days (Le Galliard et al. 2005; Bestion et al. 2014). In May 2018, after an artificial hibernation period (see **Appendix S1**), we re-installed the females in the outdoor tanks for two weeks while we captured the males.

4.3.2.2. Morphometry

Two days before the onset of the experiment, we measured SVL (0.1 mm) and mass (± 0.01 g) of each lizard with a ruler and a spring balance (Pesola, Schindellegi, Schwitzerland). Using a digital calliper (± 0.01 mm; Mitutoyo, Telford, UK) we quantified inter-limb length (ILL) in females, and two head measurements in males: length (HL) and width (HW) (Olsson et al. 2002). We also removed ~5 mm from the tail tip of each individual and preserved the tissue in 90% ethanol for genetic analyses.

4.3.2.3. Experimental enclosures and egg incubation

To study social behaviour and mating patterns in ten experimental populations of *P. muralis,* we released 180 lizards of either sex into ten experimental enclosures at the Metatron research facility (Caumont, France; Legrand et al., 2012). Within each of these enclosures, we created two types of sites that varied in structural complexity. Each site consisted of a wooden pallet (~1.2 m^2) with differing number of bricks, cinderblocks, rocks, and logs piled above, which acted both as shelter and basking sites (**Fig. 4.S3**.). We arranged high- and low-quality sites (respectively HQ and LQ) in two rows of three pallets along the N-S axis, separated by a line of six rocks (which we also considered as LQ habitat) (MacGregor et al. 2017*b*). We then surrounded the area with a plastic barrier (70 cm high) to prevent any escapes or intrusions. In total, each experimental cell had 47 m^2.

On May 23 2018, we released nine males (3O:3W:3Y) within each of the enclosures (simultaneously and always from the south-east corner). We monitored male behaviour (see below) for seven days before releasing nine females (3O:3W:3Y) within each enclosure. Due to post-hibernation mortality, the white female morph was underrepresented in two of the ten experimental enclosures (5o:1w:3y). Prior to release, we marked each lizard permanently on the ventral scales using a disposable medical cautery unit (Ekner et al. 2011) and drew a dorsal number with a toluene xylene-free permanent marker to facilitate individual recognition during behavioural observations (see **Video 4.S1** in the **Appendix S1**; Ferner & Plummer, 2016). To minimize the noise introduced by size asymmetries and prior social interactions, we allowed a maximum SVL difference of 2 mm (within-sexes) and only put lizards together in the same experimental enclosure if they had been captured at least 300 m apart.

On June 22, we released the males at their capture location (previously determined using a GPS device), and housed females individually in the laboratory until oviposition (see **Appendix S1**). We lost 22 clutches due to females laying eggs before we retrieved them from the enclosures (12 females) or because they failed to produce a clutch (10 females). These lost clutches were evenly distributed across enclosures (χ^2 = 14.667, P = 0.10) and female morphs (orange = 8, white = 8, yellow = 6). For the remaining 68 females, we counted the number of fertile and infertile eggs within each clutch by noting the presence of a calcified shell and vascularization 48 h after oviposition (Köhler 2006). We incubated the resulting 230 fertile eggs in plastic cups filled with moist coco husk (1:2 coco:water by weight) and covered with a perforated lid at a constant temperature of 28ºC (Memmert GmbH+ Co.KG incubator, Schwabach, Germany). Upon hatching, each of the 209 born juveniles was measured (SVL), weighted, sampled for DNA, permanently marked, and released at the outdoor tanks in the SETE-Moulis. For 21 embryos that died before hatching, we obtained DNA samples via dissection of the eggs. Average clutch size was 5.57 ± 0.20 eggs, average fertilization success (fertile eggs/ clutch size) was 67%, and average hatching success (hatched/ fertilized eggs) was 90%.

4.3.2.4. Behavioural observations

From May 23 to June 22, we conducted observations of spatial and social behaviour at the natural peak activity hours for the lizards (9.30-14.30; 16.30-19.30), spacing consecutive visits to the same enclosure at least 1 h and ensuring an even distribution of observations across the different time-periods. Two researchers (JA, AB) recorded the identity, position and behaviours of the lizards participating in social interactions using a behaviour sampling rule in recording sessions lasting 40 min. A social interaction was considered to occur whenever a marked lizard in our visual range directed any of the behaviours listed in **Table 4.1** towards a conspecific. During interactions, we recorded the first occurrence of the behaviours performed by each lizard. Consecutive interactions involving the same lizards were recorded as different events whenever the participants remained further than 30 cm apart for longer than 2 min. To ensure inter-observer reliability, JA and AB collected behavioural data together for the first six days of the experiment (Cohen's *kappa* ± $CI_{95\%}$ = 0.87 ± 0.05; Kaufman & Rosenthal, 2009). A third observer (OL) performed sequential rounds visiting all the enclosures every 2.5 h to collect data on the lizards' spatial behaviour. Using scan sampling, we determined the identity and location of every lizard in sight on a scale map of the enclosure that included the six wooden pallets. Each enclosure was observed from a starting position located 1 m from the plastic barrier surrounding it for 5 min, and then walking around it (randomizing direction between consecutive visits) to record lizards that were not visible from the starting position. To balance sampling effort across enclosures, scanning of a single enclosure was restricted to a maximum period of 15 min after the first lizard was spotted.

4.3.2.5. Behaviour analyses

We classified the interactions according to their socio-sexual context into four types: intra-sexual competitive and non-competitive, male-female reproductive and non-reproductive. Intra-sexual interactions were deemed competitive whenever one lizard (i.e. the loser) used fast-paced locomotion to flee from another lizard (i.e. the winner) showing display behaviour and/or physical aggression (i.e. display, bite, or chase). In males, where competitive encounters were numerous, we used the R package BradleyTerry2 to fit a Bradley-Terry model to the observed matrix of contest outcomes within each enclosure to obtain an individual index of social dominance for every male (further details in Stuart-Fox et al., 2006; Firth & Turner, 2012; Abalos et al., 2016). To examine potential non-transitive relations of dominance among male colour morphs, we also fitted three logistic mixed-models (one for each morph) on the contest outcome of heteromorphic encounters and tested whether the probability of winning against other morphs differed from even odds. Male-female interactions were classified as reproductive when the lizards engaged in sex-specific display behaviours (i.e. ♂: display; ♀: foot-shakes, tail-shake), copulatory behaviour (i.e. tail-grab, mating), or prolonged physical vicinity (i.e. co-perching). To examine the effect of morph combination on the frequency of male-female reproductive interactions, we used social network analysis on the compiled version of SOCPROG (Whitehead 2009) (**Appendix S1**).

Table 4.1.

Partial ethogram used during behavioural observations to collect data on social interactions within the experimental enclosures.

Behaviour	**Description**
Approach*	Movement toward a non-fleeing conspecific.
Display	Gular extension, back-arching, shoulders raised, head down, sagittal compression (any combination).
Bite	One or more bites to another individual (excluding tail grab)
Retreat*	Movement away from a non-chasing conspecific
Chase	Rapidly following another FLEEING lizard
Flight	Fast-paced movement to withdraw from a CHASING lizard
Foot-shakes II †	Sequence of front-leg waves in the air or onto the substrate
Tail grab	A male bites the tail or inguinal region of a female. Often followed by copulation.
Tail shake	Shaking entire tail (or its posterior portion) swiftly from side to side.
Mating	Two lizards engage in copulation
Co-perching	Two or more lizards lying together in close vicinity (<15 cm; >30 s)
Cloacal drag	Pulling body forward while keeping cloaca in contact with substrate

* We classified the mode of locomotion used as either running (fast-paced) or any other mode of locomotion (slow-paced).
† *Podarcis muralis* lizards perform four types of foot-shake displays (named I, IIa, IIb and III; see Font et al. 2012*b* and references therein), of which two (IIa and IIb) are given in a social context. We only recorded these two types of foot-shakes. Type IIa: rapid large amplitude vertical movements of front legs frequently performed by females in male-female interactions (belly-down, head-up posture). Losers of male-male agonistic interactions often perform this type of foot-shakes, which are hence considered in this context as submissive/appeasement displays (see Font and Desfilis 2002; Aragón et al. 2006 for details in other *Podarcis* lizards). Type IIb: Performed by males when approaching females (limbs extended, often displaying; Pérez i de Lanuza et al. 2016*b*).

Positional data were used to examine the putative effect of colour morph on activity, space use, and overlap with conspecifics. To account for habitat use within the enclosures, we estimated range areas by adjusting the smoothing factor in a fixed-kernel contour analysis until it matched the area of the 95% MCP (smoothing multiplier = 0.75, matrix cell number = 40; Row & Blouin-Demers, 2006; Kie, 2013; MacGregor et al., 2017). Lizards with fewer than nine sightings (*N* = *3*) were excluded from the analysis (see **Appendix S1**). For each lizard, we calculated range size and overlap with conspecifics both at the 95% (home-range) and the 50% (core-range) isopleth levels. Each lizard was assigned to a high or low-quality site based on the position where the 50% kernel estimate indicated peak density. Because of the high lizard density within the enclosures, male exclusive areas were peripheral and uninformative, so we did not conduct further analyses on them. When calculating home-range estimates, we excluded the positional data collected during the first six days of the experiment to allow for an acclimation period. All spatial analyses were conducted in Ranges 9 (Anatrack Ltd., UK; Kenward *et al.*, 2014).

4.3.2.6. Parentage analyses

We isolated DNA from tail-tip samples using the DNAeasy 96 Blood & Tissue Kit (Qiagen, Valencia, CA, USA), obtaining a final elution volume of 150 μl in AE buffer. We then combined the primers of six microsatellite loci described in *P. muralis* (Richard et al. 2012; Heathcote et al. 2014) into two different multiplexes (MPA: Pm16, Pm09, PmurC168; MPB: Pm19, Pm14, PmurC038) and ran standard PCR with 26 cycles and a final extension step of 30 min at 60ºC. Forward primers were labelled with different fluorescent dyes (FAM, NED, HEX). Diluted PCR products (1:5) were genotyped together with an internal ladder (Red ROX-500) on an ABI 3130 genetic analyser (Applied Byosystems Inc.). One researcher (HL) scored the alleles for every adult and juvenile lizard in Geneious 7.0.4 (Biomatters, available at http://www.geneious.com), which we used to conduct parentage analysis in Cervus 3.0 (Marshall et al. 1998; Kalinowski et al. 2007). We assigned paternity based on the log-likelihood statistic of each mother-father-offspring trio (LOD scores), using two confidence levels (strict: 95%, relaxed: 80%) and the nine males within each enclosure as candidate fathers. Critical LOD scores were determined by running a simulation paternity analysis based on 100,000 offspring with known mothers and nine candidate fathers. We could reliably assign paternity to every offspring examined (strict: 209 juveniles, relaxed: 229 juveniles).

To quantify individual fitness, we operationally defined two variables based on the results of the paternity analysis: mating success (i.e. the overall number of different mates with whom a lizard conceived offspring), and reproductive success (i.e. the total number of embryos/hatchlings sired). Since selection will depend on relative rather than absolute fitness, we then divided the fitness measures of each lizard by the mean for all same-sex conspecific within its enclosure. In addition, to evaluate inter-morph differences in sperm competition intensity, for each male we determined the average number of competitors with which he shared paternity of a clutch.

4.3.3. Statistical analyses

We ran linear mixed models using the *lme4* package (Bates *et al.,* 2014) in R (R Core Team, 2018) and model selection was conducted using backwards single term deletions ($P < 0.05$) of the saturated model followed by model comparisons via likelihood ratio tests (at $a = 0.05$). All numerical variables were centred and scaled before running the models (Schielzeth, 2010). We checked that all response variables conformed to homoscedasticity and normality assumptions before assuming a Gaussian distribution in model fitting. For some variables that did not conform to these assumption even after transformation, we fitted models using different distributions (**Appendix S1**).

4.3.4. Power analysis

Using G*Power (Erdfelder et al. 1996) and the methodology provided by Thalheimer and Cook (2002), we determined the effect size for an array of published morph differences detected in *U. stansburiana* and other polymorphic lizards thought to present some form of ARS (**Table 4.S1**). We then used G*power to calculate the smallest effect size that our sample size from the free-ranging population allowed us to detect (sensitivity analysis), and the sample size required to detect biologically meaningful differences among morphs in the mesocosm experiment (a priori required sample size). We chose the more conservative approach of conducting these *a priori* analyses in G*power instead of by simulation since this latter approach requires the researcher to directly determine estimates for both fixed and random effects, for which we had no previous reliable information (Green and Macleod 2016). However, to better accommodate for the mixed-model statistical design of our experiments, we additionally used the estimates obtained here to run a simulation-based analysis of power on the probability of detecting medium-sized (Cohen's $d > 0.5$) and large effects (Cohen's $d > 0.8$) with growing sample sizes (Hoenig and Heisey 2001; Haenlein and Kaplan 2004; O'Keefe 2007). We created two artificial LMMs using the *simr* package in R (Green and Macleod 2016), one corresponding to the free-ranging population, and another corresponding to the mesocosm experiment. In the former, we replicated the terms and parameters of the standardized model exploring morph differences in home-range size. In the latter, we replicated the terms and parameters of the standardized model exploring morph differences in social dominance (see **Appendix S1**). Following Green and Macleod (2016) we then modified the standardized estimate for the morph factor (i.e. effect size) to either 0.5 or 0.8, and conducted a power analysis by running 1000 simulations at 10 different levels of sample size (range = 5-50 lizards within each morph).

4.4. Results

4.4.1. Spatial behaviour in a free-ranging population of *P. muralis*

In total, we accumulated 5046 sightings of 472 different lizards. Eighty-seven lizards were observed more than one year (maximum = 3 years, 21 lizards). Out of those, 76 (87.4 %) were found on the same wall as the previous year, seven (8 %) moved between neighbouring walls, and only four (4.6 %) changed to a non-adjoining wall between years. Only 181 males and 101 females were large enough (SVL ≥ 56 mm) to be included in the analyses about morph differences (**Table 4.S2**). For each variable considered, we provide separate measures of centrality and dispersion for males and females in **Table 4.S3**. Residents represented 59.6 % of both adult male and female lizards, and no colour morph was overrepresented among resident or transient lizards (GLMM (binomial): $\chi^2 = 1.60$, $P = 0.81$). Movements between walls were similarly frequent among colour morphs (GLMM

(Gamma), $\chi^2 = 2.80$, $P = 0.59$). Colour morphs did not differ in the total number of re-sightings accumulated, the mean days elapsed between consecutive re-sightings, or the mean distance between consecutive relocations ($P > 0.28$; see **Table 4.S4** for more details and effect size).

We could calculate reliable estimates of linear home and core-ranges for 83 lizards, but decided to exclude mixed-morph lizards from the analyses due to their scarcity. The final dataset consisted of 70 lizards; 18 females and 52 males with at least 17 resightings (**Table 4.S2**). Neither sex showed significant differences in SVL among colour morphs (LMM: $\chi^2 = 6.61$, $P = 0.16$). Males had both larger linear home-ranges and core-ranges than females, and also perched higher on the stone walls ($P < 0.01$; **Table 4.S4**). Morphs did not differ in the size of their home- and core-ranges, neither in males (LMM: home-ranges: $\chi^2 = 4.31$, $P = 0.19$; core-ranges: $\chi^2 = 2.41$, $P = 0.30$), nor in females (LMM: home-ranges $\chi^2 = 0.44$, $P = 0.80$; core-ranges: $\chi^2 = 3.09$, $P = 0.21$). Similarly, mean perching height did not differ among colour morphs ($\chi^2 = 1.01$, $P = 0.60$; **Table 4.S4**). In males, we did not find significant inter-morph differences in the number of females within their linear home- or core-range (GLMM (Gamma): $\chi^2 < 1$, $P > 0.3$). Likewise, males of different colour morphs did not differ in the size of their exclusive ranges (i.e., the fraction of core-range which is not shared with any other male), or in the number of female core-ranges partially included within those ranges ($P > 0.35$; **Table 4.S4**).

4.4.2. Mesocosm experiment

4.4.2.1. Morphology and colour traits

None of the morphometric traits examined (reported to be under intra-sexual selection in male wall lizards; Baird, 2013; Pérez i de Lanuza *et al.*, 2014; While *et al.*, 2015) were found to differ among colour morphs in our sample of experimental males (**Table 4.S5**). In females, neither SVL nor ILL (both positively correlated with fecundity; Olsson *et al.*, 2002; Kratochvíl *et al.*, 2003) varied with colour morph, but white morph females (before reproduction) were found to be significantly heavier than orange females (**Table 4.S5**).

4.4.2.2. Spatial behaviour

Overall, we accumulated 7190 re-sightings of the marked lizards in 655 scan samplings. The total number of re-sightings per lizard differed significantly between sexes (males were re-sighted more often), but not among colour morphs (GLMM (negative binomial): Sex: $\chi^2 = 57.11$, $P < 0.001$; Morph: $\chi^2 = 0.81$, $P = 0.67$). Likewise, we found a strong inter-sexual difference in the ability to settle in high or low-quality sites, but no inter-morph difference (GLMM (binomial): Sex: $\chi^2 = 56.38$, $P < 0.001$; Morph: $\chi^2 = 1.37$, $P = 0.50$; **Fig. 4.3**). In fact, even though lizards were evenly distributed among sites (HQ: $N = 91$, LQ: $N = 89$), females had three times higher odds of settling in HQ sites (OR = 3.26) whereas only highly dominant males managed to occupy HQ sites (**Fig. 4.S4**). Specifically, an increase of one SD in social dominance among males meant 4.5 times higher odds of settling in HQ sites ($P < 0.001$; **Table 4.S6**). Males settled in HQ pallets did not differ in body size, weight, or head variables from males settled in LQ pallets (LMM: $\chi^2 < 1$, $P > 0.2$).

As expected, males had larger home- and core-ranges than females, and lizards settling in HQ sites occupied smaller areas than lizards in LQ sites (LMM on k50: Sex $\chi^2 = 34.95$, $P < 0.001$; Pallet quality: $\chi^2 = 7.64$, $P = 0.006$). In males, variation in home- and core-range size were significantly explained by social dominance ($P < 0.001$; **Table 4.S6**), but not by colour morph ($P > 0.20$; **Table 4.S6**). In females, we found significant differences in home- and core-range areas among female colour morphs, with white morph females showing the largest areas ($P < 0.001$; **Table 4.S7**). Male-female spatial overlap was not affected by colour morph, but was significantly associated with site quality in both sexes ($P < 0.01$; **Tables 4.S6** and **4.S7**). Males established in HQ sites overlapped with 3.0 ± 1.2 more females, and females established in LQ sites overlapped with 1.7 ± 1.0 more males.

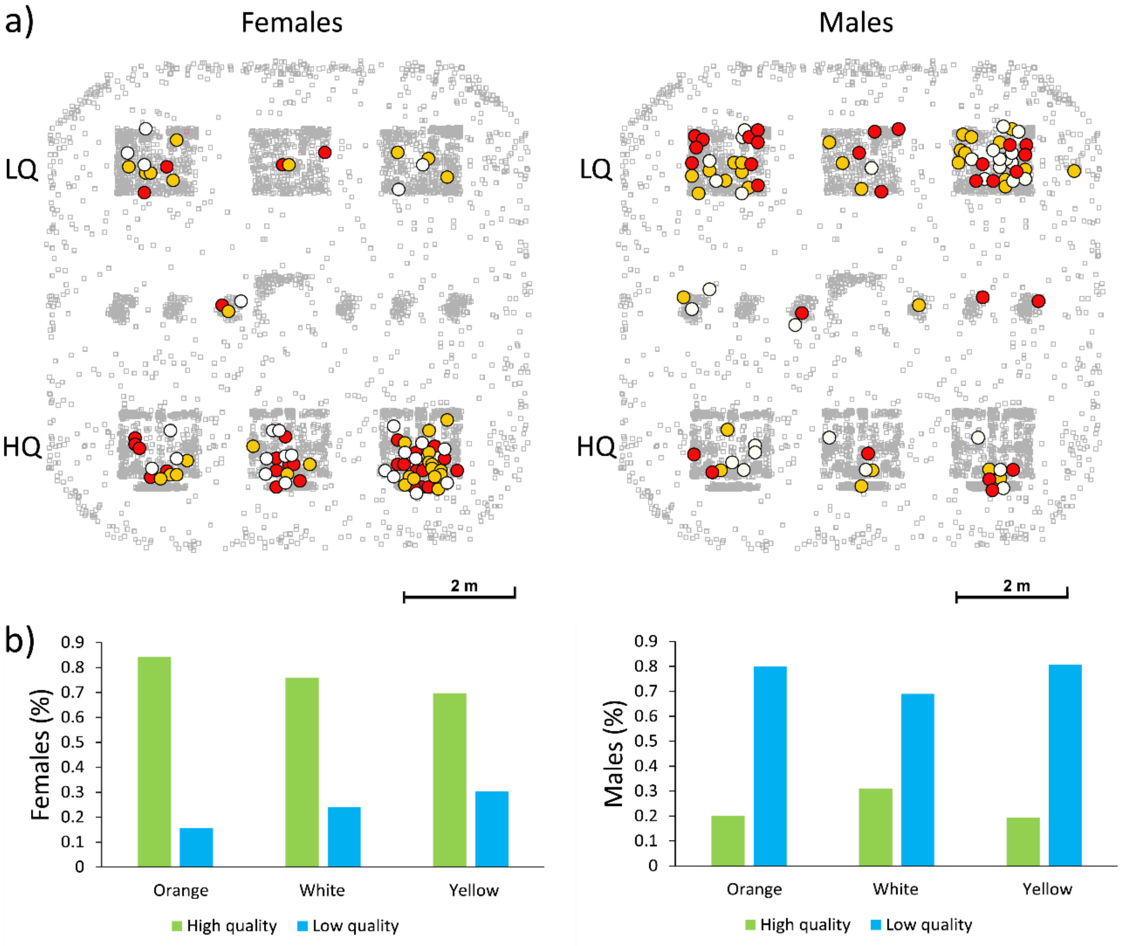


Figure 4.3. Distribution of the lizards among high and low-quality sites in the experimental enclosures. a) Position of the peak density of re-sightings for each male and female (filled circles), plotted on a background schematic diagram of an experimental enclosure obtained by pooling together every re-sighting of a lizard collected during the experiment (gray squares). The orange, white or yellow fill of the circles represent colour morph. b) Barplots showing the relative frequency of males and females of each colour morph that settled in high or low-quality sites.

4.4.2.3. Intra-sexual competition

We recorded 927 intra-sexual interactions (614 in males and 384 in females). Competitive interactions were more common among males (*N* = 543; 88% of total male-male interactions) than among females (*N* = 25; 7%), which were often observed in groups engaged in prolonged co-perching in the vicinity of a male (*N* = 338, 88%). In males, display posturing and/or foot-shakes (IIa, appeasement, **Table 4.1**) were observed in 60% of these competitive encounters, a third of them (36%) ended with a rapid chase/flight, and 16% involved physical aggression (i.e. bites). Display behaviour and bites were usually exhibited only by the winning lizard (display: *N* = 307, 91% only by winner; bite: *N* = 89, 70% only by winner), while foot-shakes were almost exclusively performed by losing males (*N* = 70, 93% only by loser) with no differences among morphs ($\chi^2 = 3.07$, *P* = 0.22). No morph combination was overrepresented among these contests ($\chi^2 = 5.63$, *P* = 0.40). We found no evidence of an inter-morph difference in the index of social dominance estimated from the Bradley-Terry model (*P* = 0.68; **Table 4.S6** and **Fig. 4.4**). After dealing with pseudo-replication (200 different pairs of rivals, **Table 4.S8**), we found no effect of morph combination on the outcome of heteromorphic contests (GLMM (Binomial): orange: $\chi^2 = 0.33$, *P* = 0.56; white: $\chi^2 = 1.83$, *P* = 0.18; yellow: $\chi^2 = 0.88$, *P* = 0.35). In fact, for either of the morphs involved in these combinations, the probability of winning did not differ significantly from even odds (**Fig. 4.4**).

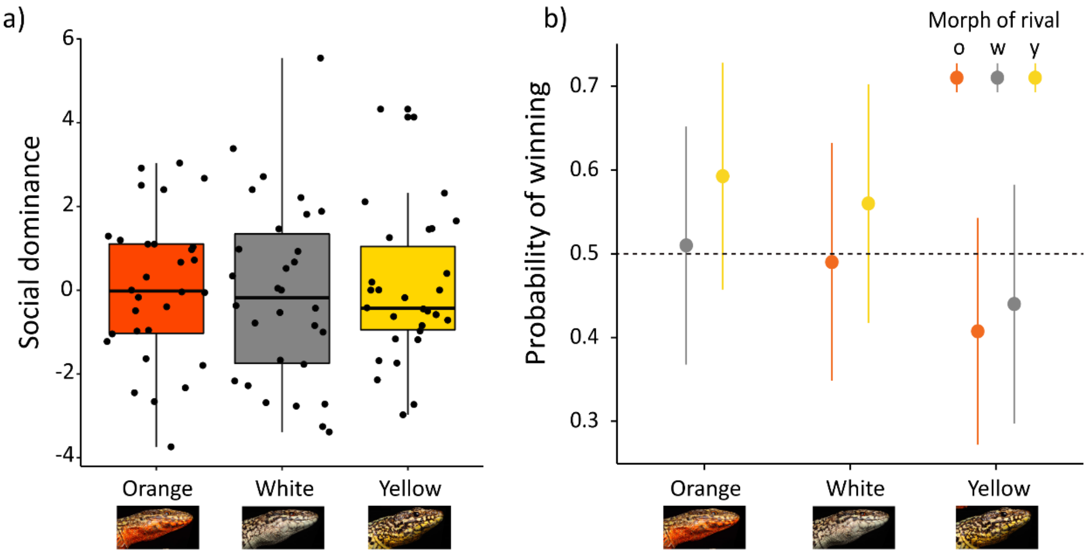


Figure 4.4. Male-male competitive interactions. a) Boxplot of social dominance by colour morph. Boxes indicate the interquartile range (IQR, 50% of data). Horizontal lines represent the median and bars extend to 1.5 times the IQR. A jittered dot cloud shows the value of the variable of interest for each lizard in our dataset. b) Mean plot showing the probability of winning for each morph combination according to the predicted values of the logistic mixed-models. Bars extend to the $CI_{95\%}$. The horizontal dotted line marks 50% probability.

4.4.2.4. Male-female interactions and parentage

In total, we recorded 1230 male-female interactions, of which 1098 were deemed as reproductive because they involved the exchange of sex-specific behaviours (441), prolonged co-perching (551), and/or copulatory behaviour (153).

Male colour morphs did not differ in the number of females with which they interacted, engaged in co-perching, or engaged in copulatory behaviour ($P > 0.57$; **Table 4.S9**). Unsurprisingly, males settled in HQ sites engaged in reproductive interactions more frequently (LMM: $\chi^2 = 36.91$, $P < 0.001$) and with a higher number of females than males settled in LQ sites ($P < 0.001$; **Table 4.S9**; **Fig. 4.5**). We found no difference in relative reproductive success, or relative mating success among male colour morphs ($P > 0.19$; **Table 4.S9**). Males settled in HQ sites showed significantly higher relative reproductive success ($P < 0.001$), but not relative mating success ($P = 0.107$; **Table 4.S9**). Sperm competition intensity faced by each individual male was also independent of colour morph ($P = 0.56$), but significantly higher in low quality sites ($P = 0.001$; **Table 4.S9**). No morph combination in male co-sirings was more prevalent than expected by chance ($\chi^2 = 2.13$, $P = 0.83$, **Table 4.S10**). Results from the analysis of male fitness are summarised in **Fig. 4.6**.

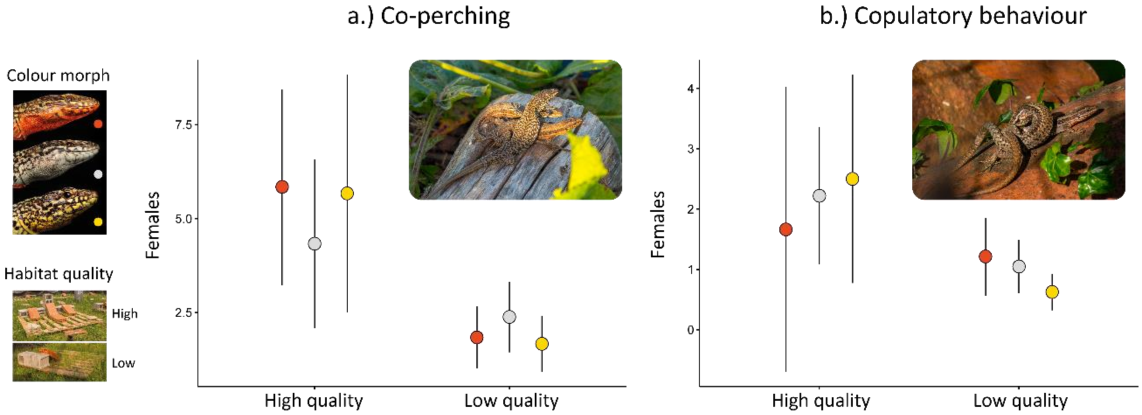


Figure 4.5. Variation in the number of different females with which males engaged in either co-perching (a) or copulatory behaviour (b, copulation and tail-grabs). Males settled in high quality pallets interacted with a significantly higher number of individual females, while male colour morphs did not differ in socio-sexual behaviour. Bars extend to the $CI_{95\%}$.

Female colour morphs did not vary in the number of males encountered in reproductive interactions, eggs produced, or fertilization success ($P > 0.11$; **Table 4.S11**). Body mass and ILL (but not colour morph; $P = 0.71$), were significantly related with laying date, with heavier and longer females laying their clutches sooner than the rest ($P = 0.014$; **Table 4.S11**). Although we found high levels of multiple paternity within the experimental enclosures (81% of clutches), female colour morphs did not differ in the number of sires fathering offspring in their clutches (LMM: $\chi^2 = 2.84$, $P = 0.24$), nor in the number of viable juveniles conceived (LMM: $\chi^2 = 4.31$, $P = 0.12$). Relative measures of fitness yielded similar results ($P > 0.16$; **Table 4.S11**). We found a significant effect of habitat quality on some aspects of female social behaviour and reproductive parameters: females established in LQ sites interacted with a higher number of males, showed higher levels of multiple paternity, and their clutches contained a smaller fraction of unfertilized eggs ($P < 0.05$; **Table 4.S11**).

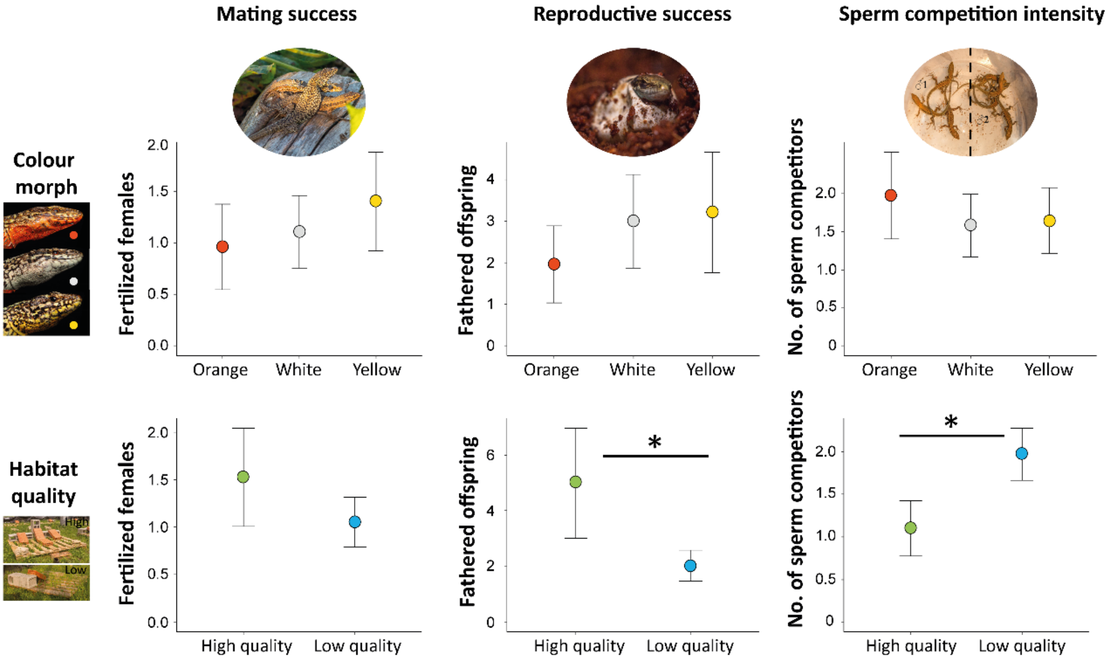
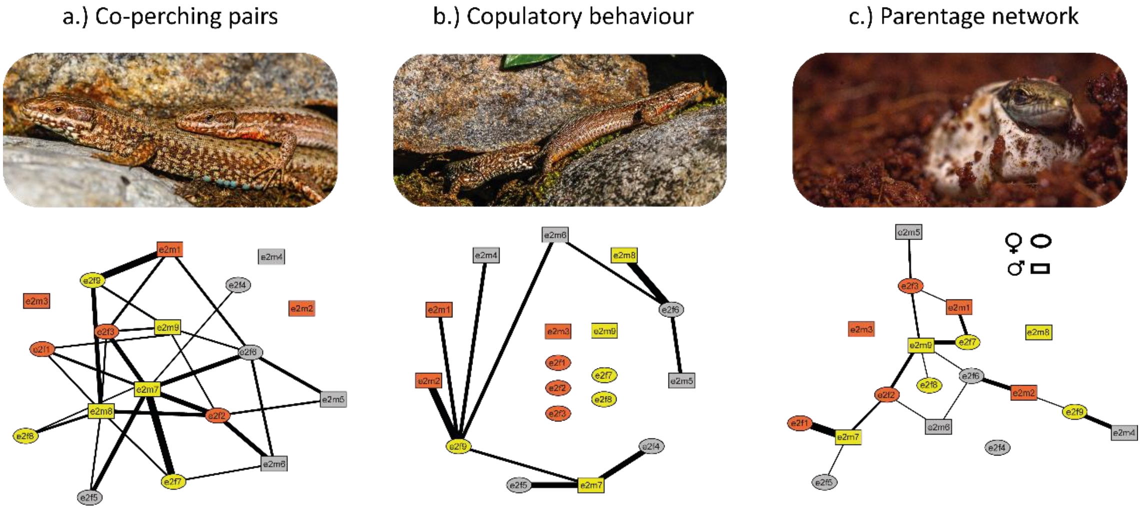


Figure 4.6. Variation in male individual fitness among alternative colour morphs (up) and between sites of different quality (below). Bars extend to the $CI_{95\%}$. Significant differences are marked with an asterisk ($P < 0.001$).

Parentage was significantly predicted across enclosures by both of the association networks based on social behaviour during male-female interactions (co-perchings: $\chi^2 = 51.91$, $P < 0.001$; copulation attempts: $\chi^2 = 45.40$, $P < 0.001$). However, neither of the behavioural association networks, nor the resulting parentage network were found to be affected by morph combination (co-perchings: $\chi^2 = 0.69$, $P = 0.69$; copulation attempts: $\chi^2 = 0.83$, $P = 0.83$; parentage: $\chi^2 = 0.32$, $P = 0.32$, **Fig. 4.7**). We found a significant interaction of the parental morph combination over juvenile body mass (LMM: $\chi^2 = 12.91$, $P = 0.012$). Splitting the dataset by female morph, we found that this result was exclusively driven by a non-significant tendency of yellow males to sire heavier offspring than orange males when coupled with white females (LMM: $\chi^2 = 6.28$, $P = 0.09$). We found no effect of male or female morph alone on juvenile mass (LMM: $\chi^2 < 1$, $P > 0.5$).

4.4.3. Power analysis

The sensitivity analysis in G*Power estimated a minimum detectable effect size of Cohen's $d = 0.46$ ($N = 181$) and Cohen's $d = 0.88$ ($N = 52$) for activity and space use differences (respectively) between male colour morphs in the free-ranging population. For the mesocosm experiment, we estimated that a sample size of 90 males and females would allow us to detect medium-sized (Cohen's *d* = 0.66) intra-sexual differences in behaviour and fitness among colour morphs with a standard statistical power of 0.80. These effect sizes are at the lower end of the range of effect sizes (Cohen's *d* = 0.49 - 2.32) which we calculated from the literature (**Table 4.S1**), suggesting that we had enough statistical power to detect even subtle but biologically meaningful differences among morphs. Accordingly, results from the two simulation-based analyses of power showed that our sample sizes were high enough to detect biologically relevant differences among colour morphs (power > 0.80 to detect medium-sized and large effect sizes). In fact, introducing the observed coefficients for the fixed and random factors in the simulations and plotting the expected increment in power at different sample sizes revealed a higher statistical power for the data presented here than the more conservative estimates obtained in G*Power (**Fig 4.S5**).



a.) Co-perching pairs

b.) Copulatory behaviour

c.) Parentage network

Figure 4.7. Example network diagrams from one of our experimental enclosures based on a) co-perching pairs, b) copulatory behaviour (i.e. interactions involving tail-grabs and/or matings), and c) the resulting parentage network. Each node represents an individual lizard, with shape and colour denoting sex and colour morph, respectively. Alphanumeric codes within the nodes correspond to the unique ID of each lizard within the enclosure. The thickness of the lines connecting nodes characterizes the number of social interactions (a, b) or offspring (c) between each dyad of lizards. Unconnected nodes represent lizards that we did not observe to engage in co-perching or copulatory behaviours (a, b), or did not reproduce (c).

4.5. Discussion

Overall, our results from both a longitudinal field study and an enclosure experiment argue against the hypothesis that *P. muralis* colour morphs reflect alternative reproductive strategies (ARS) involving differential socio-sexual behaviour and space use. In territorial species such as *Podarcis* lizards, resource holding potential, spatial behaviour, and activity are expected to vary across males employing alternative strategies (Sinervo et al. 2000*a*; Zamudio and Sinervo 2000; Sinervo and Zamudio 2001; Calsbeek and Sinervo 2002*c*, 2002*b*; Sinervo and Svensson 2002; Noble et al. 2013; Molnár et al. 2016). In this study, we did not find any evidence that colour morphs differ in resource holding potential (i.e. social dominance, agonistic behaviour, territoriality), space use (i.e. site-fidelity, home-range size, overlap with conspecifics), or activity (i.e. frequency of re-sightings, distance between consecutive re-sightings).

No colour morph was over-represented among resident or transient lizards in the field, and we did not observe differences in either inter-morph re-sighting propensity, distance between consecutive re-sightings, or inter-annual site fidelity. Furthermore, colour morphs showed similar home-range size and male-female overlap both in natural conditions and in experimental enclosures. In both sexes, alternative colour morphs obtained similar relative fitness within the enclosures (which would be necessary for their maintenance over time), but crucially this was not associated with different behavioural strategies. In line with previous evidence on the behavioural ecology of territorial lizards (Baird et al. 2003; Baird 2013), males competed fiercely to settle in high-quality sites irrespective of their colour morph, and the subset of successful dominant males (23%) engaged in co-perching with a higher number of females, experienced significantly lower levels of sperm competition, and ultimately achieved higher reproductive success. In sum, while lizards were strongly attracted to high-quality sites (both in the field and in the mesocosm experiment), we did not find any evidence that colour morph played a role in securing access to them or in the ability to exclude other conspecifics from its use. In fact, we did not find an effect of colour morph on the outcome of male-male competitive interactions. These results contrast previous evidence suggesting lower fighting ability in orange morph males during lab-staged encounters (Abalos et al. 2016), likely because any differences between size-matched morphs meeting at a neutral arena are overridden by the effect of size asymmetries and residency status when confrontations occur under more natural conditions (Stuart-Fox and Johnston 2005). Similarly, Sacchi et al. (2009) reported no effect of colour morph on aggressive behaviour during lab-staged contests when the experimental design allowed for size and residency asymmetries. Previous studies have reported larger body sizes in orange morph lizards with respect to white (Sacchi et al. 2007; Calsbeek et al. 2010), with some authors suggesting an advantage of orange morph lizards in male-male competition for preferred territories and hence, reproductive success (Calsbeek et al. 2010). The size difference, however, may result from mis-categorizing subadult lizards as pertaining to the white morph (i.e. the lizards' ventral surface appears white to the human eye before achieving sexual maturity), leading to the conflation of any possible morph difference with the expected size asymmetry between younger and older lizards. For instance, orange morph lizards from our study population in Angosutrine are only 1.7 ± 0.3 mm larger than white morph lizards in the free-ranging population of Angoustrine (1942 adult SVL > 56 mm lizards), which represents a 2.6% of the average SVL in adult lizards. There is, in fact, no evidence for biologically relevant differences among male *P. muralis* morphs neither in morphology or sex-specific coloration (i.e. UV-blue ventrolateral spots; Pérez i de Lanuza et al. 2014), and in this study we did not observe differential use of agonistic behaviours during intra-sexual competitive interactions.

The existence of ARS in a polymorphic territorial species does not necessarily imply that colour morphs must differ in territoriality or aggressive behaviour (Shuster and Wade 2003). ARS in males of polygynandrous species are often expressed as differential sexual behaviours (e.g. mate-guarding) or physiological adaptations (e.g. increased testis size) representing alternative solutions to the trade-off between securing fertilizations and acquiring new mates (Taborsky 2001; Formica et al. 2004; Shuster 2008; Taborsky and Brockmann 2010). For example, in the Australian painted dragon (*Ctenophorus pictus*), yellow morph males have larger testis and strongly outperform orange males in lab-staged sperm competition trials, despite the absence of differential territory-acquisition abilities between both morphs (Healey and Olsson 2008; Olsson et al. 2009). In contrast, *P. muralis* male morphs within experimental enclosures showed similar time allocation between guarding females and acquiring new mates, no difference in the number of mates sired, and experienced similar levels of sperm competition. In *U. stansburiana*, the interplay between the usurper, guarding, and sneaker strategies leads to morph-biased patterns of shared paternity, with yellow sneaker males obtaining almost all of their reproductive success from co-siring clutches with orange males, while blue guarding males show low overall levels of co-siring (especially with yellow males; Zamudio & Sinervo, 2000; Sinervo & Zamudio, 2001). Here we found no evidence of a similar bias, with no morph combination in co-sired clutches being more prevalent than expected by random association. In fact, given the absence of differences in pre-copulatory behaviour, the similar reproductive success achieved by males of the three colour morphs indirectly argues against the existence of physiological adaptations in the context of post-copulatory sexual selection (e.g. larger testis and ejaculates, which would have biased paternity in the absence of differential social behaviour). Further research could directly address this question by studying reproductive physiology in *P. muralis* colour morphs and staging realistic sperm competition trials across morphs.

While most research on colour polymorphism and ARS concerns males, females are also often polymorphic. Differential female breeding strategies, such as the different solutions to the trade-off between egg size and number described in the female colour morphs of *U. stansburiana* (Alonzo and Sinervo 2001), have also been suggested to occur in *P. muralis*. One study of an Italian population found that, in captivity, yellow females laid relatively larger clutches of smaller eggs than white morph females (Galeotti et al. 2013). Our results also contradict this hypothesis, as we found no difference among female morphs in clutch size or juvenile mass. Unexpectedly, white morph females roamed across larger areas than females from the other morphs. Rather than alternative strategies in space use, we think this difference may result from white morph females being heavier (and likely more advanced in their ovarian cycle) when released into the enclosures. This could have prompted exploratory behaviour in the search for suitable egg-laying sites earlier in this morph.

Whether this unexpected result is artefactual or derives from differences in the timing of reproduction among female morphs should be examined in future studies. Overall, our results constitute strong evidence against the existence of ARS concerning male-male aggression, spatial dominance, sexual behaviour, or breeding strategy in *P. muralis* colour morphs.

Even if colour morphs do not reflect ARS, non-random mating with respect to colour can contribute to the stability of polymorphic systems over time (Galeotti et al. 2003; Roulin 2004; Wellenreuther et al. 2014). Mate preferences may vary among individuals if the expected benefits derived from mating with differently coloured individuals is a function of the chooser's morph (e.g. genetic compatibility) or vary relative to other factors (e.g. time, space, population density; Roulin, 2004; Mckinnon & Pierotti, 2010; Wellenreuther et al., 2014). In polymorphic Pyrenean populations of *P. muralis,* homomorphic pairs of males and females occur more frequently than heteromorphic pairs, irrespective of local morph diversity (Pérez i de Lanuza et al. 2013*a*, 2016*b*). This assortative pairing suggests a role of colour morph in mate choice, but is not sufficient to demonstrate its existence (Roulin 2004; Roulin and Bize 2007; Wellenreuther et al. 2014). In fact, colour assortative pairing can also occur in the absence of mate choice, for example if phenotypically similar lizards tend to cluster together within populations as a consequence of similar environmental constraints or population viscosity (Roulin 2004; Wellenreuther et al. 2014). Here, we did not find evidence of morph-assortativity in the male-female social interactions observed within the enclosures. Previous research using lab-staged mate choice trials has already reported the absence of colour-assortative preferences towards differently coloured males in *P. muralis* females (Sacchi et al. 2015). However, we think that our results constitute a more realistic perspective of male-female dynamics in nature, since mounting evidence suggests that the initiation and outcome of pre-copulatory male-female interactions in lizards are almost completely under male control (Noble and Bradley 1933; Andrews 1985; Olsson and Madsen 1995; Tokarz 1995; Olsson 2001; Olsson et al. 2013; Heathcote et al. 2016). Following our results, we deem unlikely that the colour-assortative pattern observed in the wild (>60% of pairings at our study site, see Pérez i de Lanuza et al., 2013) results from the lizards actively choosing to pair with similarly coloured partners. Rather, assortative pairing could result indirectly from some form of clustering in the spatial distribution of colour morphs in natural populations, due to population viscosity or eco-physiological constraints (Svensson *et al.,* 2009; Wellenreuther et al., 2014; Svensson, 2017; Pérez i de Lanuza, Sillero, & Carretero, 2018b; Lindsay et al., 2019).

Our results also offer evidence against the existence of strong frequency-dependent effects on morph fitness. As stated before, by introducing the colour morphs in equal frequencies within the enclosures we simulated a situation which is rarely observed in any of the different *P. muralis* lineages showing colour polymorphism. Such balanced morph frequencies were never observed in natural populations from eastern Pyrenees (examined in Pérez i de Lanuza et al. 2017, 2018*b*, *N* = 116 localities), where white morph lizards usually predomínate (e.g. morph frequency ranges: orange = 0-60%; white = 27-92%; yellow = 0-25%; orange-white = 0-27%; yellow-orange = 0-13%), and only

3.45% of the localities show a morph other than white as the most common. Additionally, morph frequencies do not seem to experience substantial inter-annual variation, with the same rank order being maintained in the study population of Angoustrine for the last six years (**Fig. 4.S1**). If colour morphs are, in fact, under some form of frequency-dependent selection, the frequencies observed in natural populations may reflect a selective equilibrium where each morph obtains equal average fitness. By using a 1:1:1 morph ratio in our experimental setup we simulated a displacement from such equilibrium frequencies which should have resulted in a selective pullback, and hence higher fitness in white morph lizards (Roulin 2004; Sinervo et al. 2007; San-Jose et al. 2014; Svensson 2017). In contrast, we did not find significant differences in fitness among colour morphs, suggesting that strong frequency-dependent effects on morph fitness are unlikely to be the prime determinant of morph relative frequencies in *P. muralis* natural populations. This study is primarily aimed at detecting differences in socio-sexual behaviour among male morphs, and we acknowledge that our experimental design is not tailored to test for frequency-dependent effects on fitness. In fact, testing for a rare (NFDS) or a common morph advantage with a mesocosm design would require to introduce each morph consistently in lower or higher frequency across the enclosures (Roulin 2004; Wellenreuther et al. 2014; Svensson 2017). Additionally, selection on colour morphs is often dependent on both biotic (demography, sex-ratio) and abiotic factors (environmental conditions), as well as on the population morph composition and relative morph frequencies (Forsman et al. 2008; Gosden and Svensson 2008, 2009; McLean and Stuart-Fox 2014; McLean et al. 2015; Svensson 2017; Willink et al. 2019; Svensson et al. 2020). Future studies should examine the environmental dependence of morph fitness in populations characterized by extreme morph compositions and socio-ecological contexts (i.e. varying sex-ratio, density, and environmental conditions), for example by combining field observations with the experimental alteration of these same parameters in enclosure experiments.

The maintenance of colour polymorphism may be possible through genetic mechanisms entirely independent of socio-sexual behaviour. For instance, if heterozygosity at genes coding for colour polymorphism provides fitness benefits (i.e. overdominance), and the advantages of heterozygosity only concern viability selection (e.g. survival to adulthood), colour morphs would be maintained in the population even if morphs mated at random (Krüger et al., 2001; Roulin, 2004; Roulin & Bize, 2007; Wellenreuther et al., 2014). In a breeding experiment conducted on captive *P. muralis* lizards from Italian polymorphic populations, morph pair combination was found to affect fertilization success, hatching success and newborn quality (i.e. juvenile mass; Galeotti et al. 2013). Here, we found a weak effect of colour morph combination on juvenile mass, but the low sample size (N = 44) is insufficient to draw firm conclusions. To examine the role of genetic compatibility and overdominance on stabilising colour polymorphism in future research, we would need to estimate juvenile fitness and inter-annual survival at the genotypic (rather than the phenotypic) level, as the fitter heterozygotes could be phenotypically indistinguishable from other genotypes (Tregenza and Wedell 2000; Gratten et al. 2008; Johnston et al. 2013).

Despite drawing substantial interest from evolutionary biologists, the evolutionary causes and consequences of lacertid colour polymorphisms are still poorly understood. Alternative reproductive strategies have been suggested to occur in the Dalmatian wall lizard (*Podarcis melisellensis*), where orange males have been found to present larger body size, disproportionately large heads, and higher fighting ability in size-matched contests staged in the lab (Huyghe et al., 2007; Huyghe et al., 2009; 2012). In contrast, in the European common lizard (*Zootoca vivipara*), inter-population differences in morph composition and rapid morph cycles have been explained by the cumulative effect of two frequency-dependent mechanisms starkly different from ARS (morph biased female mate choice and offspring survival; Sinervo *et al.,* 2007; San-Jose et al., 2014). Meanwhile, differences in morph composition among island populations of the Skyros wall lizard (*Podarcis gaigeae*) have been found to be fall within that expected under neutral genetic divergence, and genetic drift could thus not be rejected as an explanation of the pattern (Runemark et al. 2010). Lastly, most of the evidence suggesting the existence of physiological or behavioural morph differences in *P. muralis* comes from studies conducted on the southern Alps sub-lineage (Galeotti et al. 2007, 2013; Scali et al. 2016; Sacchi et al. 2017*b*, 2017*a*), which is only distantly related to the western European lineage found in Pyrenees (Giovannotti et al. 2010; Schulte et al. 2012; Gassert et al. 2013). These observations, together with the high prevalence and ancient origin of colour polymorphisms in wall lizards (Arnold et al. 2007; Andrade et al. 2019; Jamie and Meier 2020), suggest the intriguing possibility that genes coding for the expression of the alternative colour morphs might become linked to genes that influence other functionally relevant traits (i.e. physiology, behaviour, life-history, development) only at times, and hence be under selection only in some environments or in some lineages (i.e. *Podarcis* species). Linkage disequilibria are expected to decay rapidly if not counteracted by strong and chronic correlational selection, and genetic drift is very effective in leading to the loss of polymorphism (especially in small populations; Sinervo and Svensson 2002; Gray and McKinnon 2007; Mckinnon and Pierotti 2010; Svensson 2017). Hence, this evolutionary scenario would cause correlations between colour and other phenotypic traits to vary either in space or time, and even lead to morph loss in some populations or lineages. Polymorphism loss has likely occurred in wall lizards. Despite their putative ancestral origin (Andrade et al. 2019), colour morphs are apparently absent in some *Podarcis* species (Arnold et al. 2002), and the polymorphic species which have been examined often show marked geographical variation in morph diversity (Runemark et al. 2010; MacGregor et al. 2017*a*; Pérez i de Lanuza et al. 2018*b*; Jamie and Meier 2020). However, due to its high genetic diversity, effective population sizes in *P. muralis* (and likely in other wall lizards) have been estimated to be sufficiently large ($Ne > 4x10^6$; Yang et al. 2020) to allow for the long-term persistence of a largely neutral trait under intermittent selection contigent on the environment. Local morph extinctions could thus be counteracted by immigration from larger populations where selectively-neutral colour expression could resist the eroding effect of genetic drift for longer periods, and inter-population differences in morph composition would be mainly driven by the environmental and genetic constraints of colour expression (Roulin et al. 2004; Gray and McKinnon 2007; Mckinnon and Pierotti 2010). Recent results showing the recessive genetic basis of orange and yellow ventral coloration in *P. muralis* with respect to white (Andrade et al. 2019) could provide a simple explanation for the marked bias towards the white morph observed in natural populations (Pérez i de Lanuza et al. 2017, 2018*b*, 2019*b*; **Fig. 4.S1**). Future research should investigate the possibility of spatially or temporally varying correlations between polymorphic colour expression and other phenotypic differences in *Podarcis* lizards, as well as evaluate the relative importance of selection and genetic drift in shaping inter-population differences in morph composition and relative frequencies (Runemark et al. 2010).

In conclusion, our results do not warrant the frequent assumption that behavioural ARS underlie the maintenance of ventral colour morphs in the European common wall lizard. In the wake of the *U. stansburiana* model, much effort has been devoted to detect inter-morph differences suggestive of behavioural ARS in polymorphic lizards (Healey et al. 2007; Calsbeek et al. 2010; Yewers et al. 2016; Fernández et al. 2018). However, these studies have often painted a much more complex picture involving several evolutionary processes, of which ARS may represent but one in many mechanisms explaining the vast diversity of lizard colour polymorphisms (Carpenter, 1995; Huyghe *et al.,* 2012; San-Jose *et al.,* 2014; McLean *et al.,* 2015). We should therefore reassess the allegedly central role of ARS in explaining the maintenance of phenotypic variability in nature, and broaden the perspective to incorporate other hitherto overlooked processes.

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4.6. Appendix S1: Expanded materials and methods

4.6.1. Additional information on the study of spatial behaviour in a free-ranging population of *P. muralis*

Lizards were identified by a combination of unique toe-clip markings, xylene-free permanent paint marks (Edding © 751, Edding Ltd, St Albans, UK), and/or by natural individual traits (see Pérez i de Lanuza *et al.,* 2014 for additional details). During the natural peak activity hours (10.00-13.30 and 15.30-19.00 local time) we carried out surveys (45 - 90 min, 650-1300 m) where we collected data on the spatial position of marked lizards (at least 2 h between consecutive rounds). We used short focus binoculars (Papilio 8.6 x 21, Pentax, Tokyo, Japan) and a laser rangefinder (Disto A6 and A8, Leica Geosystems, St. Gallen, Switzerland; m.e. = ± 1 mm) to record lizards' position with respect to mapped landmarks. Then, using Autocad 2013 (Autodesk Inc., San Rafael, Ca, USA) we created a scaled map of the study site and a simplified diagram of the lateral face of each of the stone-walls (**Fig. 4.1**). This sampling methodology provided two types of data: frequency of re-sightings, used as a proxy of activity levels (DeNardo & Sinervo, 1994; Sinervo *et al.*, 2000; Noble *et al.*, 2013), and positional data, used to estimate space use (home- and core-range size) and overlap with conspecifics (Boag 1973; Heathcote et al. 2016).

4.6.2. Additional information on female housing and hibernation before the experiment

We installed an anti-bird net covering each of the 33 tanks used, which prevented avian predators while allowing local invertebrates to get inside the tanks and serve as primary food source. To increase habitat complexity, we complemented the naturally occurring vegetation inside the tanks by providing a perforated brick to serve as shelter, two wood logs, and a water dish to collect rain-water. We visited the tanks monthly to trim the weeds and provide an additional supply of food (*Tenebrio molitor* larvae and *Acheta domesticus* adults). On February 15 2018, we re-captured the females within the outdoor tanks, installed them in individual hibernation pods consisting of a plastic tub (12 cm diameter, 8 cm high) covered with a perforated lid and filled with moist coco husk, and stored them for 10 weeks in a dark climatic chamber to mimic the conditions at their capture sites (1st week: 10ºC/ 2nd-9th weeks: 5ºC/ 10th week: 10ºC).

4.6.3. Additional information on the housing of gravid females after the experiment

We housed females individually in 55x38x28 cm high plastic terraria with a substrate of coco husk, a water dish, a shelter, and a small brick for basking over which we suspended a 40 W incandescent light (35ºC-18ºC temperature gradient). We also introduced a plastic tub filled with moist coco husk (11 cm of diameter, 8 cm deep) which the females used to lay the eggs. We set the light cycle to mimic field conditions during the reproductive season at the capture site (15L:9D), and additionally provided all lizards with full spectrum light (Reptistar 5.0: Sylvania, Danvers, MA, USA) for 2 h daily (12.00–14.00 h). We fed lizards two *Tenebrio molitor* larvae dusted with vitamins (JBL Terravit, Neuhofen, Germany) every other day. Overall, females stayed at the laboratory for less than 15 d before being released at their capture sites (i.e. after oviposition).

4.6.4. Additional information on social network analysis

To determine whether morph combination played a role in male-female social interactions, we ran Mantel permutation analyses on the compiled version of SOCPROG (Whitehead 2009) on three different association networks based on i) male-female co-perching interactions, ii) copulatory behaviour (tail-grabs and mating, **Table 4.1**), and iii) parentage. We used social networks weighted by the total number of observed interactions (or the number of offspring, for parentage) between each dyad of lizards. We also tested for significant correlation between these two behavioural networks and the resulting parentage network in all of the ten experimental enclosures using Dietz R-tests (the non-parametric analogous of Mantel tests), recommended to correct for potential outlying values. All permutation analyses were based on 10000 permutations, which achieved stability in *P*-values. For each set of analyses (co-perching, tail-grab, and paternity networks), we combined the *P*-values from each different enclosure into a single test statistic using Fisher's method (Fisher 1932).

4.6.5. Additional information on statistical analyses

4.6.5.1 Morphometry (mesocosm experiment)

We tested for differences between the colour morphs in morphometry by fitting two linear mixed models (LMMs) with body size (SVL) and weight (mass) as response variables, colour morph as fixed factors, and capture locality as random factor. In males, we used similar models to explore the existence of inter-morph differences in head length (HL) and head width (HW). In females, we tested for differential inter-limb length (ILL) among colour morphs.

4.6.5.2. Male-male competition (mesocosm experiment)

We examined potential morph-differences in social dominance using a linear mixed models with colour morph, mass, head variables (HL, HW), and pallet quality (i.e. peak density of re-sightings at a high- or low-quality site) as fixed factors. Enclosure and capture locality were included as random factors. To explore nontransitive relationships of dominance, we created three separate logistic mixed-models (one for each colour morph) on the binary outcome of heteromorphic contests (1 = win, 0 = loss). In each of these models we included the opponent's morph and identity as fixed and random factors, respectively. We also included the enclosure in which the interaction was observed as random factor. For the linear mixed-model we checked that all residuals conformed to homoscedasticity and normality assumptions. We used the glmer_dispersion function in the blmeco package of R to preclude potential problems of overdispersion in the residuals of the logistic GLMMs ($\phi \approx 1$ for every model).

4.6.5.3. Spatial behaviour and activity (free-ranging population and mesocosm experiment)

To determine the minimum sample size required to calculate a reliable estimate of home-range size, we subsampled the lizards with at least 25 sightings and graphed incremental range-observation plots showing the increase in linear home-range size as re-sightings are accumulated (Stone & Baird, 2002). For each lizard, we averaged the number of sightings when the curve reached 80% of final home-range size in four different incremental plots obtained by randomizing the order in which sightings were included (Rose, 1982; Stone & Baird, 2002). We then averaged the mean number of sightings for all the subsampled lizards, which resulted in a minimum sample size of 17 ± 1.84 sightings (□ ± SEM) for reliable linear home-range estimation in the free-ranging population of Angosutrine, and 9 ± 1 sightings for the lizards in the enclosure experiment. Lizards with fewer sightings were deemed unsuitable to reliably estimate home-range size but were included in the analysis of spatial overlap.

a) Free-ranging population

We examined potential sex- and morph-differences in activity and spatial behaviour using mixed models with the number of re-sightings and the mean days elapsed between consecutive re-sightings, as well as the linear home-range, core-range, exclusive-range size, and mean perching height of each lizard as response variables. In each of these models we included SVL, colour morph, sex, and the interaction between the last two as fixed effects, and year as random factor. To test for the differences in spatial overlap, we fitted GLMMs with the number of either 1) males, or 2) females included within the home-, core-, or exclusive-range of each lizard as response variables, and the same predictors and random factors described for space use models. Whenever a significant sex difference was found, we run two additional models examining potential inter-morph variability separately. Home- and core-range size were logged to correct a weak positive skew, and analysed assuming a Gaussian distribution. Activity and spatial overlap variables showed a strong positive skew, which we corrected by fitting a gamma distribution with log-link function to the generalized linear mixed models. For each model, we checked that all residuals conformed to homoscedasticity and normality assumptions. For lizards observed for more than one year, we averaged the value of response variables.

b) Mesocosm experiment

For the analysis of activity and spatial behaviour, we first examined inter-sexual differences using mixed models with sex, morph, and the interaction between the latter as fixed factors. We then examined inter-morph differences by creating separate models for males and females. For males, we included colour morph, mass, head variables (HL, HW), and pallet quality (i.e. peak density of re-sightings at a high- or low-quality site) as fixed factors. In models on females, we included colour morph, mass, ILL, and pallet quality as fixed factors. Capture locality and enclosure were included as random factors in every model. We analysed activity using negative binomial GLMMs with the total number of re-sightings as response variable. Home- and core-range size were normally distributed and were analysed with LMMs. Competition for habitat quality was analysed by fitting a logistic GLMM on whether the lizard's peak density of re-sightings was located at a high- (1) or low-quality (0) site. Male-female spatial overlap was analysed using GLMMs adjusted to a Poisson distribution, and the number of opposite-sex lizards showing core-core overlap as response variable. For each model, we checked that all residuals conformed to homoscedasticity and normality assumptions. We did not find over-dispersion problems in the Poisson or the negative binomial mixed-models ($\phi \approx 1$).

4.6.5.4. Male-female interactions and individual fitness

We used GLMMs fitted with a negative binomial distribution (or a Poisson distribution if the mean was equal to the variance) to explore potential inter-morph differences in the number of opposite-sex conspecifics with which the lizards were observed engaged in social interactions (i.e. co-perchings and copulations attempts). We tested both sexes separately by creating four different mixed-models. In males, we included colour morph, mass, head variables (HL, HW), and pallet quality (i.e. peak density of re-sightings at a high- or low-quality site) as fixed factors. In females we included colour morph, mass, ILL, and pallet quality as fixed factors. Regarding individual fitness, in both sexes we tested for differential reproductive and mating success using mixed-models (negative binomial distribution in males, Gaussian distribution in females) with the same predictors. We also used mixed-models to explore additional sex-specific aspects of sexual selection. In males, we estimated the average number of males with which he shared paternity of a clutch as a proxy of sperm competition intensity. We then analysed this variable with a LMM including the same predictors of the models described above. In females, we calculated fertilization success as the percentage of viable eggs from the total number of eggs laid. We tested for inter-morph differences in fertilization success using a LMM with colour morph, mass, ILL, and pallet quality as fixed factors. Capture locality and enclosure were included as random factors in every model described above. We checked that all the residuals conformed to homoscedasticity and normality assumptions, and also that they did not show over-dispersion problems in Poisson and negative binomial GLMMs ($\phi \approx 1$).

Tables

Table 4.S1. Standardized effect size (Cohen's *d*) calculated from published morph differences reported on different polymorphic species of lizards.

Species	**Morph difference**	**Cohen's *d***	**Reference**
Uta stansburiana	Contest outcome	1.98	Calsbeek and Sinervo 2002
	Endurance	2.32	Sinervo et al. 2000
	Number of re-sightings	1.66	Sinervo et al. 2000
	Testosterone	0.94	Sinervo et al. 2000
	Home range size	1.44	Sinervo et al. 2000
	Number of co-sires	0.48	Zamudio and Sinervo 2000
	Brain cortical volume	1.22	LaDage et al. 2016
Urosaurus ornatus	Mean distance between captures	0.59	Paterson and Blouin-Demers 2018
Liolaemus sarmientoi	Head height	1.14	Fernández et al. 2018
	PC2 Aggression score	0.51	Fernández et al. 2018
	Field body temperature	1.54	Fernández et al. 2018
Ctenophorus pictus	Fertilization success in sperm competition trials	0.85	Olsson et al. 2009
	Copula duration	0.94	Olsson et al. 2009
	Endurance	1.01	Tobler et al. 2012
	Reaction time against a model predator	1.72	Tobler et al. 2012
Ctenophorus decresii	Aggression against a model	0.56	Yewers et al. 2016
	Flight initiation distance	0.56	Yewers et al. 2016
Podarcis melisellensis	Bite force	0.79	Huyghe et al. 2009
	Corticosterone	0.65	Huyghe et al. 2009
Mean ± CI_{95}		**1.10 ± 0.24**	

Table 4.S2. Adult lizards studied in the free-ranging *P. muralis* population of Angoustrine (2006-2010). For reliable home-range estimation, we excluded lizards showing fewer than 17 re-sightings.

Sample	**Sex**	**Colour morph**				
		o	w	y	ow	yo
All lizards	♀	13	30	20	22	16
	♂	36	61	51	13	20
Residents	♀	9	14	14	13	11
	♂	21	35	32	9	10
>17 re-sightings	♀	6	4	8	3	3
	♂	13	19	20	2	5

Table 4.S3. Measures of centrality and dispersion for spatial behaviour variables in *P. muralis* males and females from Angoustrine.

Variable		**□ ± SEM / MED [Q1, Q3]**				**Sample size**
		♀		♂		
Resident lizards (%)		60.4 ± 4.9 %		59.1 ± 3.7 %		282 lizards (101 ♀, 181 ♂)
Walls visited (n)		**1.34 ± 0.07**		**1.72 ± 0.06**		
Re-sightings (n)		8 [2, 16]		8 [3, 22]		
Time elapsed (days)		**1.53 [2.48, 4.02]**		**2 [1.22, 3.80]**		
Distance between sightings (m)		5.89 [2.99, 14.37]		9.60 [5.26, 14.78]		
Linear home-range (m)		**13.01 [9.46, 19.52]**		**21.71 [14.61, 29.64]**		83 lizards (24 ♀, 59 ♂)
Linear core-range (m)		**2.11 [1.39, 3.32]**		**5.12 [3.31, 7.08]**		
Linear exclusive range (m)		**0.75 [0, 2.38]**		**4.70 [3.11, 6.67]**		
Mean perch height (m)		**1.10 [0.90, 1.39]**		**1.39 [1.11, 1.81]**		
		with ♀	**with ♂**	**with ♀**	**with ♂**	
Spatial overlap	**Home-core (n)**	**1.0 ± 0.2**	**2.0 ± 0.2**	**1.7 ± 0.2**	**1.5 ± 0.2**	
	Core-core (n)	**0.2 ± 0.1**	**1.2 ± 0.2**	**0.9 ± 0.1**	**0.3 ± 0.1**	
	Exclusive-core (n)	**-**	**0.2 ± 0.1**	**0.8 ± 0.1**	**-**	

□ **± SEM** = mean ± standard error. **MED [Q1, Q3]** = median [first and third quartiles]. Bold letters mark significant sex differences (α = 0.95).

Table 4.S4. Results from GLMMs examining potential differences in activity and spatial behaviour and male among colour morphs in the free-ranging population. Significant factors are highlighted in bold ($\alpha = 0.95$, $P < 0.05$). Statistics for non-significant factors are included at the point of their deletion from the model.

Model	Variable	df	χ^2	P-value	η_p^2	Effect size
Residency (Binomial)	**SVL**	**1**	**$\chi^2 = 11.60$**	**$P < 0.001$**	**0.04**	***pseudo-r^2* = 0.05**
	Morph	4	$\chi^2 = 1.60$	$P = 0.808$	0.01	
	Sex	1	$\chi^2 = 0.29$	$P = 0.592$	0.00	
	Morph*Sex	4	$\chi^2 = 2.99$	$P = 0.560$	0.01	
Re-sightings (Gamma)	**SVL**	**1**	**$\chi^2 = 29.46$**	**$P < 0.001$**	**0.06**	***pseudo-r^2* = 0.16**
	Morph	4	$\chi^2 = 5.02$	$P = 0.285$	0.02	
	Sex	1	$\chi^2 = 0.01$	$P = 0.914$	0.00	
	Morph*Sex	4	$\chi^2 = 2.01$	$P = 0.733$	0.01	
Days elapsed (Gamma)	**SVL**	**1**	**$\chi^2 = 11.44$**	**$P < 0.001$**	**0.01**	***pseudo-r^2* = 0.03**
	Morph	4	$\chi^2 = 3.19$	$P = 0.527$	0.01	
	Sex	**1**	**$\chi^2 = 4.24$**	**$P = 0.039$**	**0.01**	
	Morph*Sex	4	$\chi^2 = 1.03$	$P = 0.901$	0.00	
Distance between sightings (Gamma)	**SVL**	**1**	**$\chi^2 = 6.94$**	**$P = 0.008$**	**0.02**	***pseudo-r^2* = 0.11**
	Morph	4	$\chi^2 = 1.32$	$P = 0.857$	0.00	
	Sex	1	$\chi^2 = 8.65$	$P = 0.003$	0.00	
	Morph*Sex	4	$\chi^2 = 0.46$	$P = 0.978$	0.00	
Linear home-range (Gaussian)	SVL	1	$\chi^2 = 1.30$	$P = 0.255$	0.02	
	Morph	2	$\chi^2 = 2.20$	$P = 0.332$	0.02	
	Sex	1	**$\chi^2 = 7.27$**	**$P = 0.007$**	**0.13**	***pseudo-r^2* = 0.11**
	Morph*Sex	2	$\chi^2 = 1.25$	$P = 0.534$	0.02	
Linear core-range (Gaussian)	SVL	1	$\chi^2 = 0.64$	$P = 0.423$	0.01	
	Morph	2	$\chi^2 = 1.80$	$P = 0.406$	0.04	
	Sex	1	**$\chi^2 = 15.12$**	**$P < 0.001$**	**0.18**	***pseudo-r^2* = 0.20**
	Morph*Sex	2	$\chi^2 = 4.10$	$P = 0.128$	0.06	
Mean perch height (Gaussian)	SVL	1	$\chi^2 = 3.10$	$P = 0.080$	0.02	
	Morph	2	$\chi^2 = 1.01$	$P = 0.603$	0.00	
	Sex	1	**$\chi^2 = 10.09$**	**$P = 0.001$**	**0.05**	***pseudo-r^2* = 0.08**
	Morph*Sex	2	$\chi^2 = 1.87$	$P = 0.391$	0.01	
Overlap with females (home-range) (Gamma)	SVL	1	$\chi^2 = 0.43$	$P = 0.513$	0.00	
	Morph	2	$\chi^2 = 1.72$	$P = 0.424$	0.02	
Overlap with females (core-range) (Gamma)	SVL	1	$\chi^2 = 3.10$	$P = 0.080$	0.01	
	Morph	2	$\chi^2 = 1.01$	$P = 0.603$	0.03	
Overlap with females (exclusive-range) (Gamma)	SVL	1	$\chi^2 = 0.71$	$P = 0.400$	0.02	
	Morph	2	$\chi^2 = 2.09$	$P = 0.351$	0.05	

Table 4.S5. Results from linear models examining potential inter-morph differences in morphometric traits in our sample of experimental lizards. Bold letters mark significant differences ($\alpha = 0.95$, $P < 0.05$).

Sex	Trait	F	P-value
Males	SVL	$F_{2, 87} = 0.10$	$P = 0.903$
	Mass	$F_{2, 87} = 0.07$	$P = 0.937$
	HL	$F_{2, 87} = 0.71$	$P = 0.493$
	HW	$F_{2, 87} = 1.25$	$P = 0.293$
Females	SVL	$F_{2, 87} = 0.39$	$P = 0.680$
	ILL	$F_{2, 87} = 0.48$	$P = 0.619$
	Mass	$\boldsymbol{F_{2, 87} = 6.78}$	$\boldsymbol{P = 0.002}$

Table 4.S6. Results from GLMMs examining potential differences in dominance and spatial behaviour among male colour morphs from the mesocosm experiment. Significant factors are highlighted in bold ($a = 0.95$, $P < 0.05$). Statistics for non-significant factors are included at the point of their deletion from the model.

Model	Variable	df	Statistic	P-value	η_p^2	Effect size ± 95%CI
Social dominance (Gaussian)	Morph	2	$\chi^2 = 0.78$	$P = 0.677$	0.01	
	Mass	1	$\chi^2 = 0.00$	$P = 0.951$	0.00	
	HW	1	$\chi^2 = 0.12$	$P = 0.734$	0.00	
	HL	1	$\chi^2 = 1.08$	$P = 0.299$	0.01	
	PQ	**1**	**$\chi^2 = 25.56$**	**$P < 0.001$**	**0.24**	***Hedge's g* = 1.4 [0.7, 1.7]** ***pseudo*-r^2 = 0.25**
Pallet quality (Binomial)	Morph	2	$\chi^2 = 2.13$	$P = 0.345$	0.03	
	Mass	1	$\chi^2 = 0.84$	$P = 0.360$	0.02	
	HW	1	$\chi^2 = 1.68$	$P = 0.196$	0.03	
	HL	1	$\chi^2 = 0.25$	$P = 0.619$	0.00	
	Dominance	**1**	**$\chi^2 = 23.97$**	**$P < 0.001$**	**0.25**	***OR* = 4.5 [2.3, 11.7]** ***pseudo*-r^2 = 0.30**
Home-range size (Gaussian)	Morph	2	$\chi^2 = 2.14$	$P = 0.344$	0.03	
	Mass	1	$\chi^2 = 2.01$	$P = 0.157$	0.03	
	HW	1	$\chi^2 = 0.96$	$P = 0.327$	0.01	
	HL	1	$\chi^2 = 0.31$	$P = 0.580$	0.00	
	Dominance	**1**	**$\chi^2 = 23.97$**	**$P < 0.001$**	**0.36**	***pseudo*-r^2 = 0.30**
Core-range size (Gaussian)	Morph	2	$\chi^2 = 3.18$	$P = 0.204$	0.04	
	Mass	1	$\chi^2 = 0.03$	$P = 0.863$	0.00	
	HW	1	$\chi^2 = 0.35$	$P = 0.552$	0.00	
	HL	1	$\chi^2 = 0.43$	$P = 0.512$	0.01	
	Dominance	**1**	**$\chi^2 = 31.90$**	**$P < 0.001$**	**0.35**	***pseudo*-r^2 = 0.31**
Overlap with females (k50) (Poisson)	Morph	2	$\chi^2 = 1.46$	$P = 0.481$	0.01	
	Mass	1	$\chi^2 = 0.02$	$P = 0.898$	0.00	
	HW	1	$\chi^2 = 0.00$	$P = 0.972$	0.00	
	HL	1	$\chi^2 = 0.13$	$P = 0.715$	0.00	
	PQ	**1**	**$\chi^2 = 31.90$**	**$P < 0.001$**	**0.28**	**HQ-LQ = 3.0 ± 1.2 females** ***pseudo*-r^2 = 0.24**

Table 4.S7. Results from GLMMs examining potential differences in spatial behaviour among female colour morphs from the mesocosm experiment. Significant factors are highlighted in bold (*a* = 0.95, $P < 0.05$). Statistics for non-significant factors are included at the point of their deletion from the model.

Model	Variable	df	Statistic	P-value	η_p^2	Effect size ± 95%CI
Pallet quality (Binomial)	Morph	2	$\chi^2 = 2.95$	$P = 0.229$	0.05	
	Mass	1	$\chi^2 = 3.75$	$P = 0.206$	0.02	
	ILL	1	$\chi^2 = 0.19$	$P = 0.662$	0.00	
Home-range size (Gaussian)	**Morph**	**2**	$\boldsymbol{\chi^2 = 15.68}$	$\boldsymbol{P < 0.001}$	**0.18**	**W-O = 6.4 ± 3.5 m^2** ***pseudo-r*^{2} = 0.18**
	Mass	1	$\chi^2 = 0.06$	$P = 0.802$	0.03	
	ILL	**1**	$\boldsymbol{\chi^2 = 3.90}$	$\boldsymbol{P = 0.048}$	**0.05**	
Core-range size (Gaussian)	**Morph**	**2**	$\boldsymbol{\chi^2 = 15.54}$	$\boldsymbol{P < 0.001}$	**0.19**	**W-O = 1.7 ± 0.9 m^2** ***pseudo-r*^{2} = 0.16**
	Mass	1	$\chi^2 = 0.03$	$P = 0.866$	0.00	
	ILL	1	$\chi^2 = 1.82$	$P = 0.178$	0.01	
Overlap with males (k50) (Poisson)	Morph	2	$\chi^2 = 1.31$	$P = 0.520$	0.03	
	Mass	1	$\chi^2 = 0.02$	$P = 0.879$	0.00	
	ILL	1	$\chi^2 = 1.20$	$P = 0.273$	0.02	
	PQ	**1**	$\boldsymbol{\chi^2 = 8.42}$	$\boldsymbol{P = 0.004}$	**0.13**	**LQ-HQ = 1.7 ± 1.0 males** ***pseudo-r*^{2} = 0.08**

Table 4.S8. Male-male competitive interactions recorded within the experimental enclosures, sorted by morph combination and contest outcome.

		Loser morph			Total (heteromorphic)
		Orange	White	Yellow	
Winner morph	Orange	34 (14)	57 (26)	85 (32)	142 (58)
	White	97 (25)	44 (14)	74 (28)	171 (53)
	Yellow	52 (22)	58 (22)	42 (17)	110 (44)
	Total (heteromorphic)	149 (47)	115 (48)	159 (60)	423 (155)

Plain numbers = absolute frequency. In brackets = absolute frequency after dealing with pseudo-replication.

Table 4.S9. Results from GLMMs examining potential differences in sexual behaviour and fitness among male colour morphs from the mesocosm experiment. Significant factors are highlighted in bold ($a = 0.95$, $P < 0.05$). Statistics for non-significant factors are included at the point of their deletion from the model.

Model	**Variable**	**df**	**Statistic**	**P-value**	η_p^2	**Effect size ± 95%CI**
Females encountered in reproductive interactions (n) (NegBin)	Morph	2	$\chi^2 = 0.09$	$P = 0.958$	0.00	
	Mass	1	$\chi^2 = 0.00$	$P = 0.970$	0.00	
	HW	1	$\chi^2 = 0.12$	$P = 0.967$	0.00	
	HL	1	$\chi^2 = 0.03$	$P = 0.858$	0.00	
	PQ	**1**	$\boldsymbol{\chi^2 = 27.24}$	$\boldsymbol{P < 0.001}$	**0.30**	**HQ-LQ = 2.7 ± 0.9 females** ***pseudo-r^2* = 0.36**
Females in co-perching (n) (NegBin)	Morph	2	$\chi^2 = 0.49$	$P = 0.784$	0.00	
	Mass	1	$\chi^2 = 2.26$	$P = 0.133$	0.02	
	HW	1	$\chi^2 = 0.02$	$P = 0.891$	0.00	
	HL	1	$\chi^2 = 1.76$	$P = 0.185$	0.01	
	PQ	**1**	$\boldsymbol{\chi^2 = 29.73}$	$\boldsymbol{P < 0.001}$	**0.32**	**HQ-LQ = 3.0 ± 1.5 females** ***pseudo-r^2* = 0.32**
Females in copulatory behaviour (n) (NegBin)	Morph	2	$\chi^2 = 1.14$	$P = 0.566$	0.01	
	Mass	1	$\chi^2 = 0.00$	$P = 0.968$	0.00	
	HW	1	$\chi^2 = 2.01$	$P = 0.156$	0.01	
	HL	1	$\chi^2 = 0.02$	$P = 0.900$	0.00	
	PQ	**1**	$\boldsymbol{\chi^2 = 13.36}$	$\boldsymbol{P < 0.001}$	**0.13**	**HQ-LQ = 1.1 ± 0.7 females** ***pseudo-r^2* = 0.13**
Relative mating success (NegBin)	Morph	2	$\chi^2 = 3.32$	$P = 0.190$	0.04	
	Mass	1	$\chi^2 = 0.10$	$P = 0.751$	0.00	
	HW	1	$\chi^2 = 0.14$	$P = 0.707$	0.00	
	HL	1	$\chi^2 = 1.20$	$P = 0.274$	0.01	
	PQ	1	$\chi^2 = 2.60$	$P = 0.107$	0.03	
Relative reproductive success (NegBin)	Morph	2	$\chi^2 = 2.63$	$P = 0.268$	0.02	
	Mass	1	$\chi^2 = 0.00$	$P = 0.971$	0.00	
	HW	1	$\chi^2 = 0.00$	$P = 0.960$	0.00	
	HL	1	$\chi^2 = 1.91$	$P = 0.167$	0.01	
	PQ	**1**	$\boldsymbol{\chi^2 = 11.28}$	$\boldsymbol{P < 0.001}$	**0.12**	**HQ-LQ = 0.8 ± 0.4** ***pseudo-r^2* = 0.13**
Sperm competition intensity (Gaussian)	Morph	2	$\chi^2 = 1.15$	$P = 0.562$	0.02	
	Mass	1	$\chi^2 = 2.11$	$P = 0.147$	0.02	
	HW	1	$\chi^2 = 1.14$	$P = 0.285$	0.03	
	HL	1	$\chi^2 = 0.02$	$P = 0.875$	0.02	
	PQ	**1**	$\boldsymbol{\chi^2 = 10.60}$	$\boldsymbol{P = 0.001}$	**0.19**	**HQ-LQ = 0.8 ± 0.5 males** ***pseudo-r^2* = 0.15**

Table 4.S10. Shared paternity associations among males of different colour morphs during the enclosure experiment.

	O-O	W-W	Y-Y	O-W	Y-O	W-Y	Total
Observed	4	4	7	13	16	20	64
Expected	5.33	5.33	5.33	16	16	16	64
Probability	0.083	0.083	0.083	0.25	0.25	0.25	

Table 4.S11. Results from GLMMs examining potential differences in sexual behaviour and fitness among female colour morphs from the mesocosm experiment. Significant factors are highlighted in bold (α = 0.95, $P < 0.05$). Statistics for non-significant factors are included at the point of their deletion from the model.

Model	**Variable**	**df**	**Statistic**	**P-value**	η_p^2	**Effect size ± 95%CI**
Males encountered in reproductive interactions (n) (Gaussian)	Morph	2	$\chi^2 = 0.31$	$P = 0.856$	0.00	
	Mass	1	$\chi^2 = 3.69$	$P = 0.055$	0.07	
	ILL	1	$\chi^2 = 1.99$	$P = 0.158$	0.03	
	PQ	**1**	**$\chi^2 = 5.59$**	**$P = 0.018$**	**0.08**	**LQ-HQ = 1.1. ± 0.8 males** ***pseudo-r^2* = 0.06**
Eggs produced (n) (Gaussian)	Morph	2	$\chi^2 = 4.61$	$P = 0.110$	0.07	
	Mass	**1**	**$\chi^2 = 14.35$**	**$P < 0.001$**	**0.19**	***pseudo-r^2* = 0.18**
	ILL	1	$\chi^2 = 0.59$	$P = 0.444$	0.01	
	PQ	1	$\chi^2 = 0.06$	$P = 0.800$	0.00	
Fertilization success (%) (Gaussian)	Morph	2	$\chi^2 = 2.18$	$P = 0.335$	0.04	
	Mass	1	$\chi^2 = 0.58$	$P = 0.447$	0.01	
	ILL	1	$\chi^2 = 0.08$	$P = 0.784$	0.00	
	PQ	**1**	**$\chi^2 = 4.13$**	**$P = 0.042$**	**0.07**	**LQ-HQ = 0.29 ± 0.27 %** ***pseudo-r^2* = 0.07**
Laying date (n) (Gaussian)	Morph	2	$\chi^2 = 0.69$	$P = 0.709$	0.01	
	Mass	**1**	**$\chi^2 = 6.03$**	**$P = 0.014$**	**0.06**	***pseudo-r^2* = 0.11**
	ILL	**1**	**$\chi^2 = 6.03$**	**$P = 0.014$**	**0.00**	
	PQ	1	$\chi^2 = 0.02$	$P = 0.878$	0.07	
Relative mating success (NegBin)	Morph	2	$\chi^2 = 3.24$	$P = 0.197$	0.05	
	Mass	1	$\chi^2 = 0.90$	$P = 0.898$	0.01	
	ILL	1	$\chi^2 = 1.57$	$P = 0.210$	0.04	
	PQ	**1**	**$\chi^2 = 4.50$**	**$P = 0.034$**	**0.04**	**LQ-HQ = 0.7 ± 0.6** ***pseudo-r^2* = 0.07**
Relative reproductive success (NegBin)	Morph	2	$\chi^2 = 3.62$	$P = 0.164$	0.04	
	Mass	1	$\chi^2 = 0.00$	$P = 0.964$	0.00	
	ILL	1	$\chi^2 = 0.97$	$P = 0.326$	0.01	
	PQ	1	$\chi^2 = 0.90$	$P = 0.343$	0.01	

Figures

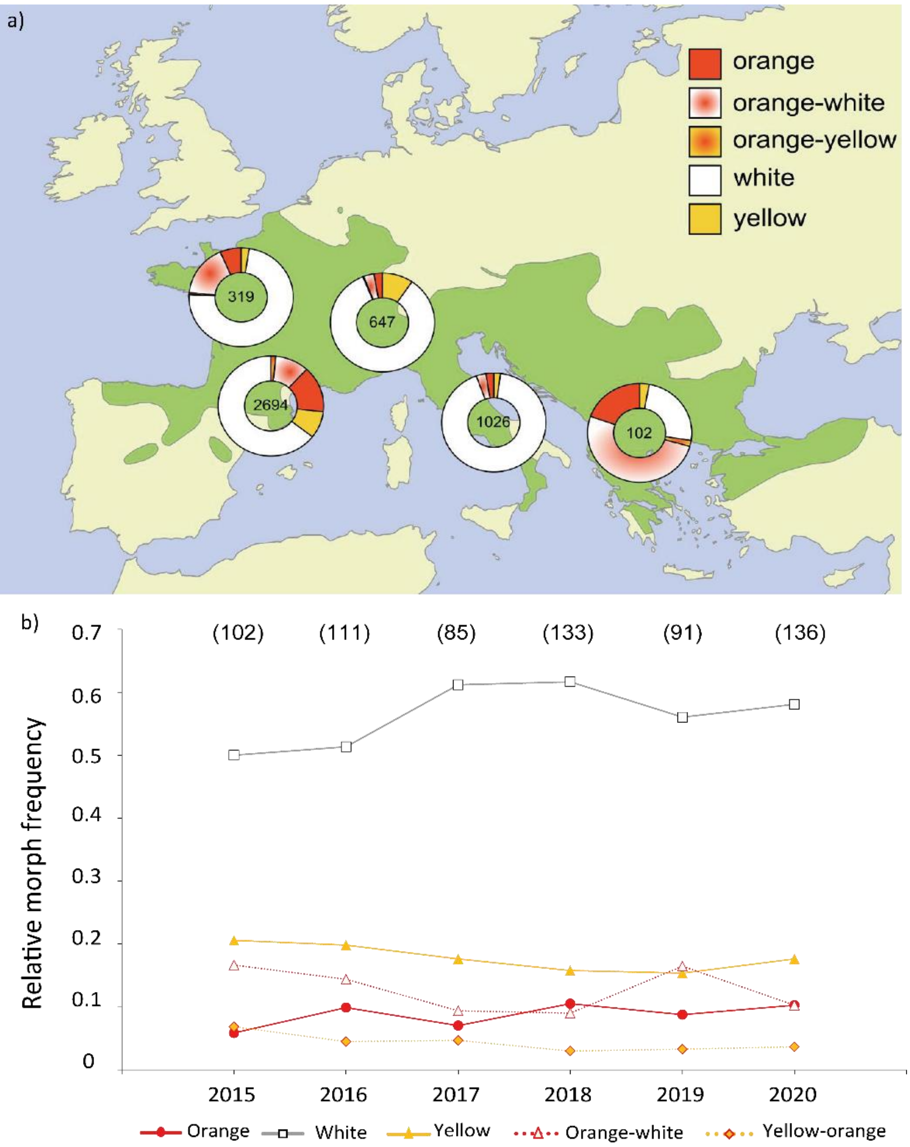

Figure 4.S1. a) Native distribution of *Podarcis muralis* (green shading) with pie charts representing the natural morph frequencies pooled by geographically distant sub-lineages. White morph lizards are usually the most common. The number of lizards sampled is indicated inside the pie charts. Figure extracted from Andrade *et al.* (2018) and reproduced here with permission from the authors. b) Variation in morph relative frequencies (pooling males and females) for a six-year period (2015-2020) in the study population of Angoustrine. Sample size for each year is provided in brackets.

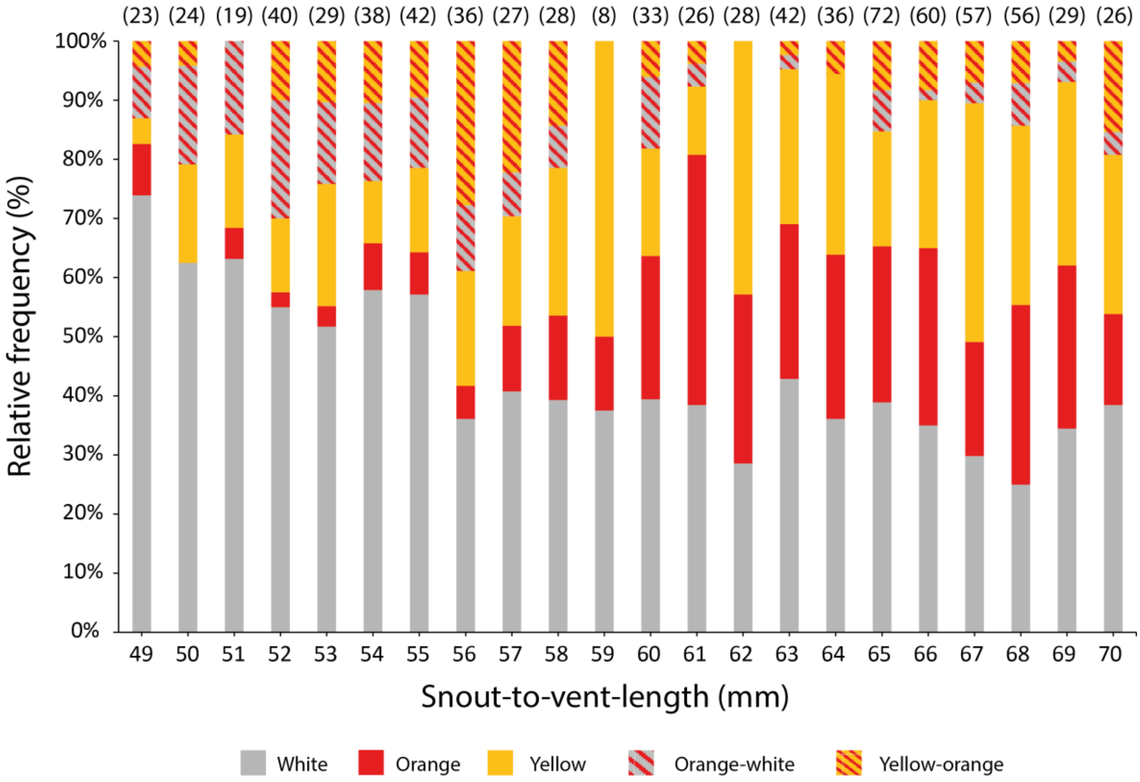

Figure 4.S2. Colour morph relative frequencies in males from Angoustrine showing different body sizes (snout-to-vent-length, SVL). Sample size for each SVL are provided in brackets. White morph frequency falls steeply toward larger body sizes with the largest change in slope occurring between 55 and 56 mm, after which colour morph frequencies tend to stabilize (as suggested by results in Pérez i de Lanuza et al., 2013). Given this result, we consider 56 mm a suitable cut-off value of SVL for reliable morph determination in the localities sampled. This cut-off, however, could vary among different populations.

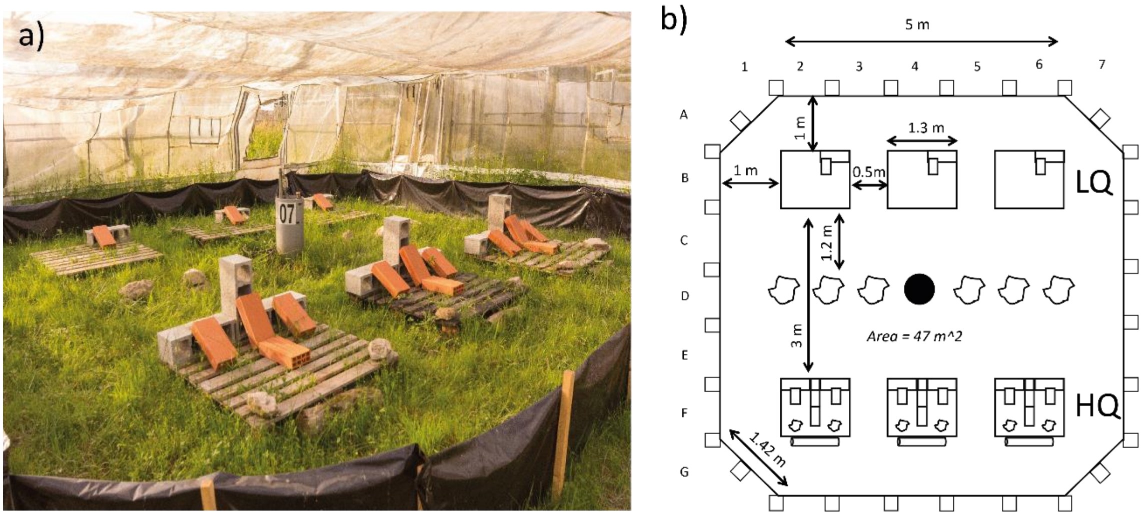

Figure 4.S3. a) Photography of one of the experimental enclosures at the Metatron research facility (CNRS; Caumont, France) associated to the Station d'Ecologie Theóretique et Experimentale (SETE, Moulis, France). b) Diagram of the spatial arrangement inside the experimental enclosures (HQ = high-quality sites, LQ = low-quality sites).

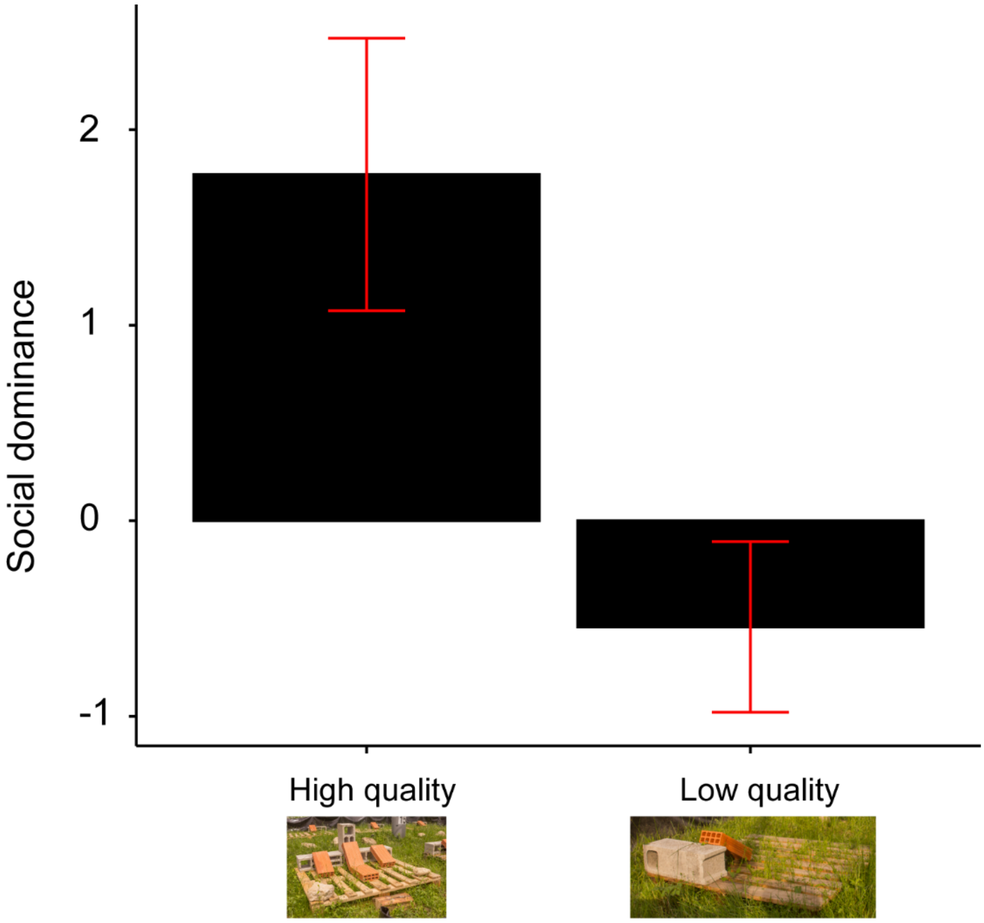

Figure 4.S4. Barplot showing the difference in social dominance between males occupying high- and low-quality sites. Red bars represent 95% CI.

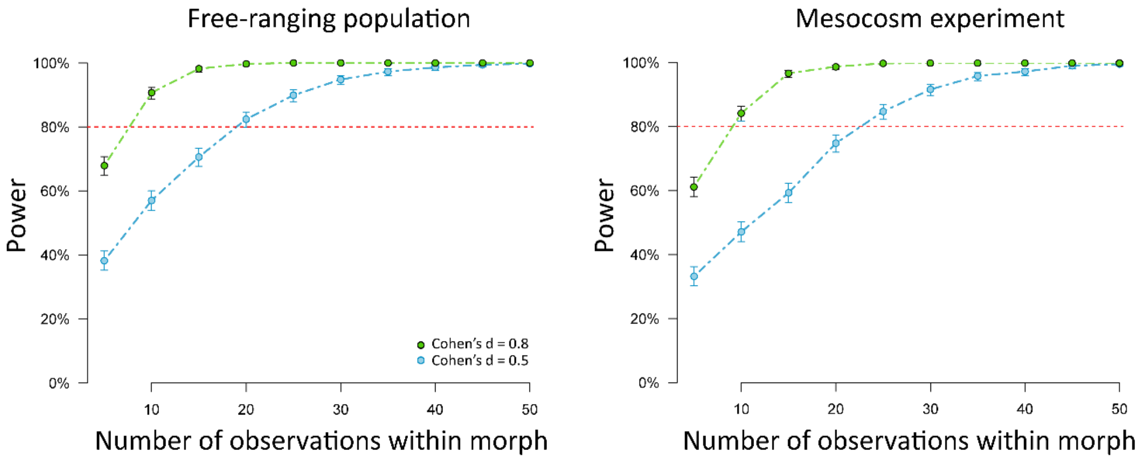

Figure 4.S5. Power (± CI_{95}) to detect a medium-sized (Cohen's *d* = 0.5, blue line) or large effect size (Cohen's *d* = 0.8, green line) for morph differences in the free-ranging population (left) or the mesocosm experiment (right), calculated over a range of sample sizes (5-50) using the powerCurve function in the *simr* package (1000 simulations). Estimates for fixed and random effects (besides morph) replicate those obtained in the LMMs exploring male morph differences in home-range size (left) or social dominance (right). The curves confirm that our mixed-model statistical designs (with 13-30 lizards per morph) were sufficiently powered to detect biologically relevant morph differences in socio-sexual behaviour.

Video recordings

Video 4.S1. Screenshot of a social interaction observed during the period of behavioural observations within the experimental enclosures. The full recording can be downloaded as a separate MP4 file.

https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1002%2Fece3.6659&file=ece36659-sup-0006-VideoS1.mp4

Chapter V

"Isn't it enough to see that a garden is beautiful without having to believe that there are fairies at the bottom of it too?"

· Douglas Adams, The Hitchhiker's Guide to the Galaxy·

This chapter reproduces entirely the manuscript:

Abalos, J., Pérez i de Lanuza, G., Bartolomé, A., F., Uller, T., and E. Font. Viability, behaviour, and colour expression in the offspring of matings between common wall lizard (*Podarcis muralis*) colour morphs. *Under review.*

Chapter V:

Viability, behaviour, and colour expression in the offspring of matings between common wall lizard (*Podarcis muralis*) colour morphs

5.1. Abstract

Colour polymorphisms are widely studied to identify the mechanisms responsible for the origin and maintenance of phenotypic variability in nature. The two main mechanisms currently thought to explain the long-term persistence of polymorphisms are correlational selection favouring alternative phenotypic optima and heterosis. These mechanisms predict differences in offspring viability and fitness arising from different morph combinations. Here, we examined the effect of parental morph combination on fertilisation success, embryonic viability, newborn quality, antipredator and foraging behaviour, as well as inter-annual survival by conducting controlled matings in a polymorphic lacertid, *Podarcis muralis*, where colour morphs are frequently assumed to reflect alternative phenotypic optima. Lizards were kept in outdoor tubs for a year in order to study inter-annual growth, survival, and the inheritance and ontogeny of colour expression. In agreement with previous literature, morph frequencies in the offspring of the experimental crosses matched the frequencies expected if orange and yellow expression depended on recessive homozygosity at two separate loci. The whitish coloration exhibited by newborn lizards reflects the near ultraviolet (UV) waveband and is likely perceived by conspecifics as a chromatically distinct colour different from any of the colour morphs expressed by adult lizards. In contrast with the predictions of correlational selection and heterosis, we found no compelling evidence of morph-combination effects on offspring viability or behaviour. We conclude that genetic incompatibilities or heterozygote advantage is of little significance for the population dynamics of colour polymorphism in *P. muralis* from the Pyrenees.

Keywords: alternative breeding strategies - colour polymorphism - controlled matings - lizard behaviour - morph ontogeny - newborn viability

5.2. Introduction

Population polymorphism refers to a situation where two or more discrete phenotypic forms coexist in sympatry among individuals of the same sex and age (i.e. morphs), with the rarer morph being too frequent to be solely the result of recurrent mutation (Ford 1945; Huxley 1955). Often, this phenotypic variation takes the form of distinct colour morphs that are genetically inherited, fixed in adults, and not condition-dependent (i.e. colour polymorphism; Galeotti et al. 2003; Roulin 2004; Mckinnon and Pierotti 2010). Explaining the maintenance of colour polymorphism in natural populations remains one of the major challenges in evolutionary biology, as it requires some form of selective balance to counteract the eroding effects of differential morph fitness and drift (Galeotti et al. 2003; Roulin 2004; Gray and McKinnon 2007).

Colour morphs often differ in features other than colour (Forsman et al. 2008; Mckinnon and Pierotti 2010). Such co-variations are thought to have originated via disruptive, correlational selection favouring optimal trait combinations in the different morphs, each representing alternative adaptive peaks (i.e. alternative strategies; Sinervo and Svensson 2002; Shuster and Wade 2003; Gray and McKinnon 2007). Consider the classical example of the Peppered moth (*Biston betularia*), with two morphs conferring a cryptic advantage in different habitats. Genetic correlations between colour morph and behaviour (i.e. habitat choice preferences) would be expected to arise, as moths that choose backgrounds matching their own colour survive, while those with the wrong preference perish (Kettlewell 1955; Sinervo and Svensson 2002; Cook et al. 2012). Colour polymorphic strategies can persist over long timescales only if they show the same average fitness over time, which may occur under balancing or spatio-temporally varying selection (Zamudio and Sinervo 2003; Roulin 2004; Gray and McKinnon 2007; Mckinnon and Pierotti 2010; Wellenreuther et al. 2014). However, the observation of alternative colour-coded strategies within a population does not mean that the polymorphism is balanced and stable in time. In fact, the evolution of alternative multi-trait strategies may have consequences for reproductive isolation, and ultimately serve as a pre-requisite for speciation by favouring the evolution of both pre- and post-zygotic reproductive barriers between the morphs (Denoël et al. 2001; Sinervo and Svensson 2002; Gray and McKinnon 2007; Pryke and Griffith 2009*a*; Svensson et al. 2009; Mckinnon and Pierotti 2010; Bastiaans et al. 2014; Svensson 2017). Correlational selection is expected to favour the evolution of morph-specific genetic mechanisms (i.e. linkage disequilibria) preventing recombination from breaking apart co-adapted gene complexes. Morph-assortative mate choice preferences are also likely to evolve under correlational selection, as disassortative mating generates sub-optimal trait combinations in the offspring. Moreover, the cumulative effect of disruptive, correlational selection, and assortative mating can further decrease inter-morph offspring fitness by adding genetic divergence (and hence post-zygotic incompatibility) between the morphs (Sinervo and Svensson 2002; Gray and McKinnon 2007; Pryke and Griffith 2009*a*; Mckinnon and Pierotti 2010; Lancaster et al. 2014). For instance, in the polymorphic Gouldian finch (*Erythrura gouldiae*), where red- and black-headed males show differential dominance (Pryke and Griffith 2006), offspring produced by genotypically-mixed mating pairs showed consistent inviability effects from egg to adulthood, which led the authors to conclude that the current morphs may represent "a snapshot in the process of speciation" (Pryke and Griffith 2009*a*).

Heterosis, in which genetically intermediate (i.e. heterozygotes) morphs show a fitness advantage, represents the opposite evolutionary scenario to correlational selection, and offers another mechanism by which colour polymorphism may be maintained over time (Roulin 2004; Roulin and Bize 2007; Johnston et al. 2013; Wellenreuther et al. 2014). Heterosis can result from heterozygote individuals being less inbred than homozygous ones and thus expressing less deleterious recessive traits, which allows them to cope with a wider range of stress-inducing factors during life (Roulin 2004). If heterozygosity at the genes coding for the colour polymorphism (or at genes linked to them) provides a fitness advantage, selection is likely to favour the evolution of disassortative mating preferences. However, it is worth noting that if the heterozygous advantage concerns only viability selection (i.e. survival to adulthood), the colour polymorphism may be maintained even if the morphs show random or even maladaptative mating preferences (Krüger et al. 2001; Roulin and Bize 2007). Hence, the relative balance between the maintenance of genetic polymorphisms and the evolution of reproductive isolation depends largely on the interplay between, on one hand, assortative mating and correlational selection building reproductive barriers between the morphs, and, on the other hand, disassortative mating preferences, gene flow and balancing selective regimes (i.e. heterosis, NFDS, RPS, and fluctuating selection) preventing any of the alternative morphs from going extinct (Sinervo and Svensson 2002; Roulin 2004; Roulin et al. 2004; Chunco et al. 2007; Gray and McKinnon 2007; Roulin and Bize 2007; Mckinnon and Pierotti 2010; Wellenreuther et al. 2014; Svensson 2017; Iversen et al. 2019).

Lizards are an excellent model group in which to study the evolutionary dynamics associated with colour polymorphism. Many phylogenetically distant taxa have convergently evolved discrete colour morphs which are heritable (often with a relatively simple genetic architecture – few autosomal genes with major effects; Olsson et al. 2007; Sinervo et al. 2010; Rankin et al. 2016), functionally relevant to crypsis or thermoregulation, and/or correlated to other physiological or behavioural traits under selection (Zamudio and Sinervo 2003; Olsson et al. 2009; Baird 2013; Lattanzio and Miles 2014; Ortega et al. 2015; Yewers et al. 2016; Yewers 2017; Fernández et al. 2018; Stuart-Fox et al. 2020). Furthermore, polymorphic lizards sometimes represent different points in the balance between colour polymorphism maintenance and incipient speciation. While there is compelling evidence for the long-term maintenance of colour polymorphism via frequency-dependent selection in some cases (i.e. *Uta stansburiana;* Sinervo and Lively 1996; Sinervo et al. 2000), colour-assortative pairing, limited gene flow and weak post-zygotic barriers to inter-morph breeding have also been described in other cases (Rosenblum 2006; Gray and McKinnon 2007; McLean and Stuart-Fox 2014; Mclean et al. 2014*a,b*; Dong et al. 2019; McLean et al. 2020). For instance, differential selection for crypsis in light and dark habitats of the White Sands Ecotone has convergently favoured the evolution of a genetic melanin-based colour polymorphism in three lizard species, with inter-morph genetic and phenotypic divergence within each species being directly related to the level of gene flow between the patches of habitat where they are found (Rosenblum et al. 2004; Rosenblum 2006). In the side-blotched lizard (*Uta stansburiana*), where three morph-specific sexual behaviours (in males) and two alternative breeding strategies (in females) persist cycling in frequency over time (Sinervo and Lively 1996; Sinervo et al. 2000*b*), females have been found to show morph-assortative mate choice preferences and decreased egg viability in disassortative pairings (Bleay and Sinervo 2007; Lancaster et al. 2014).

In wall lizards (genus *Podarcis*), a widely distributed group present in most of Europe, northwestern Africa, and the Mediterranean islands, several species exhibit a striking ventral colour polymorphism (Arnold et al. 2007; Huyghe et al. 2007; Sacchi et al. 2007; Runemark et al. 2010; Marshall and Stevens 2014; Brock et al. 2020*a*, 2020*b*). One such species (which is also the most widely distributed) is the European common wall lizard (*Podarcis muralis*). In this species, adults of both sexes may show up to five alternative ventral colour morphs (**Fig. 5.1**): three uniform (pure) morphs, i.e. orange (o), white (w) and yellow (y), and two mixed-morph mosaics combining orange and white (ow) or yellow and orange (yo) (Sacchi et al. 2007, 2013; Pérez i de Lanuza et al. 2013, 2019). Adult ventral colours are fixed at maturity, with published spectra showing that chromatic differences between pure colour morphs are due mainly to variation in reflectance between 400 and 600 nm (i.e. discriminable by humans), and further empirical evidence confirming that the morphs are perceived as chromatically distinct by conspecifics (Pérez i de Lanuza et al. 2014, 2018*a*). No study has established morph inheritance, but recent research suggests that orange and yellow colour expression are caused by recessive homozygosity at two separate loci in the regulatory regions of two genes associated with pterin (SPR) and carotenoid (BCO2) metabolism, respectively (Andrade et al. 2019). Notably, newborn ventral coloration (which is perceived as identical to the white morph by human observers; Pérez i de Lanuza et al. 2013) has never been objectively characterized. Because of their ventral position and conspicuousness to the species' visual system (Pérez i de Lanuza and Font 2015; Pérez i de Lanuza et al. 2018*a*), at least the pure colour morphs of *P. muralis* have often been thought to represent the visible mark of an underlying set of alternative phenotypic optima (e.g. reproductive or life-history strategies; Sinervo et al. 2007; Calsbeek et al. 2010; Scali et al. 2013, 2016; Sacchi et al. 2017*a*; Mangiacotti et al. 2019). Although recent evidence from a mesocosm experiment does not support the existence of alternative strategies concerning socio-sexual and spatial behaviour (Abalos *et al.,* 2020), research on *P. muralis* colour morphs has detected inter-morph differences in several (mostly physiological) traits relevant to fitness such as immune response, hormonal profile, and prevalence of infection by parasites, hence suggesting the existence of alternative trade-offs in resource allocation and life-history strategies in the different morphs (Galeotti et al. 2007, 2010;

Calsbeek et al. 2010; Zajitschek et al. 2012; Sacchi et al. 2017*b*, 2017*a*). Female colour morphs from Italian polymorphic populations have also been found to show differential breeding investment, and clutch viability and newborn mass were also found to vary depending on parental morph combination (Galeotti et al. 2013). Lastly, field observations suggest an assortative morph bias in pair formation (68% of couples observed in the wild are homomorphic; Pérez i de Lanuza et al. 2013, 2016).

Taken together, these findings suggest that disruptive, correlational selection coupled with assortative mating may have favoured different co-adapted trait complexes in each morph while introducing some degree of genetic divergence between the colour morphs. If this is the case, we would expect to find decreased embryonic viability, newborn quality, and survival to adulthood in the offspring of disassortative matings. By contrast, higher viability and overall performance in the offspring of disassortative pairings would suggest the existence of a heterozygote advantage in the genes coding for the expression of *P.muralis* polymorphic coloration. Morph combination effects on behaviours directly related to juvenile survival to adulthood are key to evaluate the existence of heterosis, as a strong heterozygote advantage in viability selection could counteract the effects of assortative pairing and help to explain the maintenance of *P. muralis* colour polymorphism over time (Roulin 2004; Gray and McKinnon 2007; Mckinnon and Pierotti 2010).

Here, we tested for such effects using controlled matings among all-pair combinations of *P. muralis* pure colour morphs. Our objective was four-fold: a) first, to compare primary reproductive parameters (i.e. clutch size and juvenile mass) among female morphs in order to evaluate the existence of alternative breeding strategies, b) second, to evaluate the existence of genetic incompatibilities or heterozygote advantage between colour morphs at both the pre- and post-zygotic levels by comparing fertilisation success, embryonic viability, and newborn quality in matings between different parental morph combinations, c) third, to test for morph combination effects on viability selection (i.e. survival to adulthood) by raising the juveniles in a common garden and keeping track of their inter-annual growth and survival, and d) fourth, to describe the inheritance and ontogeny of colour expression in the year-old juveniles.

Figure 5.1. Colour variation in the ventral surface of adult *Podarcis muralis* lizards. Both sexes may show up to three pure colour morphs (white, orange, yellow), or two intermediate mosaics combining orange and white or yellow and orange scales (i.e. mixed-morphs). In males, the colour covers the entire ventral surface while in females it is sometimes restricted to the thoat.

5.3. Materials and methods

5.3.1. Lizard capture and female hibernation

In total, we captured 135 female and 44 male adult lizards by noosing from 14 polymorphic localities in the eastern Pyrenees. In each of these localities we captured lizards showing each of the three pure colour morphs. We only captured lizards with a snout-to-vent length (SVL) larger than 56 mm, a reliable cut-off point for adult morph expression in *P. muralis* polymorphic populations from eastern Pyrenees (Pérez i de Lanuza et al. 2013*a*). We captured 60 females at the end of the 2018 breeding season (September 5-8) and transferred them to the Station d'Ecologie Théorique et Expérimentale (SETE, Moulis, France), where they were kept under natural conditions in 45 outdoor plastic tubs (170 cm diameter, 60 cm high; **Fig. 5.S1**). Each tub housed a group of 3-5 females coming from the same locality. Tubs were covered with anti-bird netting to prevent predation while allowing local invertebrates to get inside the tubs and serve as a primary food source for the lizards. To increase habitat complexity, in addition to the naturally occurring vegetation inside the tubs, we provided a perforated construction brick to serve as shelter, two wood logs, and a water dish to collect rain-water. We visited the tubs monthly to trim the weeds and provide additional food (*Tenebrio molitor* larvae and *Acheta domesticus* adults). As long-term sperm storage does not occur in *P. muralis* (Pellitteri-Rosa et al. 2012; While and Uller 2017), on March 2019 we captured 75 additional females from the same localities, re-captured the females within the outdoor tubs, and installed all of them in individual hibernation pods consisting of a plastic bowl (12 cm diameter, 8 cm high) covered with a perforated lid and filled with moist coco husk. Hibernation pods were stored for 9 weeks in a dark climatic chamber to mimic the conditions at their capture sites (1st week: 10ºC/ 2nd-8th weeks: 5ºC/ 9th week: 10ºC). In May 8 2019, we took the females out of hibernation and re-installed them into the outdoor tubs for acclimation while we captured the males at the same localities as the females. Two days before the onset of the experiment, we measured SVL (0.1 mm) and mass (± 0.01 g) of each lizard with a ruler and a spring balance (Pesola, Schindellegi, Switzerland). We also removed ~5 mm from the tail tip of each individual and preserved the tissue in 90% ethanol for genetic analyses.

5.3.2. Staged mating trials and juvenile husbandry

To study the effect of parental morph combination on reproductive output and juvenile viability, we carried out staged mating trials by releasing 131 females (45o, 44w, 42y) and 44 males (14o, 15w, 15y) into 44 outdoor plastic tubs (**Fig. 5.S1**). In each tub, we released three females (1o: 1w: 1y) followed five days later (May 14 2019) by one male showing either orange, white, or yellow ventral coloration (*N* = 14 tubs for each male ventral color). Due to female post-hibernation mortality, the yellow female morph was absent in two of the 44 experimental tubs (a: 2o♀, 1w, 1w♂; b: 1o♀, 1w♀, 1y♂). To reduce any possible effects of female size asymmetry, time of capture, and prior social interactions, we allowed a maximum SVL difference of 2 mm among females housed in the same tub, minimized co-habitation of females captured at different times, and only put lizards together in the same experimental tub if they had been captured at least 300 m apart. Prior to release, we marked females with a non-toxic permanent marker to facilitate individual recognition (**Fig. 5.S2**; Ferner and Plummer 2016; Ngo et al. 2016). In total, the males and females spent 30 and 35 days, respectively, inside the experimental tubs. During this period, we monitored male-female interactions by visiting the outdoor tubs twice per day and recording the identity of the male-female pairs we sighted either i) co-perching (i.e.lying in physical contact), or ii) engaged in copulatory behaviour (i.e. intertwined in the donut-shaped mating posture typical of lacertid lizards; Weber 1957; Carpenter and Ferguson 1977; in Den Bosch and Zandee 2001).

On June 15 2019, we released the males at their capture location (previously determined using a GPS device), and housed females in the laboratory until oviposition (see **Appendix S1**). Nine females did not produce a clutch and we lost 10 clutches due to failure to retrieve the female from the enclosures before oviposition. For the remaining 112 females, we counted the number of fertile (452) and infertile (105) eggs within each clutch by noting the presence of a calcified shell and vascularization 48 h after oviposition (Köhler 2006). We incubated the 452 fertile eggs in plastic cups filled with moist coco husk (1:2 coco:water by weight) and covered with a perforated lid at a constant temperature of 28ºC in an incubator (Memmert GmbH + Co.KG, Schwabach, Germany). Upon hatching, each of the 424 born juveniles was measured (SVL), weighted, sampled for DNA, temporarily marked, and housed in 55 x 38 x 28 cm high glass terraria according to their mother's tub assignment. For 28 embryos that died before hatching, we obtained DNA samples via dissection of the eggs. Juvenile lizards were fed small live prey (pinhead *Acheta domesticus* and flightless *Drosophila hydei*) dusted with vitamins. To confirm exclusive paternity from the experimental male and determine parentage in some clutches found within the experimental tubs, we conducted genetic analyses of paternity based on six known microsatellite loci described in *P. muralis* (Richard et al. 2012; Heathcote et al. 2014). We could reliably assign paternity to every offspring examined (strict: 271 juveniles, relaxed: 41 juveniles; see **Appendix S1**). On August 18 2019, after conducting lab-staged behavioural tests on a subset of the juveniles (see below), we released 372 juveniles (52 juveniles died shortly after hatching) in groups of 10-12 unrelated individuals into 33 outdoor plastic tubs for their long-term maintenance. Prior to release, we permanently marked the juveniles on the ventral scales using a disposable medical cautery unit (Ekner et al. 2011), and obtained spectrophotometric measurements of throat reflectance (see **Appendix S1**).

5.3.3. Juvenile behavioural tests

To evaluate prospective fitness, 3-6 days after hatching we conducted lab-staged behavioural tests on two juveniles from each clutch. Offspring mass being often considered a proxy of newborn quality (Stamps 1988; Civantos and Forsman 2000; Galeotti et al. 2013), we chose the heaviest and lightest from each clutch to encompass the offspring mass range of each female. These tests were repeated one year later for the 50 surviving juveniles found in the outdoor tubs in July 2020. Each test comprised three stages each lasting 5 min: acclimation period, antipredator trial, and foraging trial (see **Video 5.S1** in the **Appendix S1**). We conducted tests during the lizards' natural daily period of activity (10:00–13:30 and 16:00–19:00 h local time). In total, we conducted 247 behavioural tests. We started each test by transferring a juvenile lizard inside a plastic cup into an experimental arena consisting of a 50 x 25 x 40 cm high glass terrarium with all except the front wall covered with cardboard, and a piece of filter paper provided with a 4 cm grid as substrate. After the 5 min acclimation period, in which the lizard could move freely across the arena, we simultaneously introduced a shelter in the arena and simulated the presence of an avian predator using an artificial plastic model with the size, shape and coloration of an Eurasian magpie (*Pica pica*), a generalist native predator that feeds on wall lizards (Martin and López 1990). The model magpie was placed in front of the arena, at a distance of 5 cm from the arena's front wall and at the end of a 75 cm pole. The shelter (consisting of two superimposed tiles separated along the edges by thin pieces of felt on three sides so as to leave a single entrance to a 90 x 90 x 12 mm cavity) was positioned in the middle and in contact with the back wall, with its entrance facing the front wall (**Fig. 5.S3**). After 5 min, we removed the magpie model and introduced a novel prey item (one < 8 mm *T. molitor* larvae) into the arena. In every trial, we positioned the larvae in the juvenile's field of vision and at a distance of 4-8 cm from its head (or the shelter's entrance, whenever the juvenile was sheltered). We stopped the trial 5 min after the prey item was introduced. To minimize the impact of human presence on the lizard's behaviour, all trials were filmed remotely with a photographic camera (Canon®, EOS 7D, Tokyo, Japan). During trials, the arena was illuminated by two 40 W lamps (providing a uniform 25ºC temperature in the arena), and a full spectrum lamp (Reptistar 5.0) equipped with a high frequency ballast (Quicktronic, Osram, Munich, Germany). The experimental arena and shelter were thoroughly cleaned with alcohol and rinsed with water after every test. We played back each filmed test and a single researcher recorded: a) the latency to enter the shelter once presented with the magpie model, b) the latency to bite the novel prey item, and c) the time elapsed between prey capture and consumption (prey handling time).

5.3.4. Inter-annual survival, morph inheritance and colour expression

Because of the global COVID-19 crisis, we had to postpone the monitoring of juvenile growth, survival, and colour expression (initially planned for March 2020) to early July 2020, when borders between Spain and France were reopened. We re-captured the juvenile lizards installed in our outdoor tubs. We also searched for marked lizards in adjacent patches of lizard habitat (approximately 1 km from the study site) to account for any potential escape, but none were found. For each recaptured juvenile, we measured SVL, mass, and obtained spectrophotometric measurements of throat reflectance (see **Appendix S1**). We assigned each of the 50 surviving lizards to a colour morph according to their throat reflectance spectra. We then used the spreadsheet available in Rankin et al. (2016) to calculate the morph frequencies expected in the offspring of each morph combination and compare them to the observed frequencies. Following Andrade et al. (2019), we considered two different loci each with two alleles (O/o, Y/y), and a recessive homozygous base for orange and yellow colour expression. We assumed that phenotypically white parents had equal odds of being homozygous or heterozygous. To increase sample size, we added data from 17 juvenile lizards of known parental morph combination which were conceived during a previous experiment (see Abalos *et al.* 2020) and also maintained in the same outdoor tubs for a similar period (September 2018- July 2019). We compared observed and expected offspring morph frequencies for each parental morph combination using G likelihood-ratio tests and combined the P-values into a single test statistic using Fisher's method (Fisher 1932). We also used visual modelling tools in the R package PAVO (Maia et al. 2013) to determine quantitatively the chromatic distance between the ventral colour exhibited by each recaptured individual 3-4 weeks after hatching, and one year later (September 2019 vs. July 2020; see **Appendix S1**). The chromatic distances between paired spectra from the same individual were expressed as just noticeable differences (JND). Chromatic distances of < 1 JND are generally considered not discriminable, values between 1 and 3 JND represent colours that are discriminable under good lighting, and values above 3 JND represent easily discriminable colours (Siddiqi et al. 2004; Marshall and Stevens 2014; Pérez i de Lanuza et al. 2018*a*).

5.3.5. Statistical analyses

To account for the random factors in our experimental design, we ran generalised linear mixed models (GLMMs) using the *lme4* package (Bates *et al.,* 2014) in R (R Core Team, 2019) and model selection was conducted using backwards single term deletions (P < 0.05) of the saturated model followed by model comparisons via likelihood ratio tests (at $a = 0.05$). All numerical variables were centred and scaled before running the models (Schielzeth, 2010). For each fitted model, we calculated r^2 (following Nakagawa et al. 2017), checked the existence of multicollinearity among predictors, influential data points, and graphically explored that residuals conformed to homoscedasticity and normality assumptions using the performance package in R (Lüdecke et al. 2020). For some variables that did not conform to these assumptions even after transformation, we fitted models using different distributions. For details on the statistical analyses, see **Appendix S1**.

5.4. Results

5.4.1. Effect of parental morph combination on reproductive output

There was no morph bias in body size or mass in the adult lizards (**Table 5.S1**). In the experimental tubs, males and females were frequently observed lying in close proximity (n = 1002) or engaged in copulatory behaviour (n = 102), but colour morph played no role on the patterns of male-female association (co-perching: $\chi^2 = 8.33$, $P = 0.40$; copulatory behaviour: $\chi^2 = 1.64$, $P = 1.0$; **Fig. 5.S4**).

The levels of fertilization success (i.e. fertilized eggs/ clutch size) was 81.4% and embryonic viability (i.e. viable juveniles/ fertilised eggs) was 93.7 %. Egg incubation lasted 37.7 ± 0.4 (□ ± CI$_{95}$ henceforth) days with a mean clutch size of 4.6 ± 0.2. Clutch size correlated significantly with female weight and juvenile mass (LMM: female weight: $\chi^2 = 15.88$, $P < 0.001$; juvenile mass: $\chi^2 = 20.23$, $P < 0.001$; *pseudo*-$r^2 = 0.32$), but not with female morph, male morph, or the interaction between the two ($P > 0.2$; **Table 5.S2**). Similarly, we found no difference in clutch size between homomorphic and heteromorphic pairings ($P > 0.2$; **Fig. 5.S5**, **Table 5.S3**). The absolute number of viable juveniles produced was also independent of parental morph and morph combination (**Table 5.S2** and **5.S3**). However, we found a significant morph bias in fertilisation success, (GLMM binomial: female morph x male morph: $\chi^2 = 19.50$, $P < 0.001$; **Table 5.S2**) which was mainly driven by the large number of infertile clutches laid by yellow females paired with orange males (**Fig. 5.2**). Eggs produced by white homomorphic pairs (which showed the highest fertilisation success) were 1.61 (odd ratio CI$_{95}$ = 0.99, 2.79) times more likely to be fertile than eggs produced by YO (♀♂) pairs. We also found significantly lower fertilisation success in yellow morph females with respect to the other morphs (GLMM binomial: $\chi^2 = 17.36$, $P < 0.001$; **Table 5.S2**) and in heteromorphic pairs with respect to homomorphic matings (GLMM binomial: $\chi^2 = 9.46$, $P = 0.002$; **Table 5.S3**), although the odds ratios for these differences were small (OR (CI$_{95}$): W♀/Y♀ = 1.21 (0.89, 1.65); homomorphic/heteromorphic = 1.11 (0.86, 1.44)). Embryonic viability was unaffected by parental morph combination (**Fig. 5.2**), and showed only a weak negative relationship with female mass (GLMM binomial: female morph x male morph: $P > 0.5$; female mass: $P = 0.012$, **Tables 5.S2** and **5.S3**). Juvenile mass was negatively correlated with clutch size (GLMM: $\chi^2 = 21.75$, $P < 0.001$, *pseudo*-$r^2 = 0.21$; **Table 5.S3**) but unrelated to parental morph or morph combination ($P > 0.4$; **Tables 5.S2** and **5.S3**; **Fig. 5.3**). According to our model, each additional egg layed by a female entailed a decrease of 0.02 ± 0.01 g in the average juvenile mass of its clutch, approximately a 5% of the average mass of juveniles in our sample (0.38 ± 0.01 g).

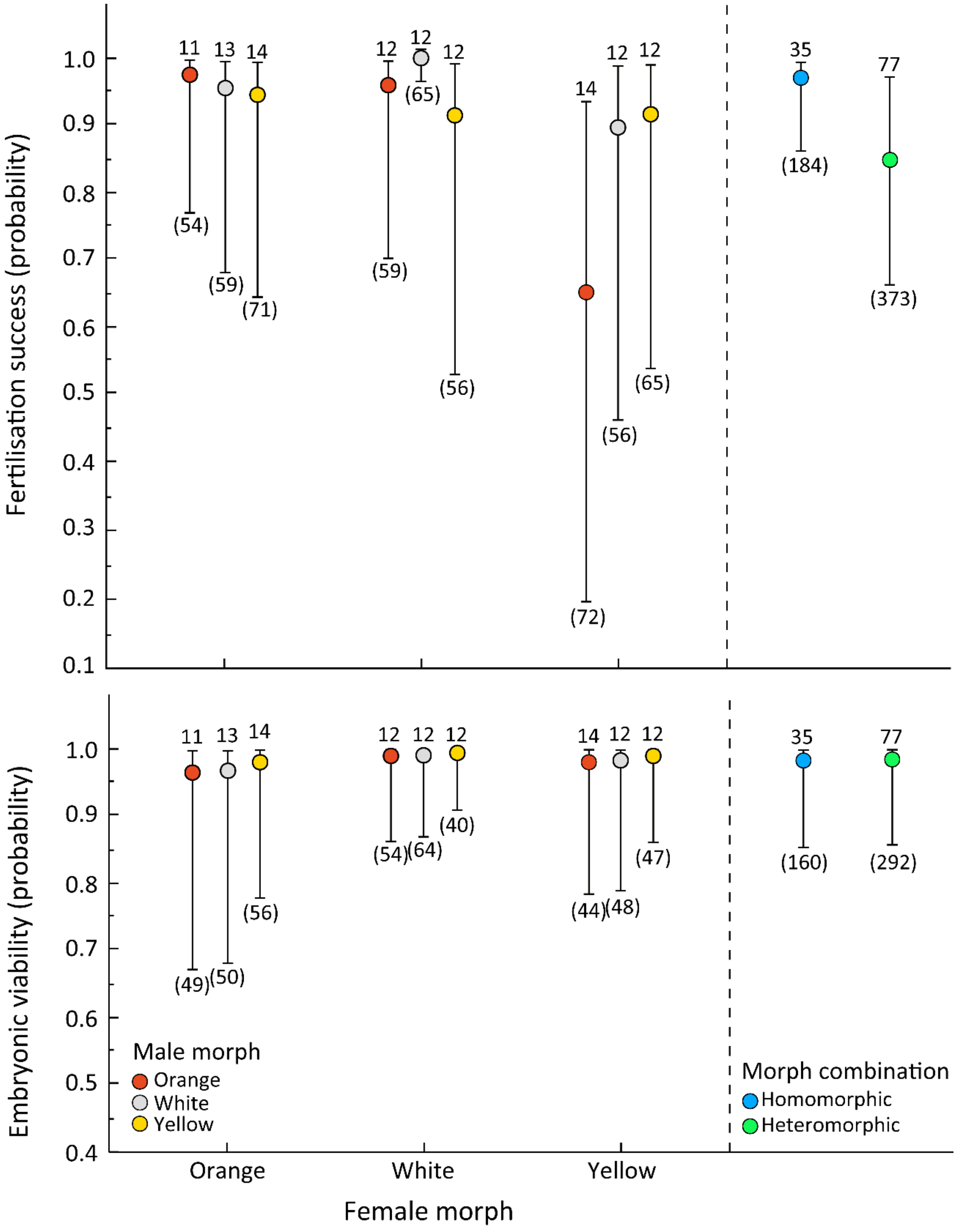

Figure 5.2. Above, predicted probability that an egg is fertilised (i.e. fertilisation success) according to morph combination. Below, predicted probability that a fertilised egg turns into a viable juvenile (i.e. embryonic viability). Circles mark the position of the mean and bars extend to the CI_{95}. Numbers and numbers within brackets correspond to the number of females and eggs within each category, respectively.

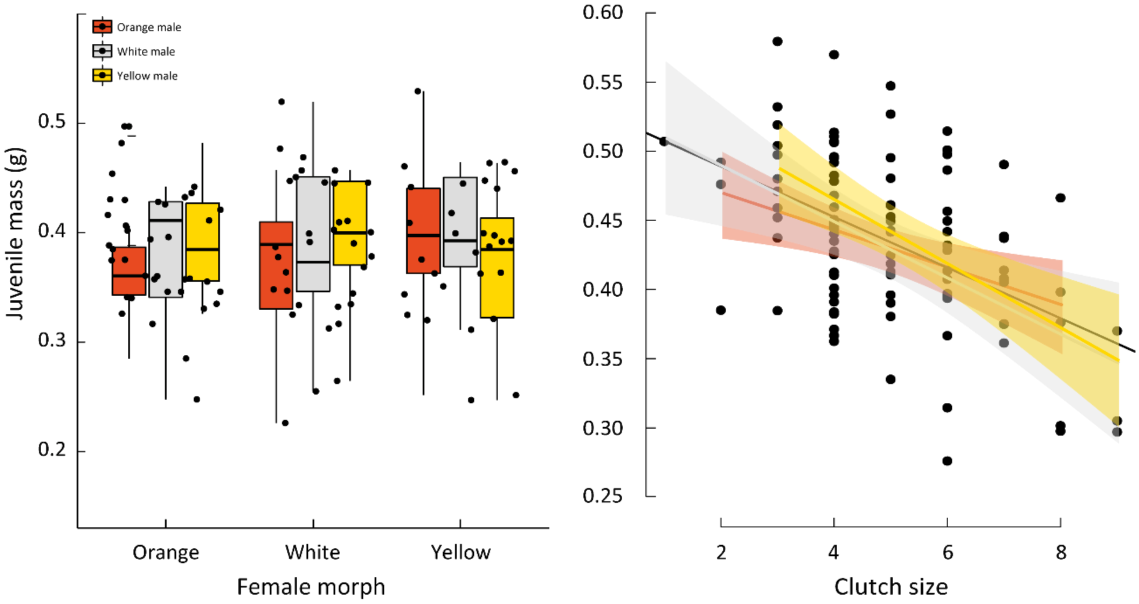

Figure 5.3. Left, boxplots showing average juvenile mass by female (g) according to morph combination. Boxes indicate the interquartile range (IQR, 50% of data). Horizontal lines represent the median and bars extend to 1.5 times the IQR. A jittered dot cloud represents each value in our dataset. Right, scatter plot showing the negative relationship between average juvenile mass and clutch size. Coloured lines and shaded areas correspond to the linear regression slope and CI_{95} for each female morph.

5.4.2. Effect of parental morph combination on juvenile behaviour

In 2019, we run 197 behavioural tests on the offspring of 99 different females representing 42 of the outdoor experimental tubs (see **Fig. 5.4** for information on sample size per morph combination). Nearly half of the juveniles entered the shelter upon presenting them with the magpie model (98 juveniles, 49.7%, mean latency = 133 ± 19 s), while two thirds of the juveniles captured and consumed the novel prey item (133 juveniles, 67.5%, mean latency = 57 ± 11 s). We observed a positive relationship between juvenile mass and performance in the viability tests: the probability that a juvenile entered the shelter doubled with each 0.2 g increase in juvenile mass (GLMM binomial: $\chi^2 = 4.12$, $P = 0.042$, *pseudo*-$r^2 = 0.03$; **Table 5.S4**, **Fig. 5.4**), while latency until the novel prey item was captured (GLMM: $\chi^2 = 22.67$, $P < 0.001$, *pseudo*-$r^2 = 0.16$; **Table 5.S4**, **Fig. 5.4**) and consumed (GLMM: $\chi^2 = 22.10$, $P < 0.001$, *pseudo*-$r^2 = 0.16$; **Table 5.S5**) decreased exponentially with growing juvenile mass. However, we did not find any significant effect of parental colour morph (or morph combination) on the juveniles' antipredator response or foraging ability ($P > 0.2$, **Tables 5.S4** and **5.S5**). In July 2020, we run 50 behavioural tests on the offspring of 34 different females representing 23 of the outdoor experimental tubs. Yearling lizards entered the shelter and ate the prey more frequently than the animals tested few days after hatching (GLMM binomial: enter: 73.9%, $\chi^2 = 9.43$, $P = 0.004$; eats: 87.8%, $\chi^2 = 7.23$, $P < 0.007$; **Fig. 5.S6**). They also showed longer latencies to enter the shelter (GLMM: $\chi^2 = 16.16$, $P < 0.0001$), but no difference in the latency to consume the prey

(**Fig. 5.S6**). Again, we did not find any significant effect of parental morph combination on the juvenile's antipredator response or foraging ability ($P > 0.05$, **Table 5.S6**).

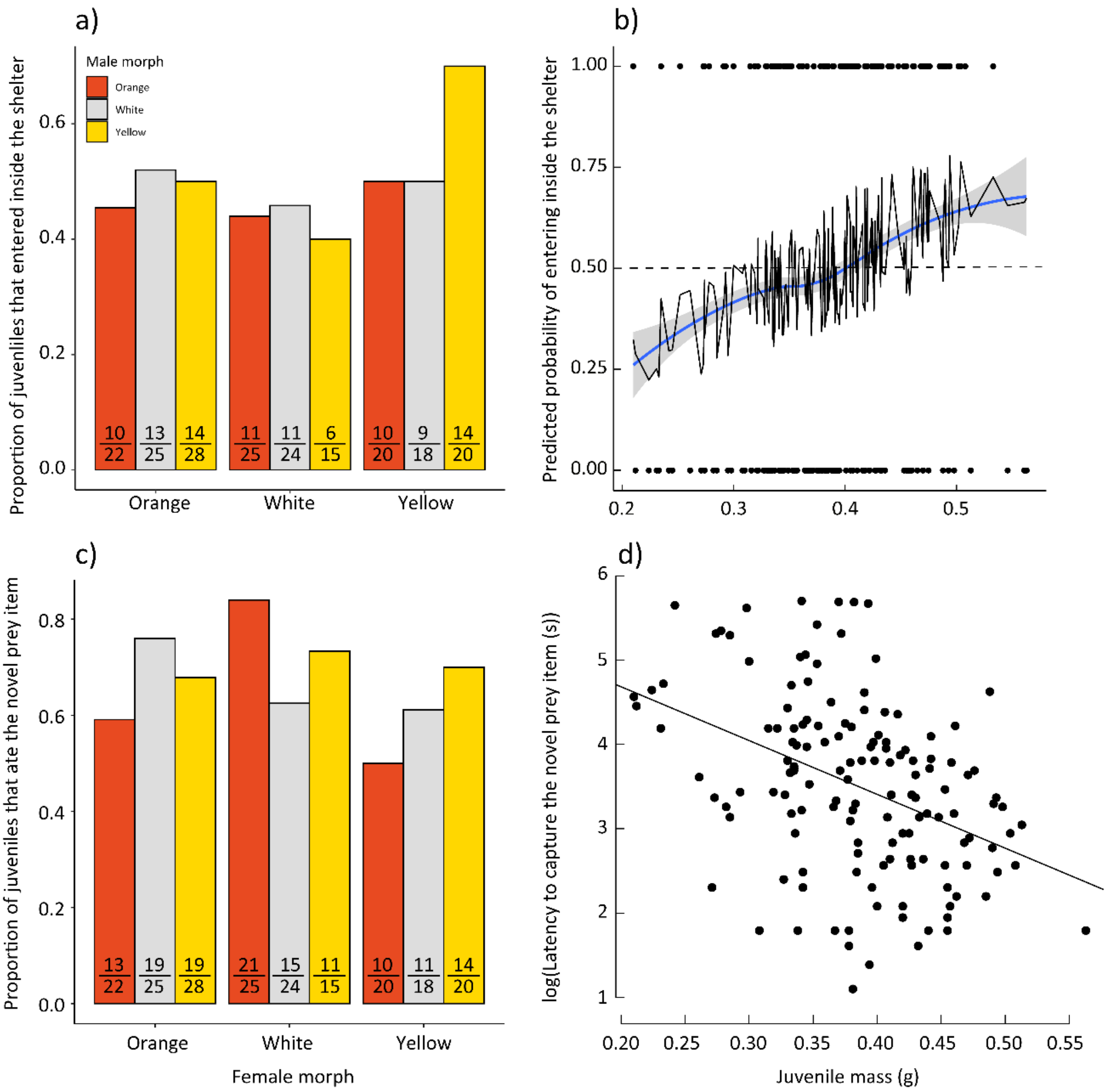

Figure 5.4. a) Barplot showing the proportion of juveniles that entered the shelter during antipredator trials grouped by parental morph combination. Numbers inside the bars correspond to sample size (e.g. 10 out the 22 juveniles fathered by orange homomorphic pairs entered inside the shelter). b) Positive relationship between the probability that a juvenile entered the shelter and juvenile mass. Black line represents individual probabilities predicted by the logistic mixed model. Blue line and shaded area correspond to the smoothed tendency line and its CI_{95}. c) Barplot showing the proportion of juveniles that captured and ate the novel prey item (i.e. a small *Tenebrio molitor* larva), grouped by parental morph combination. Numbers inside the bars correspond to sample size. d) Scatterplot showing the negative relationship between juvenile mass (g) and the time elapsed from the introduction of the novel prey item until the juvenile bit the larva (i.e. latency in seconds).

5.4.2. Survival, inter-annual growth, and colour expression

Short-term survival (3-4 weeks after hatching) averaged 87.7% (372 lizards out of 424) and was positively related with juvenile mass (GLMM binomial: $\chi^2 = 9.39$, $P = 0.002$, *pseudo*-$r^2 = 0.09$), but not with parental morph ($P > 0.1$; **Table 5.S7**; **Fig. 5.5**). Inter-annual survival (one year after hatching) averaged 13.4% (50 lizards out of 372) and was signficantly related with juvenile mass and male colour morph (GLMM binomial: juvenile mass: $\chi^2 = 10.43$, $P = 0.001$; male morph: $\chi^2 = 8.57$, $P = 0.014$; **Table 5.S7**; **Fig. 5.5**). Namely, offspring sired by yellow males had 1.44 (odd ratio $CI_{95} = 0.49, 3.17$) higher odds of staying alive until July 2020 than juveniles fathered by orange morph males (**Fig. 5.5**). Lastly, juvenile lizards gained an average of 2.19 ± 0.24 g in one year, but growth was unrelated to any of the examined predictors (**Table 5.S7**).

Observed morph frequencies in subadult lizards were largely congruent with the frequencies expected for each morph combination (assuming two loci each with two alleles and a recessive, homozygous base for orange and yellow expression; $P = 0.110$, **Table 5.S8**, **Fig. 5.6**). Spectra from the white throat coloration exhibited by 99% of the hatchlings in September 2019 showed higher UV reflectance than any of the incipient colour morphs expressed by subadult lizards (**Fig. 5.7**), and was hence termed ultraviolet-white (UV-white). While ontogenetic changes in throat reflectance for orange and yellow morph subadults encompassed almost the entire visual range of *P. muralis* (300-600 nm), spectral changes in white morph subadults were mainly located in the UV waveband (300-400 nm; **Fig. 5.7**). Chromatic distances between paired spectra from the same individual averaged 4.62 ± 0.66 JND ($N = 46$ pure morph juveniles), and were significantly greater than 3 JND (hence, easily discriminable colours for the lizard visual system) for each of the pure colour morphs (orange: □ ± $CI_{95} = 6.52 \pm 3.50$, $t(6) = 2.36$, $P = 0.024$, yellow: 4.72 ± 0.75, $t(20) = 4.80$, $P < 0.0001$, white: 3.76 ± 0.78, $t(17) = 2.05$, $P = 0.028$; **Fig. 5.7**).

5.5. Discussion

In this study, we examined the effect of parental morph combination on fertilisation success, embryonic viability, newborn mass, and behaviour (i.e. antipredator response and foraging ability) in common wall lizards (*P. muralis*), a species where ventral colour morphs are often thought to reflect alternative life-history trade-offs (Calsbeek et al. 2010; Sacchi et al. 2017*b*, 2017*a*; Mangiacotti et al. 2019). By keeping the juveniles in outdoor tubs for approximately one year, we could also evaluate the effect of parental morph on inter-annual growth and survival, as well as describe ontogenetic changes in colour expression. Overall, we found no evidence of parental morph biases that would suggest the existence of strong advantages to either morph-assortative or disassortative mating (i.e. genetic incompatibilities). In fact, although we did find a higher fertilisation success of females when paired assortatively, parental morph combination was mostly irrelevant to the different stages of development we examined (i.e. pre-hatching, post-hatching, survival to adulthood).

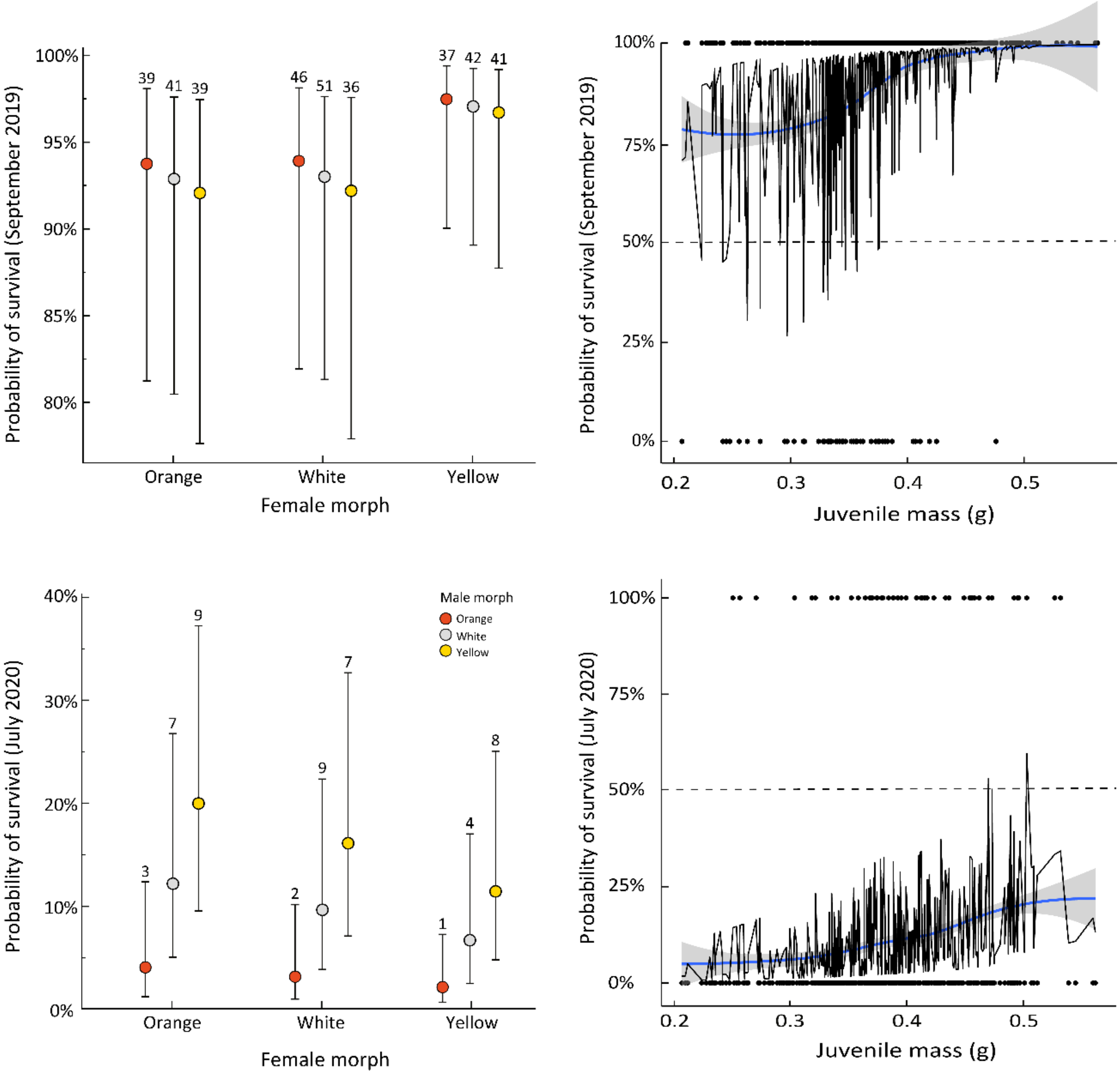

Figure 5.5. Predicted probability that a juvenile survived according to parental morph combination (left) and mass at eclosion (right), measured at two different points in time: 3-4 weeks after hatching (September 2019), and one year later (July 2020). Circles mark the position of the mean and bars extend to the CI_{95}. Numbers on top of the error bars correspond to the surviving number of juveniles in each morph combination category. Black line represent individual probabilities predicted by the logistic mixed model. Blue line and shaded area correspond to the smoothed tendency line and its CI_{95}. There is a positive relationship between survival and growing juvenile mass. In addition, juveniles sired by orange male lizards showed significantly lower levels of inter-annual survival than juveniles sired by yellow morph lizards (although mortality and dispersion could have been conflated in our experimental tubs).

Both pre-copulatory (e.g. inability to copulate) and post-copulatory (e.g. sperm rejection) barriers to reproduction may result in biased fertilisation. However, no sex/morph combination was underrepresented in our recording of inter-sexual interactions, which argues against the former mechanism as an explanation for our results. Adaptive sperm-sorting strategies whereby females handicap the sperm of less-preferred male morphs have been invoked to explain morph-biased patterns of fertilisation in *U. stansburiana* (Calsbeek and Sinervo 2002*a*; Lancaster et al. 2014). However, this explanation seems unlikely in the case of *P. muralis* for three reasons. First, the difference we found in fertilisation success was mainly driven by the high prevalence of infertile clutches in yellow females mated to orange males (five out of a total of nine infertile clutches), in contrast with the almost perfect fertilisation success of white homomorphic pairs. The converse crossing (i.e. orange female with yellow male) showed no abnormal levels of fertilisation success, as expected if limited gene flow between these two morphs hampered hybridization (Gray and McKinnon 2007; Pryke and Griffith 2009*a*; Lancaster et al. 2014). Second, due to the recessive genetic basis of orange and yellow coloration (Andrade et al. 2019), the offspring of white homomorphic pairings ought to be the most genotypically diverse and thus finding the highest levels of fertilisation success in this crossing (instead of in orange or yellow homomorphic crossings) argues against the existence of an homozygote advantage. Third, when sympatric colour morphs undergo correlational selection, the genetic architecture protecting co-adapted gene complexes from recombination is predicted to raise mainly post-zygotic barriers and hence significantly decrease embryonic viability (which was not observed in our experiment) rather than fertilisation success (Sinervo and Svensson 2002; Gray and McKinnon 2007; Mckinnon and Pierotti 2010). For instance, conducting controlled crosses in *U. stansburiana* (in which colour morphs reflect multi-trait reproductive strategies in both males and females), Lancaster et al. (2014) reported that disassortative pairing entails lower probabilities of hatching in fertilised eggs, which they interpret as a form of outbreeding depression causing increased embryonic mortality. Likewise, significantly lower hatchability (34% reduction) was documented in the fertilised eggs of disassortative pairings between two of the colour morphs exhibited by the Gouldian finch (*Erythrura gouldiae*) (Pryke and Griffith 2009*a*). In both of these species, the combined effect of assortative mating preferences and linkage disequilibria between co-adapted gene complexes within each morph (i.e. correlational selection) seems to have introduced significant genetic divergence and reproductive isolation between morphs (Bleay and Sinervo 2007; Pryke and Griffith 2007). In contrast, our results suggest that despite the high prevalence of morph-assortative pairings observed in wild populations of *P. muralis* (Pérez i de Lanuza et al. 2013*a*, 2016*b*), inter-morph breeding remains entirely viable. This aligns well with the recent finding of only minor genetic divergence between colour morphs in polymorphic *P. muralis* populations from eastern Pyrennees, which suggests inter-morph gene flow must be sufficiently high to prevent the build-up of strong genetic differentiation between the morphs (Andrade et al. 2019). In short, although we found a weak disadvantage of heteromorphic mating in fertilisation success, the bias (which remains to be confirmed using a larger sample) involves only one of six heteromorphic crossings examined (Y♀-O♂), is not mirrored in the converse crossing (O♀-Y♂), and does not fit well with predictions of how correlational selection may lead to incipient reproductive isolation between morphs. Hence, our results suggest that colour polymorphism is not promoting speciation in *P. muralis* lizards from eastern Pyrennees, while arguing against a fitness advantage of homomorphic matings as an explanation for the high prevalence of morph-assortative pairings in natural populations.

These results are in stark contrast with those of a previous mating experiment in *P. muralis* from northern Italy where parental morph combination was found to significantly impact reproductive output, and female colour morphs were found to show alternative breeding investment strategies (Galeotti et al. 2013). In that study, orange and yellow females showed an overall higher fertilisation success and embryonic viability when paired assortatively, while white morph females performed better when paired with orange males. In addition, yellow morph females tended to produce many light offspring and white females produced few heavy juveniles. Similar morph-specific strategies have also been found in females of the common lizard (*Zootoca* vivipara), a close relative of the genus *Podarcis* (Vercken et al. 2007; Vercken and Clobert 2008; but see Cote et al. 2008). While our results agree with these studies on the negative relationship between juvenile mass and clutch size, we failed to replicate any of their results concerning colour polymorphism. It is possible that these contrasting results are biologically meaningful. Most of the evidence suggesting physiological or behavioural morph differences in *P. muralis* comes from studies on the Southern Alps lineage, which is only distantly related to the Western European lineage of the Pyrenees (Giovannotti et al. 2010; Schulte et al. 2012; Gassert et al. 2013; Salvi et al. 2013; Yang et al. 2018). Recent studies in polymorphic organisms have reported correlations between local environmental factors and morph population frequencies (McLean and Stuart-Fox 2014; McLean et al. 2014*a,b*, 2015), incorporating climate and other abiotic components of natural selection into the study of the adaptive value of colour polymorphism (Roulin 2004; Svensson 2017; Svensson et al. 2020). In *P. muralis*, both climatic and demographic factors (i.e. sex ratio) may affect morph composition, suggesting the worth to investigate the environmental dependence of morph fitness in future research (Pérez i de Lanuza et al. 2017, 2018*b*; Pérez i de Lanuza and Carretero 2018). In fact, we deem the finding that *P. muralis* colour morphs do not show strong genetic incompatibilities an important motivation for new studies tackling the environmental dependence of morph fitness in natural populations (see also Abalos et al. 2020).

Although we did not find evidence of a strong morph combination effect on reproductive output, we found lower inter-annual survival in the offspring of orange morph males. Calsbeek *et al.* 2010 reported lower levels of inter-annual survival for free-roaming orange morph lizards inhabiting a stone church and a cemetery at < 1 km from where our experimental tubs were positioned. Having also found larger body sizes and higher levels of parasite infection in orange morph lizards, Calsbeek et al. (2010) argued that selection may have favoured a higher investment on dominance and reproduction in the orange morph, at the cost of a weaker immune function and shorter lifespan than the other morphs. Alternative life-history strategies subject to environmentally-dependent fitness have been described in other polymorphic systems such as the tawny owl (*Strix aluco*), where grey-coloured individuals live longer and produce more offspring than the brown morph under cold environmental conditions, but the situation is reversed under warmer conditions (Brommer et al. 2005). Unfortunately, both ours and Calsbeek *et al.* (2010) results may conflate mortality with dispersal, as no experimental constrain to dispersal was established when monitoring the free-roaming population, and restrictions to fieldwork during the COVID-19 lockdown may have allowed some of our juveniles to escape the outdoor tubs through the overgrown vegetation. Although in both cases the study site was surrounded by unsuitable lizard habitat and researchers failed to find marked lizards in the adjacent area, these results do not allow us to disentangle the relative effects of escape and mortality on the observed pattern of inter-annual re-sighting. Still, the fact that two independent studies found a similar bias in inter-annual re-sighting concerning the orange morph suggests the potential value of investigating differences in life-history traits such as life expectancy, longevity, or dispersal among *P. muralis* colour morphs in future research.

Our monitoring of colour expression in the surviving lizards yielded important information regarding morph inheritance and ontogeny. Although based on a limited sample size, the observed morph frequencies among the subadult lizards allow us to reject certain mechanisms of inheritance that control morph expression in other polymorphic lizards. For instance, a mechanism based on a single locus with three co-dominant alleles (e.g. *U. stanburiana;* Sinervo et al. 2010*; Ctenophorus pictus;* Olsson et al. 2007) entails that yellow-orange offspring can only result from YO matings and that WW crossings should always produce white morph lizards, two conditions that are not met in our sample (**Fig. 5.6**). Likewise, a mechanism based on two separate loci (each with two alleles) where presence of the dominant O and Y allele results in colour expression (*Ctenophorus decresii*; Rankin et al. 2016) would restrict the outcome of certain inter-morph matings in a way incompatible with our observations (e.g. WY crossings could never result in orange offspring). By contrast, observed morph frequencies were congruent with those expected under the assumption that yellow and orange coloration are controlled by two separate loci (each with two alleles) where recessive homozygosity of the y and o alleles results in colour expression (Andrade et al. 2019). In fact, orange and yellow subadults were primarily produced in OO and YY homomorphic crossings (respectively), while heteromophic and WW homomorphic crossings resulted in a wider range of differently-coloured offspring (**Fig. 5.6**). Several corollaries follow these results. First, white coloration showing genetic dominance over orange and yellow offers a simple explanation for the overall higher prevalence of this morph in natural *P. muralis* populations (Pérez i de Lanuza et al. 2017, 2018*b*; Andrade et al. 2019). Second, recessive expression of orange and yellow coloration may have important implications on colour polymorphism maintenance, as recessivity lowers exposure to selection hence allowing for the expression of alternative (e.g. specialist) phenotypes only under particular conditions. In turn, this highlights the need to genotypically characterize *P. muralis* lizards when studying morph frequencies and selection in natural populations. Lastly, these results evidence taxonomic diversity in the genetic architecture of lizard colour polymorphism. Assesing the evolutionary causes and consequences of such diversity may be a fruitful avenue of research for future comparative studies (Stuart-Fox et al. 2020).

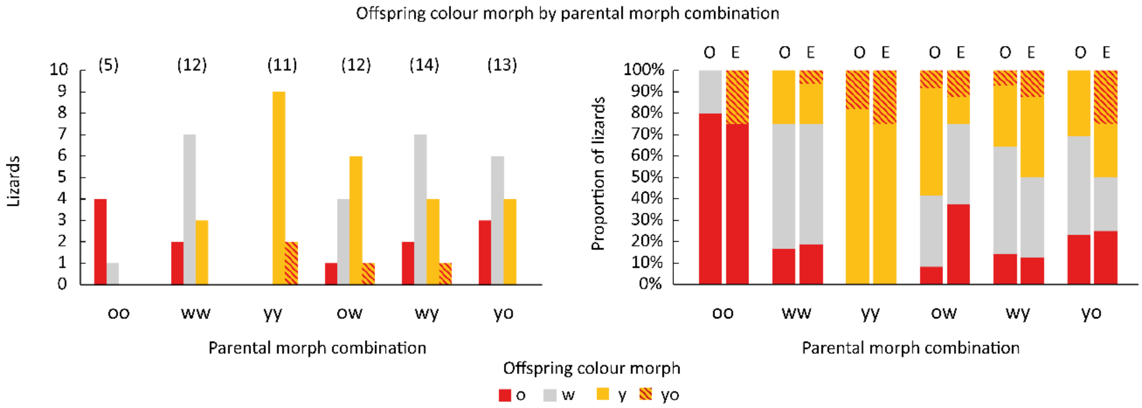

Figure 5.6. Left) Morph assignment for 67 subadult *P. muralis* lizards one year after hatching. Numbers correspond to sample size per morph combination. Right) Barplot comparing the observed morph frequencies (O) per parental morph combination with the frequencies expected (E) if the expression of orange and yellow (instead of white) ventral coloration required recessive homozygosity at two separate loci, each with two alleles.

Concerning morph ontogeny, the spectrophotometric analysis of throat coloration revealed that the white coloration expressed by 99% of newborn lizards presents increased reflectance in the UV waveband relative to subadult lizards showing incipient morph expression (**Fig. 5.7**). Further, within-individual chromatic distances suggest that all colour morphs are likely perceived by conspecifics as chromatically distinct from the UV-white coloration expressed by newborn lizards. This observation carries interesting implications for our understanding of the ontogeny of ventral colour polymorphism in *P. muralis* and other lacertid lizards, as white morph lizards have been often assumed to retain the juvenile ventral coloration during growth (in contrast with orange, yellow, and mixed-morph lizards) (e.g. Pérez i de Lanuza et al. 2013). Our results suggest instead that hatchlings express a newborn-specific colour that is chromatically distinct from either of the colour morphs expressed in the throat by adult lizards. Interestingly, similar UV-reflecting white coloration has been documented in the belly (but not the throat) of many female *P. muralis* lizards from eastern Pyrenees suggesting that, in these populations, females may retain the newborn coloration in their bellies through adulthood (see **Fig. 2** in Pérez i de Lanuza and Font 2015). This unexpected result expands the scope for a role of *P. muralis* ventral coloration in a communication context (Pérez i de Lanuza et al. 2013*a*, 2016*b*; Pérez i de Lanuza and Font 2015). For instance, UV-white throat coloration (typically exhibited by newborn lizards) may act as a chromatic signal conveying information about its owner's young age and sexual immaturity during social interactions. Juvenile coloration has been found to reduce aggression from adult conspecifics in some vertebrate species (Hill 1989; Ochi and Awata 2009), including lizards (Clark and Hall 1970; Hawlena et al. 2006; Fresnillo et al. 2015).

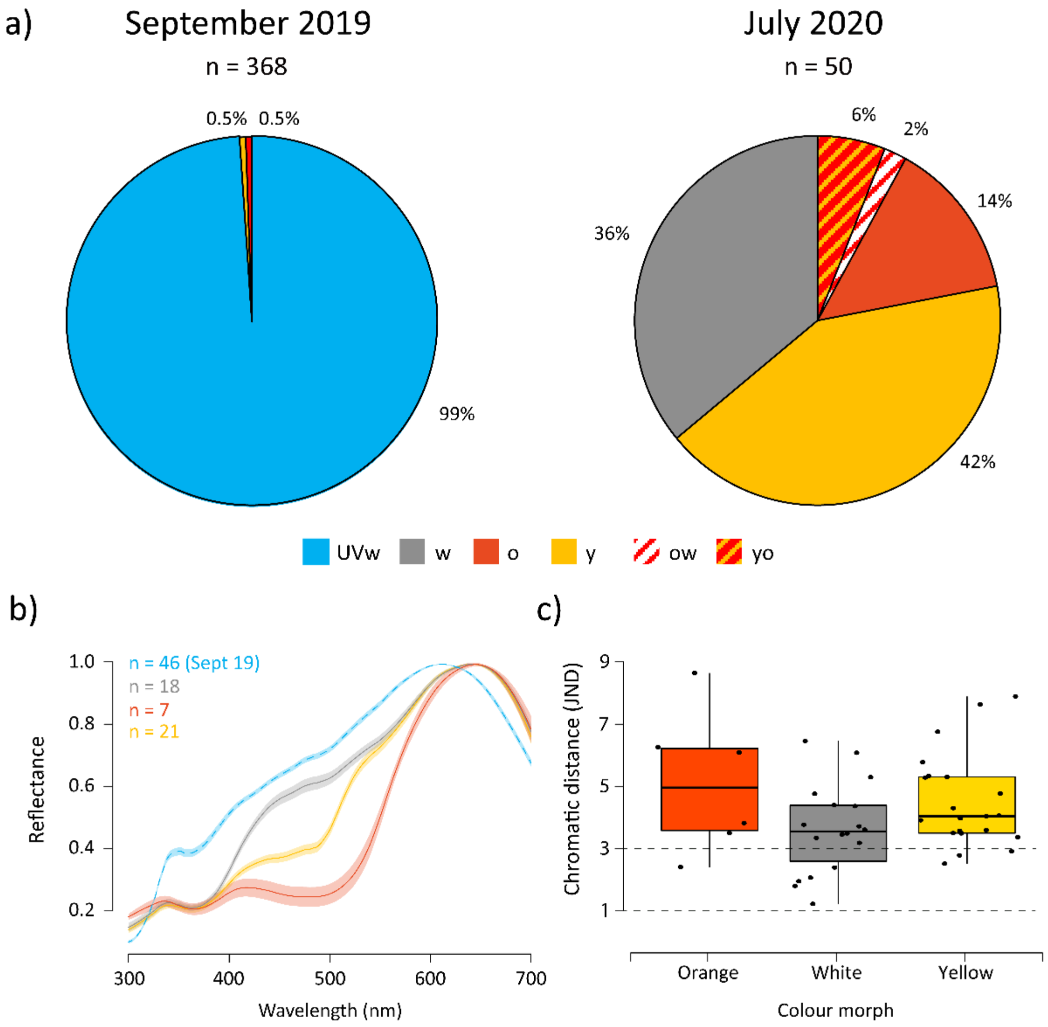

Figure 5.7. Ontogenetic change in throat coloration. a) Pie charts represent the fraction of juveniles showing different throat colours (UVw = UV-white, w = white, o = orange y = yellow, ow = orange-white, yo = yellow-orange) in September 2019 (3-4 weeks after hatching), and July 2020 (one year after hatching). b) Reflectance spectra from the 46 surviving juveniles showing pure colour morphs in July 2020 (solid lines and shaded area represent means±1 SEM). The dashed blue line represents the mean UVw spectra shown by these same lizards when first measured in September 2019. For clarity, the spectra have been normalized by making the reflectance at all wavelengths proportional to the maximum reflectance. c) Boxplots showing chromatic distances between paired measurements of throat reflectance in the surviving lizards of each colour morph. Horizontal dotted lines indicate the discriminability thresholds of 1 just noticeable difference (JND; values above 1 JND indicate pairs of colours that are discriminable under good illumination conditions) and 3 JND (values above 3 JND indicate easily discriminable pairs of colours).

Altogether, our results suggest that, at least in the examined populations (i.e. eastern Pyrenees), *P. muralis* colour morphs do not reflect alternative breeding investment strategies in females and parental morph combination does not influence offspring viability, behaviour, and survival. Although we found lower fertilisation success in YO crossings, we list several reasons why this result should be confirmed in new studies before inferring its possible consequences for the long-term maintenance of colour polymorphism. In contrast with the widely held assumption that *P. muralis* morphs reflect complex suites of co-adapted gene complexes (i.e. alternative phenotypic optima) evolved by correlational selection, overall our results suggest that inter-morph breeding remains entirely viable in *P. muralis* while maintaining the uncertainty about which mechanism is preventing any of the colour morphs from going extinct.

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Author contributions: Conceptualization and experimental design: J.A., A.B., G.P.L., F.A., T.U., E.F.; Investigation: J.A., A.B.; Statistical analysis: J.A.; Visualization: J.A.; Writing - original draft: J.A.; Writing - review & editing: J.A., G.P.L., A.B., F.A., T.U., E.F.; Supervision: G.P.L., E.F.

Conflict of interests: The authors declare no conflict of interests

Data archiving: The datasets used and analysed in this study will be made available at Dryad upon acceptance.

5.6. Appendix S1: Expanded materials and methods

5.6.1. Additional information on the housing of gravid females in the lab

We housed females individually in 55 x 38 x 28 cm high plastic terraria with a substrate of coco husk, a water dish, a shelter, and a small brick for basking over which we suspended a 40 W incandescent light (18-35ºC temperature gradient). We also introduced a plastic bowl filled with moist coco husk (11 cm of diameter, 8 cm deep) which the females used to lay the eggs. We set the light cycle to mimic field conditions during the reproductive season at the capture site (15L:9D), and additionally provided all lizards with full spectrum light (Reptistar 5.0: Sylvania, Danvers, MA, USA) for 2 h daily (12.00–14.00 h). We fed lizards two *Tenebrio molitor* larvae dusted with vitamins (JBL Terravit, Neuhofen, Germany) every other day. Overall, females stayed in the laboratory for less than 15 days before being released at their capture sites (i.e. after oviposition).

5.6.2. Additional information on spectrophotometry and visual modelling

To characterize the spectral properties of juvenile *P. muralis* throats, we obtained reflectance spectra using a USB 2000 portable diode-array spectrometer equipped with a QP200-2-UV/VIS-BX reading-illumination probe and a PX-2 Xenon strobe light for full spectrum illumination (Ocean Optics Inc., Dunedin, FL, USA; Font et al. 2009). Measurements encompassed the 300–700 nm range to cover the entire visual spectrum of *P. muralis* (Pérez i de Lanuza and Font 2014; Martin et al. 2015*a.b*).

We used Vorobyev and Osorio's (1998) receptor noise model and the R package PAVO (Maia et al. 2013) to determine quantitatively the chromatic distance (i.e. discriminability) between the throat colour exhibited by each recaptured individual 3-4 weeks after hatching, and one year later (September 2019 vs. July 2020). We assumed a cone abundance ratio of 1:1:1:4 (corresponding to the UV-, short-, middle- and long-wavelength sensitive cones, previously used in lacertids; Marshall and Stevens 2014; Martin et al. 2015*a,b*; Pérez i de Lanuza et al. 2018), and a Weber fraction of 0.05 for the long-wavelength sensitive cone (see Pérez i de Lanuza et al. 2018 for further details). Cone absorbance spectra of *P. muralis* were obtained from the literature (Martin et al. 2015*a,b*).

5.6.3. Additional information on parentage analysis

We isolated DNA from tail-tip samples using the DNAeasy 96 Blood & Tissue Kit (Qiagen, Valencia, CA, USA), obtaining a final elution volume of 150 µl in AE buffer. We then combined the microsatellite primers into two different multiplexes (MPA: Pm16, Pm09, PmurC168; MPB: Pm19, Pm14, PmurC038) and ran standard PCR reactions with 26 cycles and a final extension step of 30 min at 60ºC. Forward primers were labelled with different fluorescent dyes (FAM, NED, HEX). Diluted PCR products (1:5) were genotyped together with an internal ladder (Red ROX-500) on an ABI 3130 genetic analyser (Applied Byosystems Inc.). One researcher (JA) scored the alleles for every adult and juvenile lizard in Geneious 7.0.4 (Biomatters, available at http://www.geneious.com), which we used to conduct paternity analyses in Cervus 3.0 (Marshall et al. 1998;

Kalinowski et al. 2007). We assigned paternity based on the log-likelihood statistic of each mother-father-offspring trio (LOD scores), using two confidence levels (strict: 95%, relaxed: 80%) and the male within each tank as candidate father. Critical LOD scores were determined by running a simulation paternity analysis based on 100,000 offspring with known mothers and nine candidate fathers.

5.6.4. Additional information on statistical analysis

We examined the effect of parental morph and morph combination on reproductive output by fitting linear mixed models (LMMs) with the total number of eggs laid (clutch size) and the number of viable juveniles as response variables, and juvenile mass, female mass, as well as male and female colour morph (and the interaction between the two) as fixed factors. We also included female capture locality, experimental tank, and whether the female was captured at the end of the previous season or not as random factors (capture year). To evaluate the possible effect of parental morph combination on fertilisation success (1) and embryonic viability (2), we fitted two logistic mixed models (GLMM binomial) with the number of fertilised and unfertilised eggs (1), or the number of viable and unviable embryos (2) conceived by each female as dependent variables, female mass, clutch size, and the interaction between male and female colour morph as fixed factors, and female capture locality, year, and experimental tank as random factors. Likewise, to investigate the effect of parental morph on survival we fitted two logistic mixed models with the number of deceased and surviving lizards 3-4 weeks after hatching (when the juveniles were introduced in the outdoor tanks, September 2019), and approximately one year after hatching (in July 2020) as dependent variables, and the same factors, covariates and interactions described above. We also tested for morph differences in juveniles mass at birth (i.e. a proxy of newborn quality) and inter-annual growth by fitting a linear mixed model including the same explanatory variables.

To analyse juvenile behavioural tests we first coded the performance of each lizard in the antipredator trial as 1 if the lizard entered the shelter in the given time (5 min) or 0 if the lizard failed to do so. Likewise, we coded the performance of each lizard in the foraging ability trial as 1 if the juvenile captured and consumed the prey during the 5 min period, or 0 if it did not. We then fitted two generalized mixed models with a binomial distribution on juvenile performance, including female mass, juvenile mass, clutch size, and the interaction between male and female colour morph as fixed factors, as well as female identity, capture year, locality, and experimental tank as random factors. Restricting the analysis to the juveniles which either entered into the shelter or bit the novel prey item, we further assessed the existence of morph combination biases in antipredator response and foraging ability by fitting two separate linear mixed models with latency as the dependent variables, and the same factors, covariates and random factors as above.

Additionally, to specifically address the hypothesis that genetic incompatibilities between colour morphs (leading to an heterozygote or homozygote advantage) may contribute to the maintenance of *P. muralis* colour polymorphism over time, for each of the models described above we run another model in which we included the factors, covariates and interactions, but substituted the interaction between male and female colour morph with a dichotomous factor differentiating homomorphic from heteromorphic pairings.

Tables

Table 5.S1. Results from linear models examining potential inter-morph differences in morphometric and male colour traits in our sample of experimental lizards.

Sex	**Trait**	**Orange**	**White**	**Yellow**	***F***	***P*-value**
Males	SVL	67.6 ± 1.6	66.5 ± 1.8	67.8 ± 2.3	$F_{2, 42} = 0.65$	$P = 0.526$
	Mass	6.7 ± 0.3	6.4 ± 0.4	6.9 ± 0.5	$F_{2, 42} = 2.05$	$P = 0.143$
Females	SVL	63.4 ± 1.2	63.2 ± 1.0	63.6 ± 0.9	$F_{2, 129} = 0.75$	$P = 0.476$
	Mass	5.3 ± 0.4	5.3 ± 0.3	5.6 ± 0.3	$F_{2, 129} = 0.67$	$P = 0.517$

Table 5.S2. Results from GLMMs examining potential differences in reproductive performance among different parental morph combinations. Significant predictors are highlighted in bold (*a* = 0.95, $P < 0.05$). Statistics for non-significant factors are included at the point of their deletion from the model.

Response variable	**Predictors**	**df**	χ^2	***P*-value**	***pseudo-r*²**
Clutch size	Male morph	2	$\chi^2 = 0.32$	$P = 0.851$	**0.32**
	Female morph	2	$\chi^2 = 1.03$	$P = 0.598$	
	Female mass	**1**	$\boldsymbol{\chi^2 = 15.88}$	$\boldsymbol{P < 0.001}$	
	Juvenile mass	**1**	$\boldsymbol{\chi^2 = 20.23}$	$\boldsymbol{P < 0.001}$	
	Male morph*Female morph	4	$\chi^2 = 3.07$	$P = 0.547$	
Viable clutch size	Male morph	2	$\chi^2 = 0.17$	$P = 0.921$	**0.05**
	Female morph	2	$\chi^2 = 5.02$	$P = 0.081$	
	Female mass	1	$\chi^2 = 2.17$	$P = 0.141$	
	Juvenile mass	**1**	$\boldsymbol{\chi^2 = 5.66}$	$\boldsymbol{P = 0.017}$	
	Male morph*Female morph	4	$\chi^2 = 2.23$	$P = 0.694$	
Fertilisation success	Male morph	2	$\chi^2 = 2.18$	$P = 0.337$	**0.19**
	Female morph	**2**	$\boldsymbol{\chi^2 = 17.36}$	$\boldsymbol{P = 0.001}$	
	Female mass	1	$\chi^2 = 0.83$	$P = 0.361$	
	Clutch size	1	$\chi^2 = 0.01$	$P = 0.942$	
	Male morph*Female morph	**4**	$\boldsymbol{\chi^2 = 19.50}$	$\boldsymbol{P < 0.001}$	
Embryonic viability	Male morph	2	$\chi^2 = 1.95$	$P = 0.376$	**0.07**
	Female morph	2	$\chi^2 = 2.70$	$P = 0.259$	
	Female mass	**1**	$\boldsymbol{\chi^2 = 6.36}$	$\boldsymbol{P = 0.012}$	
	Clutch size	1	$\chi^2 = 0.44$	$P = 0.507$	
	Male morph*Female morph	4	$\chi^2 = 2.80$	$P = 0.591$	
Juvenile mass	Male morph	2	$\chi^2 = 0.98$	$P = 0.707$	**0.21**
	Female morph	2	$\chi^2 = 0.79$	$P = 0.674$	
	Female mass	1	$\chi^2 = 0.14$	$P = 0.711$	
	Clutch size	**1**	$\boldsymbol{\chi^2 = 21.75}$	$\boldsymbol{P < 0.001}$	
	Male morph*Female morph	4	$\chi^2 = 3.63$	$P = 0.459$	

Table 5.S3. Results from GLMMs examining potential differences in reproductive performance among assortative and disassortative pairings. Significant factors are highlighted in bold (*a* = 0.95, $P < 0.05$). Statistics for non-significant factors are included at the point of their deletion from the model.

Response variable	**Predictors**	**df**	χ^2	***P*-value**	***pseudo-r²***
Clutch size	Homo/hetero-morphic	1	$\chi^2 = 0.357$	$P = 0.550$	**0.32**
	Female mass	**1**	$\boldsymbol{\chi^2 = 15.88}$	$\boldsymbol{P < 0.001}$	
	Juvenile mass	**1**	$\boldsymbol{\chi^2 = 20.23}$	$\boldsymbol{P < 0.001}$	
Viable clutch size	Homo/hetero-morphic	1	$\chi^2 = 2.39$	$P = 0.243$	**0.05**
	Female mass	1	$\chi^2 = 2.38$	$P = 0.123$	
	Juvenile mass	**1**	$\boldsymbol{\chi^2 = 5.66}$	$\boldsymbol{P = 0.017}$	
Fertilisation success	**Homo/hetero-morphic**	**1**	$\boldsymbol{\chi^2 = 9.46}$	$\boldsymbol{P = 0.002}$	**0.03**
	Female mass	1	$\chi^2 = 0.11$	$P = 0.737$	
	Clutch size	1	$\chi^2 = 0.14$	$P = 0.704$	
Embryonic viability	Homo/hetero-morphic	1	$\chi^2 = -0.07$	$P = 1.000$	**0.07**
	Female mass	**1**	$\boldsymbol{\chi^2 = 6.36}$	$\boldsymbol{P = 0.012}$	
	Clutch size	1	$\chi^2 = 0.46$	$P = 0.497$	
Juvenile mass	Homo/hetero-morphic	1	$\chi^2 = 0.08$	$P = 0.772$	**0.21**
	Female mass	1	$\chi^2 = 0.04$	$P = 0.848$	
	Clutch size	**1**	$\boldsymbol{\chi^2 = 21.75}$	$\boldsymbol{P < 0.001}$	

Table 5.S4. Results from GLMMs examining the effect of parental morph combination on juvenile antipredator response and foraging ability. Significant factors are highlighted in bold ($a = 0.95$, $P < 0.05$). Statistics for non-significant factors are included at the point of their deletion from the model.

Response variable	**Predictors**	**df**	χ^2	P**-value**	***pseudo-*** r^2
Juvenile enters the shelter (yes, no)	Male morph	2	$\chi^2 = 0.36$	$P = 0.837$	**0.03**
	Female morph	2	$\chi^2 = 1.41$	$P = 0.495$	
	Female mass	1	$\chi^2 = 0.08$	$P = 0.776$	
	Juvenile mass	**1**	$\boldsymbol{\chi^2 = 4.12}$	$\boldsymbol{P = 0.042}$	
	Clutch size	1	$\chi^2 = 0.06$	$P = 0.811$	
	Male morph*Female morph	4	$\chi^2 = 3.89$	$P = 0.421$	
Latency to enter inside the shelter	Male morph	2	$\chi^2 = 2.34$	$P = 0.310$	
	Female morph	2	$\chi^2 = 3.31$	$P = 0.191$	
	Female mass	1	$\chi^2 = 0.05$	$P = 0.825$	
	Juvenile mass	1	$\chi^2 = 0.793$	$P = 0.208$	
	Clutch size	1	$\chi^2 = 0.03$	$P = 0.859$	
	Male morph*Female morph	4	$\chi^2 = 5.91$	$P = 0.206$	
Juvenile eats the novel prey item (yes, no)	Male morph	2	$\chi^2 = 0.38$	$P = 0.828$	
	Female morph	2	$\chi^2 = 2.57$	$P = 0.278$	
	Female mass	1	$\chi^2 = 0.32$	$P = 0.572$	
	Juvenile mass	1	$\chi^2 = 0.507$	$P = 0.477$	
	Clutch size	1	$\chi^2 = 0.64$	$P = 0.423$	
	Male morph*Female morph	4	$\chi^2 = 8.32$	$P = 0.081$	
Latency to capture prey	Male morph	2	$\chi^2 = 1.53$	$P = 0.465$	**0.16**
	Female morph	2	$\chi^2 = 0.06$	$P = 0.971$	
	Female mass	1	$\chi^2 = 0.18$	$P = 0.672$	
	Juvenile mass	**1**	$\boldsymbol{\chi^2 = 22.67}$	$\boldsymbol{P < 0.001}$	
	Clutch size	1	$\chi^2 = 0.00$	$P = 0.992$	
	Male morph*Female morph	4	$\chi^2 = 3.57$	$P = 0.467$	
Latency to consume prey	Male morph	2	$\chi^2 = 0.47$	$P = 0.789$	**0.16**
	Female morph	2	$\chi^2 = 1.51$	$P = 0.470$	
	Female mass	1	$\chi^2 = 0.66$	$P = 0.415$	
	Juvenile mass	**1**	$\boldsymbol{\chi^2 = 22.10}$	$\boldsymbol{P < 0.001}$	
	Clutch size	1	$\chi^2 = 0.35$	$P = 0.553$	
	Male morph*Female morph	4	$\chi^2 = 6.27$	$P = 0.180$	

Table 5.S5. Results from GLMMs examining the effect of assortative and disassortative pairing on juvenile antipredator response and foraging ability three-four weeks after hatching. Significant factors are highlighted in bold (*a* = 0.95, *P* < 0.05). Statistics for non-significant factors are included at the point of their deletion from the model.

Response variable	**Predictors**	**df**	χ^2	***P*-value**	***pseudo-r*^{2}**
Juvenile enters the shelter (yes, no)	Homo/hetero-morphic	1	$\chi^2 = 0.59$	$P = 0.443$	**0.03**
	Female mass	1	$\chi^2 = 0.02$	$P = 0.900$	
	Juvenile mass	**1**	$\chi^2 = 4.11$	$P = 0.042$	
	Clutch size	1	$\chi^2 = 0.09$	$P = 0.760$	
Latency to enter inside the shelter	Homo/hetero-morphic	1	$\chi^2 = 3.22$	$P = 0.073$	
	Female mass	1	$\chi^2 = 0.34$	$P = 0.559$	
	Juvenile mass	1	$\chi^2 = 0.35$	$P = 0.552$	
	Clutch size	1	$\chi^2 = 0.09$	$P = 0.760$	
Juvenile eats the novel prey item (yes, no)	Homo/hetero-morphic	1	$\chi^2 = 1.08$	$P = 0.299$	
	Female mass	1	$\chi^2 = 0.82$	$P = 0.366$	
	Juvenile mass	1	$\chi^2 = 0.35$	$P = 0.556$	
	Clutch size	1	$\chi^2 = 1.07$	$P = 0.301$	
Latency to capture prey	Homo/hetero-morphic	1	$\chi^2 = 1.15$	$P = 0.283$	**0.16**
	Female mass	1	$\chi^2 = 0.04$	$P = 0.842$	
	Juvenile mass	**1**	$\chi^2 = 22.67$	$P < 0.001$	
	Clutch size	1	$\chi^2 = 0.00$	$P = 0.984$	
Latency to consume prey	Homo/hetero-morphic	1	$\chi^2 = 2.75$	$P = 0.097$	**0.16**
	Female mass	1	$\chi^2 = 0.56$	$P = 0.453$	
	Juvenile mass	**1**	$\chi^2 = 22.67$	$P < 0.001$	
	Clutch size	1	$\chi^2 = 0.40$	$P = 0.526$	

Table 5.S6. Results from GLMMs examining the effect of assortative and disassortative pairing on juvenile antipredator response and foraging ability one year after hatching. Significant factors are highlighted in bold ($a = 0.95$, $P < 0.05$). Statistics for non-significant factors are included at the point of their deletion from the model.

Response variable	**Predictors**	**df**	χ^2	***P*-value**	***pseudo-r²***
Juvenile enters the shelter (yes, no)	Male morph	2	$\chi^2 = 1.07$	$P = 0.587$	**0.18**
	Female morph	2	$\chi^2 = 1.00$	$P = 0.607$	
	Homo/hetero-morphic	1	$\chi^2 = 0.27$	$P = 0.606$	
	Female mass	**1**	$\boldsymbol{\chi^2 = 5.37}$	$\boldsymbol{P = 0.020}$	
	Juvenile mass	1	$\chi^2 = 1.23$	$P = 0.268$	
Latency to enter inside the shelter	Male morph	2	$\chi^2 = 4.88$	$P = 0.087$	-
	Female morph	2	$\chi^2 = 1.91$	$P = 0.385$	
	Homo/hetero-morphic	1	$\chi^2 = 0.62$	$P = 0.432$	
	Female mass	1	$\chi^2 = 0.16$	$P = 0.689$	
	Juvenile mass	1	$\chi^2 = 0.69$	$P = 0.406$	
Juvenile eats the novel prey item (yes, no)	Male morph	2	$\chi^2 = -0.79$	$P = 1.00$	-
	Female morph	2	$\chi^2 = 4.61$	$P = 0.100$	
	Homo/hetero-morphic	1	$\chi^2 = 0.02$	$P = 0.898$	
	Female mass	1	$\chi^2 = 1.81$	$P = 0.178$	
	Juvenile mass	1	$\chi^2 = 0.24$	$P = 0.623$	
Latency to capture prey	Male morph	2	$\chi^2 = 2.03$	$P = 0.362$	-
	Female morph	2	$\chi^2 = 0.42$	$P = 0.809$	
	Homo/hetero-morphic	1	$\chi^2 = 0.01$	$P = 0.911$	
	Female mass	1	$\chi^2 = 0.00$	$P = 0.959$	
	Juvenile mass	1	$\chi^2 = 1.38$	$P = 0.240$	
Latency to consume prey	Male morph	2	$\chi^2 = 1.80$	$P = 0.406$	**0.14**
	Female morph	2	$\chi^2 = 1.20$	$P = 0.549$	
	Homo/hetero-morphic	1	$\chi^2 = 0.04$	$P = 0.848$	
	Female mass	1	$\chi^2 = 1.76$	$P = 0.184$	
	Juvenile mass	**1**	$\boldsymbol{\chi^2 = 5.47}$	$\boldsymbol{P = 0.019}$	

Table 5.S7. Results from GLMMs examining potential differences in survival and inter-annual growth among different parental morph combinations. Significant factors are highlighted in bold (*a* = 0.95, $P < 0.05$). Statistics for non-significant factors are included at the point of their deletion from the model.

Response variable	**Predictors**	**df**	χ^2	***P*-value**	***pseudo-r²***
Survival (3-4 weeks)	Male morph	2	$\chi^2 = 0.12$	$P = 0.942$	**0.09**
	Female morph	2	$\chi^2 = 3.94$	$P = 0.139$	
	Female mass	1	$\chi^2 = 0.03$	$P = 0.854$	
	Juvenile mass	**1**	$\boldsymbol{\chi^2 = 9.39}$	$\boldsymbol{P = 0.002}$	
	Male morph*Female morph	4	$\chi^2 = 1.74$	$P = 0.782$	
Survival (1 year)	**Male morph**	**2**	$\boldsymbol{\chi^2 = 8.57}$	$\boldsymbol{P = 0.014}$	**0.21**
	Female morph	2	$\chi^2 = 0.33$	$P = 0.327$	
	Homo/hetero-morphic	1	$\chi^2 = 0.65$	$P = 0.421$	
	Female mass	1	$\chi^2 = 2.29$	$P = 0.131$	
	Juvenile mass	**1**	$\boldsymbol{\chi^2 = 10.43}$	$\boldsymbol{P = 0.001}$	
Inter-annual growth	Male morph	2	$\chi^2 = 1.28$	$P = 0.527$	-
	Female morph	2	$\chi^2 = 0.56$	$P = 0.758$	
	Homo/hetero-morphic	1	$\chi^2 = 0.55$	$P = 0.456$	
	Female mass	1	$\chi^2 = 0.97$	$P = 0.325$	
	Juvenile mass	1	$\chi^2 = 0.02$	$P = 0.887$	

Table 5.S8. Likelihood ratio tests for observed and expected morph frequencies in subadult lizards of know parental morph combination. To calculate the expected frequencies we considered two separate loci (each with two alleles) where recessive homozygosity of the y and o alleles results in the expression of yellow and orange coloration, respectively. Bold letters mark significant deviations from the expected frequencies. Deviations may derive from adult colour morphs not being yet expressed at the moment of measurement.

			Offspring expressed colour				**G df =3**	**P**
			W	**O**	**Y**	**YO**		
Morph combination	**WW**	**Observed**	7	2	3	0	1.58	0.663
		Expected	6.75	2.25	2.25	0.75		
	WO	**Observed**	4	1	6	1	**10.46**	**0.015***
		Expected	4.50	4.50	1.50	1.50		
	WY	**Observed**	7	2	4	0	0.70	0.874
		Expected	5.25	1.75	5.25	1.75		
	OO	**Observed**	1	4	0	0	-	-
		Expected	0	3.75	0	1.25		
	OY	**Observed**	6	3	4	0	**8.02**	**0.046***
		Expected	3.25	3.25	3.25	3.25		
	YY	**Observed**	0	0	9	2	0.27	0.965
		Expected	0	0	8.25	2.75		

Figures

Figure 5.S1. Outdoor plastic tubs at the Statión d'Ecologie Théorique et Expérimentale (SETE, Moulis, France).

Figure 5.S2. Male-female pair of *P. muralis* lying in physical contact (i.e. co-perching) inside one of the experimental tanks during the staged mating trials. A white paint mark for individual identification can be seen on the female's dorsal surface.

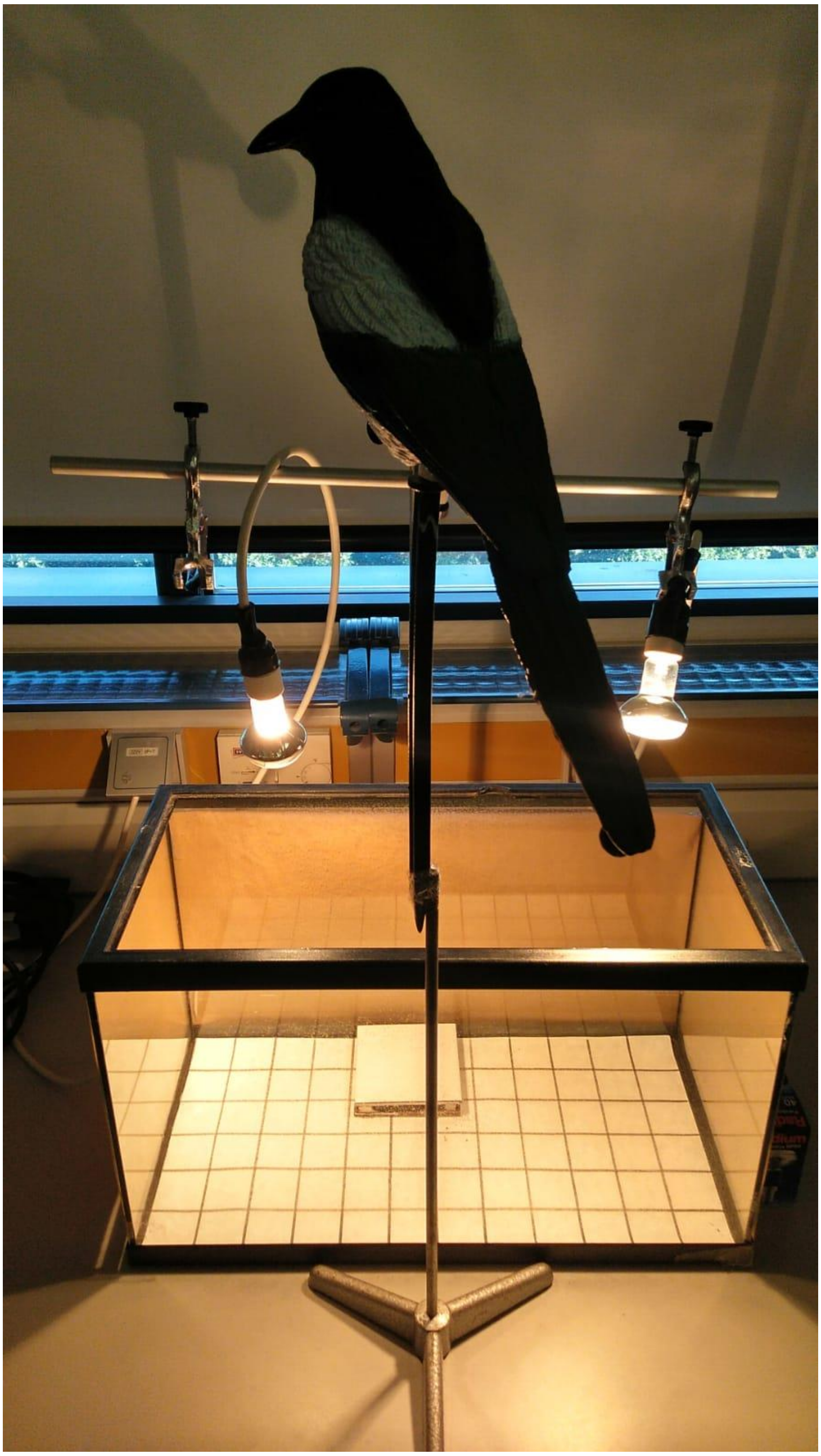


Figure 5.S3. Experimental setup for the lab-staged viability tests in which we examined the antipredator response and foraging ability of newborn lizards with differing parental morph combination.

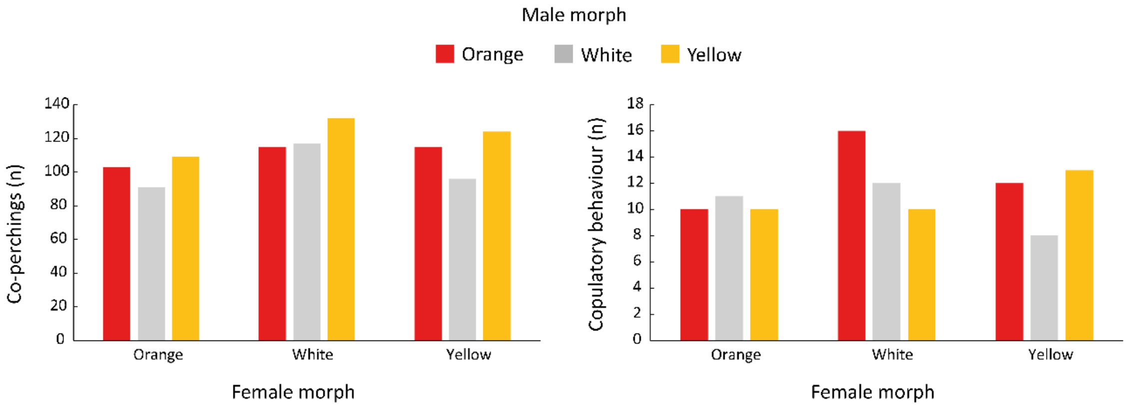


Figure 5.S4. Barplots showing the absolute frequencies of male-female pairs observed either lying in physical contact (i.e. co-perchings) or engaged in copulatory behaviour, grouped by morph combination.

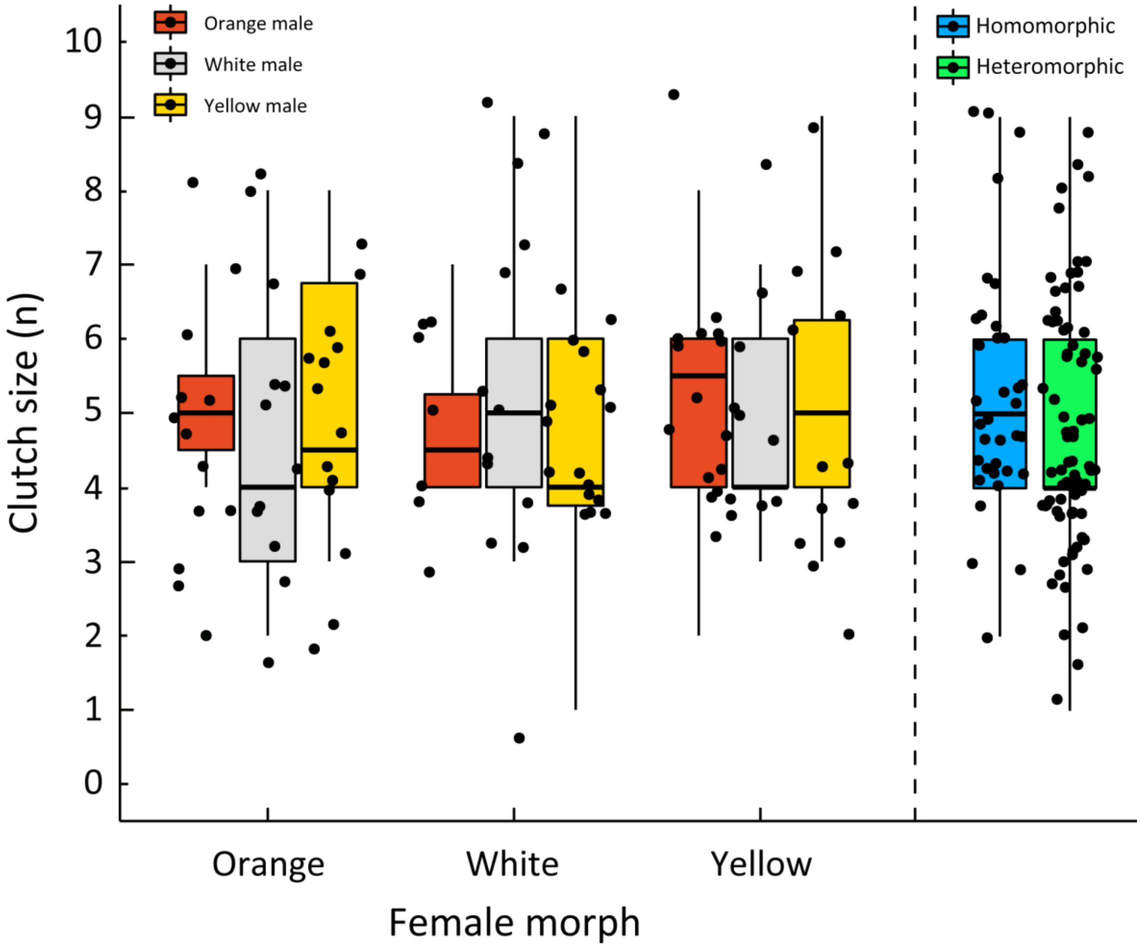


Figure 5.S5. Boxplots showing the total number of eggs (i.e. clutch size) according to morph combination. Boxes indicate the interquartile range (IQR, 50% of data). Horizontal lines represent the median and bars extend to 1.5 times the IQR. A jittered dot cloud represents each value in our dataset.

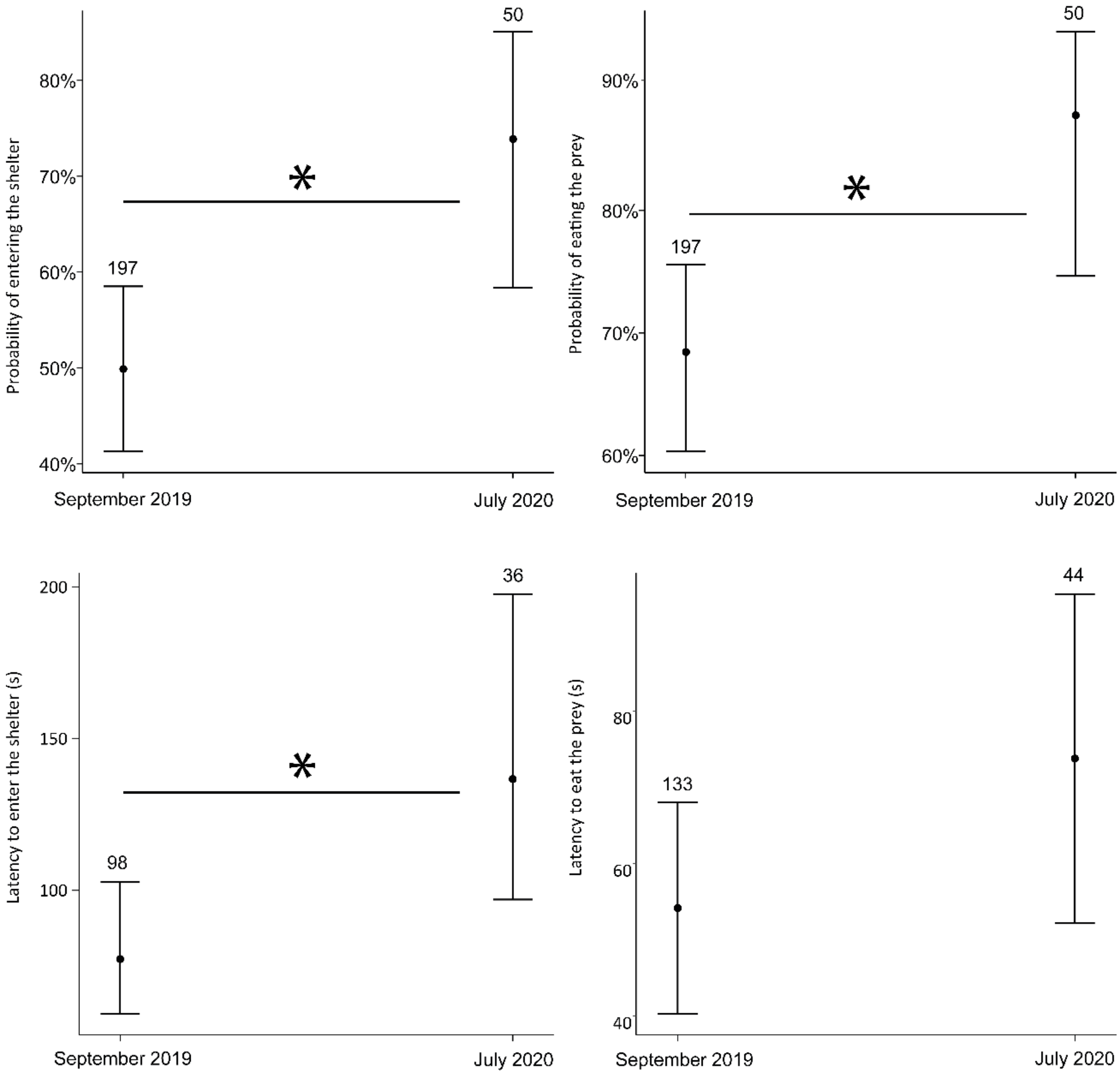
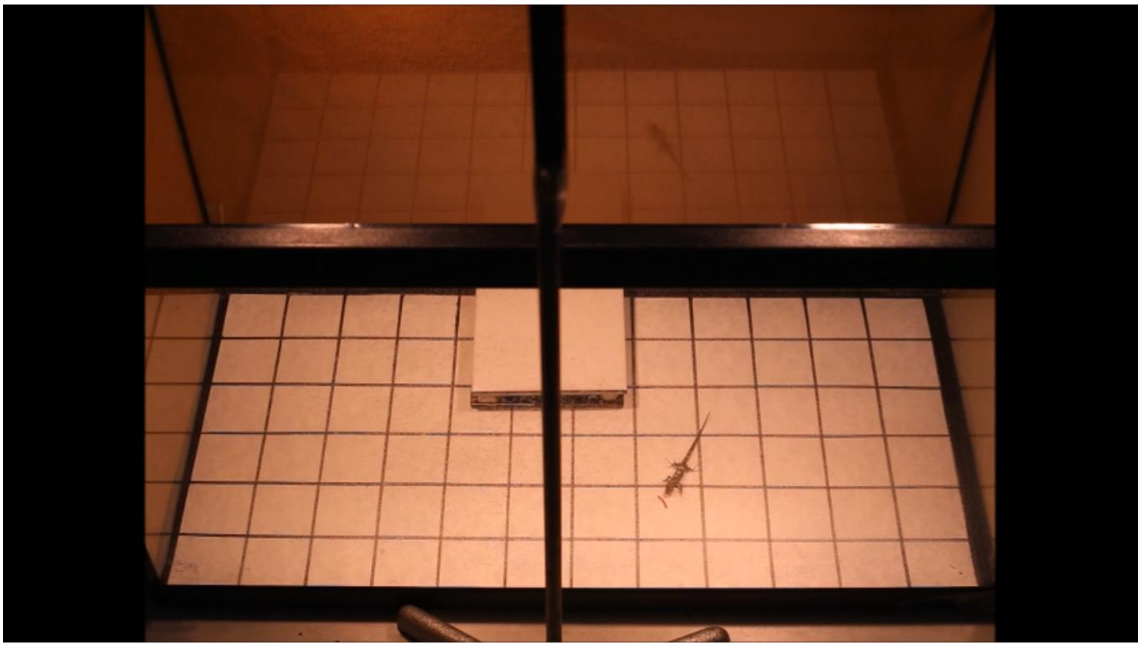


Figure 5.S6. Above, predicted probability that juveniles enter the shelter (left), or eat the prey item (right), calculated at two different points in time: 3-4 weeks after hatching (September 2019), and one year later (July 2020). Below, latency to enter into the shelter (left), or eat the prey (right) measured in the tested juveniles at the same two different points in time. Circles mark the position of the mean and bars extend to the CI_{95}. Numbers on top of the error bars indicate sample size. Asterisks represent significant ($p < 0.05$) mean differences.

Video recordings



Video 5.S1. Screenshot of a newborn lizard approaching a novel prey item during the lab-staged behavioural assays. The full recording (30x accelerated) will be available for download as a separate MP4 file.

Chapter VI

"The enemy of science is not religion. The true enemy is the substitution of thought, reflection, and curiosity with dogma."

·Frans de Waal, Are we smart enough to know how smart animals are? (2016)·

"All right then, keep your secrets…"

· Frodo Baggins, The Fellowship of the Ring (2001)·

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Chapter VI:

General discussion

6.1. No evidence for alternative strategies in *P. muralis* colour morphs

This thesis explores the functional significance of colour polymorphism in the European common wall lizard (*Podarcis muralis*). We have addressed frequently overlooked (or inadequately studied) aspects regarding the link between behaviour, fitness, and colour polymorphism to ascertain whether *P. muralis* ventral colours may function as social signals reflecting a set of alternative behavioural or life-history strategies evolved by correlational selection, as is frequently assumed in the literature (Sinervo et al. 2007; Calsbeek et al. 2010; Sacchi et al. 2017*b*; Coladonato et al. 2020). In addition, we have tested predictions of another evolutionary mechanism (i.e heterosis) which could generate balancing selection and explain the maintenance of colour polymorphism over time. Lastly, the results we present here contribute to our (still limited) knowledge of the ontogeny and heritability of colour morphs in this species. In this general discussion we will provide overall conclusions and suggest directions for future work.

A necessary condition for the putative function of *P. muralis* colour morphs as chromatic signals mediating social interactions is that the lizards themselves can perceive them as categorically distinct (Endler 1990; Maynard-Smith and Harper 2003; Tibbetts et al. 2017). However, for many polymorphic species, chromatic variation between morphs has not been quantified objectively, and morph classification is solely based on human perception (Stuart-Fox et al. 2020). In **Chapter II** we used reflectance spectrophotometry, visual modelling, and a discrimination experiment to establish that *P. muralis* lizards perceive chromatic variation among colour morphs as discrete, and can be trained to discriminate these colours even if at the lower range of perceptual distances generated by natural colour variation (see **Fig. 2.6** in **Chapter II**). Adding an achromatic control to each chromatic discrimination test and comparing the number of errors involving these controls against other incorrect chromatic stimuli, we could reasonably conclude that the discrimination exhibited by the trained lizards was based on the chromatic properties of colour (i.e. hue, chroma) rather than luminance differences (Kelber et al. 2003). In addition, we found evidence that chromatic distances between morphs vary depending on the morphs compared (orange and yellow morphs being the most similar combination, white and orange the most different), which may have implications for detectability and discrimination. Our results concerning newborn throat coloration in **Chapter V** further highlight the importance of spectrophotometry and visual modelling in the study of colour patches exhibited by species with more sophisticated visual systems than our own. Based on human colour perception, white morph lizards have been commonly assumed to retain the juvenile ventral coloration (newborn lizards appear white to our eyes) during growth (e.g. Pérez i de Lanuza et al. 2013). In contrast, we show here that newborn throat coloration reflects light in the near ultraviolet (UV-A) waveband and is likely perceived by conspecifics as a chromatically distinct colour different from any of the colour morphs expressed by adult lizards. This observation adds to our understanding of colour morph ontogeny while suggesting a possible role for juvenile UV-white coloration as a chromatic signal conveying information about its owner's young age and sexual immaturity during social interactions (i.e. reducing aggression from conspecifics). Although these results suggest that there is scope for a potential function of ventral coloration in intraspecific communication, it is possible that the alternative ventral colours have no effect on receiver behaviour during social interactions. Despite the popularity of this hypothesis, few studies have tackled the putative signalling role of ventral coloration experimentally (e.g. Healey et al. 2007). Future studies should investigate the relative importance of throat coloration as a social signal in *P. muralis* and other lizards where colour morphs are presumed to mediate social encounters (e.g. social dominance in *Uta stansburiana* and *Podarcis melisellensis*, Sinervo and Lively 1996; Calsbeek and Sinervo 2002*a*, 2002*b*; Huyghe et al. 2007; mate choice in *Zootoca vivipara,* Vercken and Clobert 2008; San-Jose et al. 2014) by recording the behaviour elicited by lizards prior to and after being painted to mimic a colour morph different than their own.

Our findings suggest novel avenues for studies of the interaction between visual ecology, behaviour, and colour polymorphism in lacertid lizards. For instance, the ability of lizards to accurately discriminate between stimuli matching their own ventral colour variation may depend on how the stimuli are presented. Recent studies on human face colour change (i.e. skin-reddening) suggest that we detect perceptually smaller colour differences when the stimuli are presented on faces rather than on non-faces, and that colour differences are subjectively perceived as greater in magnitude when displayed on faces (reviewed in Thorstenson 2018). Similarly, lizards may show more fine-tuned colour discrimination when stimuli are displayed on the throats of conspecifics lizards rather than on circular stickers surrounding a well, a possibility that should be investigated in future studies. Another problematic aspect of morph categorization in lizards is that most studies have focused on the "pure" colour morphs, with mixed-morphs combining two different colours being either included within one of the pure colour morphs without justification, or entirely neglected from analysis (Carpenter 1995*b*; Sinervo and Lively 1996; Zamudio and Sinervo 2000; Galeotti et al. 2010; Pérez i de Lanuza et al. 2014; Pérez i de Lanuza and Font 2015; Taylor and Lattanzio 2016; Sacchi et al. 2017*a*). For a better understanding of colour polymorphism, whether these mixed morphs are perceived by conspecifics as categorically distinct or clustered together with one of the pure morphs should be evaluated in future research testing morph discrimination by conspecifics across different contexts and using different morph groupings to examine model fit (Teasdale et al. 2013; Yewers et al. 2016; Stuart-Fox et al. 2020). Finally, although numerous studies across distantly related taxa have documented the importance of perceptual biases and sensory ecology in shaping the chromatic properties of social signals (Endler 1992; Hebets and Papaj 2005; Schaefer and Ruxton 2009; Stevens 2013; Lindsay et al. 2019), no study has yet explored the possible existence of colour preferences in polymorphic lizards, which could help clarify the functional significance (if any) of lizard colour polymorphism in a communicative context (but see Wagner 1933; Benes 1969; Hodgkinson and Still 1980).

Perhaps the most important results included in this thesis concern our experimental assessment of whether alternative reproductive strategies, evolved by correlational selection and concerning male socio-sexual behaviour and/or female reproductive parameters, characterize the pure colour morphs of *P. muralis*. To correct the relative neglect of quantitative behavioural analysis in the literature on *P. muralis* colour polymorphism, in **Chapter III** and **Chapter IV** we put the focus on key aspects of the species' behaviour and mating system which had received little attention. In **Chapter III** we found evidence that orange males tend to lose dyadic contests against other male morphs when confronting size-matched rivals in a neutral arena, which could result from the existence of a subordinate non-territorial strategy in this morph. However, a detailed analysis of these results and contrasting evidence from more recent studies argue against this interpretation (and the existence of alternative reproductive strategies in male morphs at large). To begin, in **Chapter III** lower levels of fighting ability were found in males showing either orange throat coloration and/or a smaller proportion of black coloration in their OVS, with our data being insufficient to disentangle the relative importance of each colour patch in determining contest outcome. Moreover, our results in **Chapter IV**, obtained both in a free-ranging population and in ten experimental mesocosm enclosures, strongly refute the hypothesis that alternative socio-sexual behaviour or space use strategies underlie colour morphs when lizards interact under more natural conditions. No colour morph was overrepresented among resident or transient lizards in the free-ranging population, and we did not observe morph differences in re-sighting propensity, inter-annual site-fidelity, distance between consecutive re-sightings, home-range size, or male-female spatial overlap. Lizards in the mesocosm experiment acclimated nicely and showed natural behaviour in accordance with our knowledge of the behaviour of free-ranging territorial lizards (Baird et al. 2001, 2003; Zamudio and Sinervo 2003; Baird 2013). Across colour morphs, social dominance was found to be the strongest predictor of male fitness. This was expected because social dominance in male lizards is typically associated with the ability to gain exclusive access to resources such as territories containing suitable basking/sheltering sites, which have a positive effect on the ability to thermo-regulate and are also attractive to females (Baird 2013; Whiting and Miles 2019). In addition, experimentally improved territories typically become smaller after treatment (e.g. by rock addition) while increasing female density (Calsbeek and Sinervo 2002*c*). Thus, the defence of a territory showing high microhabitat complexity (such as the high-quality sites in our enclosures) was expected to positively affect male reproductive success via i) improving their ability to achieve a body temperature that maximizes physiological performance, ii) decreasing the energy expenditure of territory-defence, and iii) facilitating both mate-searching and mate-guarding (Edsman 1990; Calsbeek and Sinervo 2002*c*; Whiting and Miles 2019). Accordingly, males engaged frequently in chases and physical aggression against other males, and only a subset of highly dominant males (23%, all morphs being equally represented) succeeded in settling in high-quality sites. Females were similarly attracted to high-quality sites (and indirectly, males) but showed much lower levels of intra-sexual competition. Later in the experiment we observed durable associations of one male with one or more females lying in close vicinity (i.e. co-perching), a natural behaviour that has been interpreted in lizards as both a form of mate-guarding (While *et al.*, 2015; Heathcote *et al.*, 2016; Olsson *et al.*, 2019), and a deterrent of male harassment (Censky, 1997; Drummond & Zaldívar-Rae, 2007). As expected, males in high-quality sites engaged in co-perching with a higher number of females, had smaller home-ranges, experienced significantly lower levels of sperm competition, and ultimately achieved higher reproductive success than males settled in low-quality sites. However, male colour morphs did not differ in their ability to establish themselves in high quality sites, win agonistic confrontations, or engage in either copulation or co-perching interactions with females. Likewise, male morphs did not differ in the number of females sired, offspring produced, or males with which they shared paternity (i.e. sperm competitors). Further, although the existence of alternative post-copulatory strategies in *P. muralis* male morphs should be specifically addressed in future studies, the observed alignment between socio-spatial dominance and paternity indirectly refutes the existence of physiological adaptations in any of the male morphs such as larger testis or ejaculates.

Our results from **Chapter IV** and especially **Chapter V** also call into question the existence of alternative strategies in *P. muralis*, although this time in the form of differential breeding investment by female morphs. In neither of these studies we found evidence of a differential solution to the trade-off between egg number and size among female morphs. Heavier females were found to lay larger clutches irrespective of their colour morph, and the negative relationship between clutch size (i.e. egg number) and offspring quality (i.e. newborn mass) showed a similar slope in orange, white, and yellow females. The absence of differences in the viability, behaviour, and survival of juveniles resulting from either assortative or disassortative pairings in **Chapter V** further argues against the hypothesis that alternative strategies, evolved by correlational selection, may have favoured the build-up of reproductive barriers among *P. muralis* colour morphs similar to those described in other polymorphic species (Gray and McKinnon 2007; Pryke and Griffith 2009*a*; Lancaster et al. 2014). These results run counter the expectation of most research on the functional significance of lizard colour polymorphism (Sinervo et al. 2007; Olsson et al. 2013; Stuart-Fox et al. 2020), and are also in contrast with some previous studies with *P. muralis* (Galeotti et al. 2007, 2013; Sacchi et al. 2009, 2017*b*; Calsbeek et al. 2010; Zajitschek et al. 2012; Scali et al. 2013; Mangiacotti et al. 2019). In trying to publish these results, we have met with resistance from both editors and reviewers which have, one way or another, judged our work based on how well the results matched the current zeitgeist rather than on the scientific soundness of our methodology. Some doubted the interest of "negative results" to their journal's readership, others expressed their inconvenience (one reviewer even calling our results "disappointing"), while others observed that, by definition, it is statistically impossible to verify the null hypothesis (i.e. absence of differences) and hence questioned the relevance of our results. Much to the contrary, we think that they constitute an important contribution to the scientific conversation on lizard colour polymorphism. Our understanding of nature depends on the weighting of evidence for and against each hypothesis, a process recently described as "cumulative science" (Csada et al. 1996; Scargle 2000; Jennions and Møller 2002). Any form of publishing bias distorting the unbiased accumulation of supporting and contrasting evidence is potentially lethal for science reliability (Scargle 2000; Thornton and Lee 2000). Unfortunately, science-makers are as susceptible to unconscious bias as any other person, and humans show a tendency to search for, interpret, favour, and recall information in a way that confirms or supports one's prior beliefs or values (confirmation bias, Nickerson 1998). In his 1605 book "The advancement of learning", Francis Bacon alludes to this particular bias by pointing out that it is human nature for "the affirmative or active to effect more than the negative or privative. So that a few times hitting, or presence, countervails oft-times failing or absence" (Petticrew 1998). We deem necessary to warn here against the establishment of a publication bias favouring "positive" or confirmatory results, as it will inevitably hinder our understanding of how nature works by giving rise to "echo chambers" where popular fit-for-all explanations may benefit doubly, first from the overrepresentation of supporting evidence in the literature, and second from the neglect of contrasting evidence (either through author self-censorship or editorial resistance; Rosenberg 2005; Pautasso 2010; Parker et al. 2016).

Despite drawing substantial attention from evolutionary biologists, the evolutionary causes and consequences of lacertid colour polymorphism are still far from being resolved. After decades of study, the available evidence casts serious doubts about the generality of the *Uta stansburiana* model, and instead paints a much more complex picture with several evolutionary processes at stake and geographic variation playing a role in explaining the vast diversity of colour polymorphism in lacertid lizards (Huyghe et al. 2007, 2009*a*; Sinervo et al. 2007; Calsbeek et al. 2010; Runemark et al. 2010; Runemark and Svensson 2012; Galeotti et al. 2013; San-Jose et al. 2014; Sacchi et al. 2017*b*; Brock et al. 2020*a*). With respect to *P. muralis*, in **Chapter IV** and **Chapter V** we contend that the disagreement between our results and previous studies may prove biologically meaningful if carefully examined. This species shows the widest distribution of its genus, and most of the evidence suggesting physiological or behavioural morph differences comes from studies of the Southern Alps lineage, which is only distantly related to the Western European lineage found in the Pyrenees (Giovannotti et al. 2010; Schulte et al. 2012; Gassert et al. 2013; Yang et al. 2018). These observations, together with the high prevalence and ancient origin of colour polymorphism in wall lizards, suggest the intriguing possibility that genes coding for the expression of alternative colour morphs may become linked to genes that influence other traits relevant to selection (i.e. physiology, behaviour, life-history, development) only at times, and hence be under selection only in some environments or in some lineages. This new perspective seems to better accommodate our current knowledge of lacertid colour polymorphism. Indeed, loss of the ancestral polymorphic condition seems to have occurred repeatedly within the genus *Podarcis,* and morph composition often shows considerable geographic variation even at relatively small scales. Therefore, we find it reasonable to investigate the possibility of spatially (and/or temporally) varying correlations between polymorphic colour expression and other phenotypic differences in *Podarcis* lizards, as well as to evaluate the relative importance of selection and genetic drift in shaping inter-populational differences in morph composition.

Although this thesis tries to remedy some of the most frequently assumed (and inadequately studied) aspects of the link between behaviour, fitness, and colour polymorphism in *P. muralis*, a host of interesting questions remain unanswered. For instance, in these studies we have focused mainly on the three "pure" colour morphs of *P. muralis*, while neglecting the two mixed-morphs exhibited by a smaller fraction of lizards in many polymorphic populations. The existence of mixed-morphs has interesting implications regarding the possible role of alternative strategies and game theory in explaining the maintenance of *P. muralis* colour polymorphism. When viewed by conspecifics, the two mixed morphs of *P. muralis* could be confused with some of the pure colour morphs, or perceived as a categorically distinct morph (which could, in turn, affect receiver behaviour in ways relevant to fitness; Teasdale et al. 2013; Yewers et al. 2016; Stuart-Fox et al. 2020). While there are clear predictions and empirical evidence for the stability of systems with two or even three morphs (Maynard-Smith 1982; Alonzo and Calsbeek 2010; Kokko et al. 2014), an evolutionary stable strategy for a five-morph system proves challenging (due to the increased number of potential interactions). Therefore, we need to devote more attention to these mixed morphs, and come up with explanations for the functional significance of *P. muralis* colour polymorphism which accommodate the existence of intermediate colorations (i.e. considering adaptive, neutral, and maladaptive hypothesis for these mixed morphs). An interesting possibility, given the apparently identical genetic basis of orange and orange-white coloration and the fact that the latter constitutes a transient stage in the ontogeny of the former (Pérez i de Lanuza et al. 2013*a*; Andrade et al. 2019), is that the expression of orange-white coloration may reflect poor developmental conditions (i.e. nutritional deficiencies) in adult lizards, and perhaps even convey information about its owner's quality in social interactions. Our results from **Chapter V** partially refute this hypothesis since we did not observe any negative effect on the viability, behaviour, growth rate, or survival of the offspring produced in orange-white matings. However, new studies should take a closer look at this question by comparing the match between condition, social behaviour, and life-long fitness in "pure" and mixed-morphs in natural populations of *P. muralis*.

6.2. Shifting our perspective on the functional significance of *P. muralis* colour polymorphism

In the wake of Sinervo's pioneer work with *Uta stansburiana*, the hypothesis that lizard colour morphs may reflect underlying alternative reproductive strategies involving morph-specific behaviour or life-history traits has come to dominate the field. Based on our results, we find it reasonable to reassess the allegedly central role of alternative reproductive strategies subject to frequency-dependent selection in explaining the maintenance of phenotypic variability in lizards. In the following paragraphs we will expand on three important reasons why we should shift our perspective.

First, there are other contexts (besides morph-specific physiology or sexual behaviour) where colour polymorphism may play a functionally relevant role. As ethologists, here we will expand on a hypothesis concerning social behaviour, but many other unrelated with behaviour can be formulated. While most researchers have assumed that ventral coloration may influence social interactions by conveying information about behavioural strategies (Sinervo et al. 2007; Mangiacotti et al. 2019; Coladonato et al. 2020), the possible role of polymorphic colour variation in individual recognition has been generally overlooked. Individual recognition requires individuals to uniquely identify their social partners based on phenotypic variation, and show an individual-specific behavioural response (Tibbetts and Dale 2007; Tibbetts et al. 2008). Most territorial lizards studied to date show evidence of a "dear enemy" effect by which competing neighbours (after some initial confrontations to establish territories) direct low-intensity aggressive behaviour toward familiar males, but fiercely attack non-resident male strangers with which they have no previous experience (Qualls and Jaeger 1991; Olsson 1994*b*; Whiting 1999; López and Martín 2002; Husak and Fox 2003; Baird 2013). Moreover, recent research on wall lizards suggests that males are able to discriminate between individual rivals of similar characteristics and familiarity solely on the base of their scent marks, remembering the spatial location of scent marks and behaving more aggressively toward males that consistently marked in the core of their experimental terrarium (Carazo et al. 2008; Font et al. 2012*a*). During the last two decades, research carried by Tibbetts and colleagues has shown how visual cues may play a role at least as important as that of chemical compounds in individual recognition. Polymorphic colour variation in facial features has been found to mediate inter-individual aggression by allowing individual recognition of familiar and unfamiliar conspecifics in *Polistes* paper wasps (Tibbetts 2002; Tibbetts et al. 2018, 2019, 2020). Further, by comparing *Polistes* species differing in the range of their colour variation, social systems, and ability for individual recognition, Sheehan and Tibbetts (2010) found support for the hypothesis that reduced aggression in social interactions may favour the evolution of distinctive phenotypes (i.e. identity signals) to facilitate efficient recognition. Although traditionally considered as mainly chemosensory (Mason and Parker 2010), wall lizards show both a sophisticated colour vision system and conspicuous colour patches (ventral colour polymorphism adding further complexity in some species), which makes it reasonable to think that visual (as well as chemical) signals might be involved in individual recognition (Olsson 1994*b*; López and Martín 2001; Husak and Fox 2003).

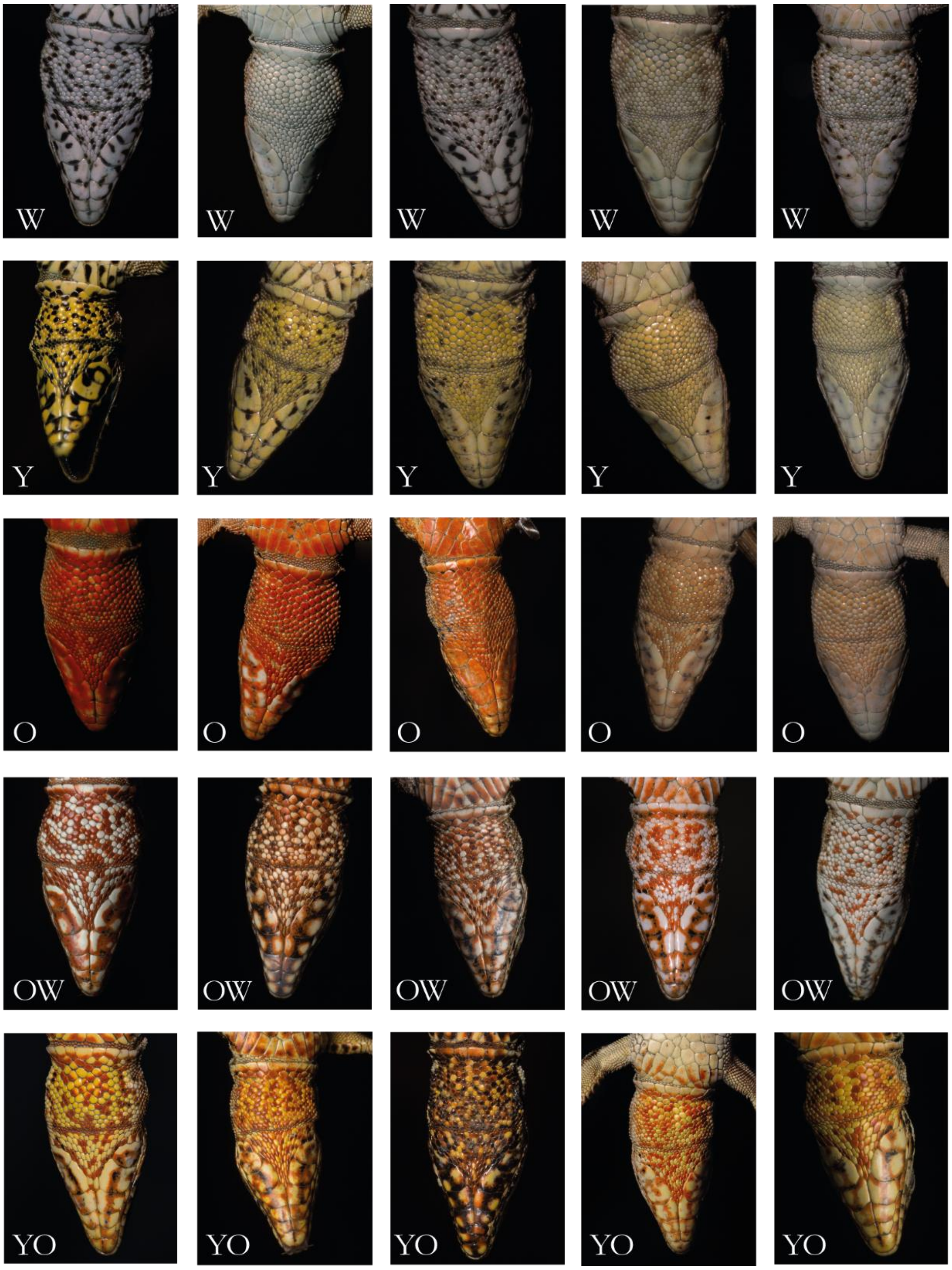

Figure 6.1. Throat colour variation within *P. muralis* colour morphs (the figure includes both males and females). Although the orange, white, and yellow ventral colours are perceived as discrete by the species visual system, there is considerable inter-individual variation (chromatic and achromatic) within each colour morph. Wall lizards possess a sophisticated visual system allowing them to discriminate subtle colour differences (that would go unnoticed to us humans), which makes it plausible to think that this variation could play a functionally relevant role in the context of individual recognition. Y = yellow, W = white, O = orange, OW = orange-white, YO = yellow-orange.

Even though chromatic variation among *P. muralis* colour morphs is discrete to the species' visual system, there is also considerable variation (both chromatic and achromatic) within each morph (**Fig. 2.6**. and **6.1**). This spectral variability (which *P. muralis* visual system is well-equipped to perceive) may play an overlooked role settling territorial disputes in *P. muralis* and other polymorphic lacertids by allowing individuals to identify their competing neighbours and adjusting their behaviour accordingly (Whiting 1999; Husak and Fox 2003; Font et al. 2012*a*). Three caveats against this hypothesis are worth emphasizing, which can be used to formulate testable predictions. First, it requires that individual recognition via visual signals may confer a selective advantage strong enough to promote the evolution of an additional individual recognition system partially redundant with the one based on chemical signals. Second, it requires inter-individual variation in ventral coloration to match the size of the typical *P. muralis* social network, so as to ensure that each of the social partners typically encountered by a lizard during the breeding season are sufficiently distinct to allow for efficient individual recognition (Tibbetts and Dale 2007; Carazo et al. 2008; Sheehan and Tibbetts 2010; Font et al. 2012*a*). Third, perceptual limitations (i.e. such as visual acuity) must be considered when formulating hypotheses about the possible function of ventral coloration (see Box 1). Recent studies on *Anolis* visual acuity suggests that fine-scale details (e.g. dotted colour patterns in male dewlaps) may be visible to conspecifics only at distances of 0.5 m or shorter (i.e. during close-range social interactions; Fleishman et al. 2017, 2020). Due to the positive relationship between acuity and eye length, smaller lizard species (such as lacertid lizards) are expected to experience even lower visual acuity. Hence, while the fine-scaled details of *P. muralis* ventral coloration could possibly convey information about individual identity when observed at close proximity, it is likely that inter-individual differences are simply not visible to conspecifics when observed at greater distances (Fleishman et al. 2020). Despite these caveats, future studies should investigate the potential role of ventral coloration in individual recognition, for example by assessing whether aggression declines towards lizards with experimentally altered ventral coloration as these novel colorations become familiar to their opponents (Tibbetts 2002; Tibbetts et al. 2008, 2017). Another promising approach would be to investigate whether the range of chromatic variation within each species of *Podarcis* lizards (with and without ventral colour polymorphism) may correlate positively with their performance in an individual recognition task (Sheehan and Tibbetts 2010). We think that much insight can be gained from testing this and other possible functions of *P. muralis* colour polymorphism in contexts different from reproductive strategies.

Second, other evolutionary forces (besides those favouring alternative reproductive strategies) can generate frequency-dependent selection and prevent any of the morphs from becoming extinct (Roulin 2004; Roulin and Bize 2007; Svensson 2017). These include some forms of apostatic selection such as the existence of a rare prey/predator advantage (Lindström et al. 2001; Olendorf et al. 2006; Halpin et al. 2008; Lawrence et al. 2019), or a rare morph social advantage (in the outcome of agonistic confrontations or mate choice trials; (Gosden and Svensson 2009; Dijkstra et al. 2010; Willink et al. 2019), which may explain the persistence of colour polymorphism in the absence of behavioural differences among the alternative colour morphs. Balancing selective regimes may also result from all morphs following the same behavioural rule (e.g. mate with the most uncommon morph, or mate with a morph different than own). Reproductive success is particularly difficult to measure in natural populations of *Podarcis* lizards. Therefore, no study to date has explicitly attempted to test frequency-dependent selection in natural populations of any *Podarcis* species showing colour polymorphism. However, we think that a mesocosm experiment such as the one in **Chapter IV** could allow researchers to investigate these questions in a semi-natural environment by introducing one morph consistently in lower frequency across the enclosures while also being able to assign parentage for all the resulting offspring.

Third, other mechanisms different from frequency-dependent selection may also result in balancing selective regimes (Roulin 2004; Roulin and Bize 2007; Wellenreuther et al. 2014; Svensson 2017). One of our aims in **Chapter V** was to investigate the existence of heterozygote advantage affecting viability selection (i.e. survival to adulthood) in the offspring of matings between *P. muralis* colour morphs, which could help explain the persistence of colour polymorphisms despite the assortative mating bias observed in natural populations (Pérez i de Lanuza et al. 2013*a*, 2016*b*). Our results do not support this hypothesis, yet, in light of new information on the genetic basis of *P. muralis* colour polymorphism (see Andrade et al 2019 and **Chapter V**), it would be interesting to re-assess the potential existence of morph combination effects (that could have gone unnoticed at the phenotypic level we examined) in future studies focused at the genotypic level. Another mechanism which could explain the maintenance of *P. muralis* colour polymorphism is spatio-temporally varying selection (see **Introduction**). Several recent studies have reported correlations between local environmental factors and morph population frequencies in polymorphic organisms (McLean and Stuart-Fox 2014; McLean et al. 2014*a,b*, 2015), incorporating climate and other abiotic components of natural selection into the study of the adaptive significance of colour polymorphism (Roulin 2004; Svensson 2017; Svensson et al. 2020). In *P. muralis*, both climatic and demographic factors (i.e. sex ratio) may affect morph composition (Pérez i de Lanuza et al. 2017, 2018*b*; Pérez i de Lanuza and Carretero 2018). Such correlations could be explained by a direct relationship between colour morph and local environmental factors (e.g. if morphs represent adaptations to different habitats; Ahnesjö and Forsman 2006; Gray and McKinnon 2007; Forsman et al. 2008; McLean and Stuart-Fox 2014). Interestingly, in eastern Pyrenees both yellow and orange-yellow lizards have been found to be geographically restricted to a subset of populations showing relatively higher annual precipitation and marked seasonality, thus suggesting that differential ecophysiology may influence morph fitness in different environments (Pérez i de Lanuza et al. 2018*b*). Alternatively, the relationship between climate and morph composition could indirectly result from the effect of climatic factors on morph-specific social interactions (e.g. non-random pairing) (McLean and Stuart-Fox 2014; McLean et al. 2015; Pérez i de Lanuza and Carretero 2018). In *P. muralis*, morph richness has been found to be associated with male-biased sex ratio in males but not in females, suggesting a relationship between the intensity of male-male competition and colour polymorphism (Pérez i de Lanuza et al. 2017). In light of these results, we think that much insight could be gained from investigating the environmental dependence of morph fitness in populations characterized by extreme morph compositions and socio-ecological contexts, as well as disentangling the relative influence of genetic drift, spatially varying selection and gene flow to geographic variation in *P. muralis* colour polymorphism.

To conclude, in this thesis we have hopefully presented compelling arguments to shift our perspective on the functional significance of lacertid colour polymorphisms, suggested promising lines of investigation for future research, and (more broadly) contributed to our growing understanding of the mechanisms maintaining intra-specific variation in natural populations at large.

6.3. Conclusions

Chapter II

Although ventral colours are often believed to act as a social signal in lizards, morph classification by its putative intended receivers (i.e. conspecifics) has rarely been assessed objectively. We used visual modelling and a discrimination experiment to show that at least the pure colour morphs of *Podarcis muralis* are likely perceived as chromatically distinct colours, and can also be behaviourally discriminated by adult conspecifics. This constitutes a necessary requisite for a potential signalling role of colour polymorphism in intraspecific interactions, yet future studies should evaluate the categorization of lizards showing mixed morphs (i.e. orange-white or yellow-orange) according to conspecifics.

Chapter III

We assessed the role of male coloration in agonistic encounters and found that both the dark melanin-based ventrolateral spots and the polymorphic ventral coloration correlate with contest outcome. We also found that winners were more aggressive when opponents showed matching proportions of their OVS covered in black, suggesting that black patches play a role in rival assessment and fighting rules. While we found a trend by orange males to lose confrontations against other morphs, we also observed relatively smaller OVS area covered in black in this morph, and hence, our results did not allow us to disentangle the relative importance of both types of coloration. These results suggest a role for male coloration in mediating intra-sexual confrontations, at least when the rivals are size-matched and meet in a neutral arena. Future studies should evaluate the interplay between morphology, colour signals, performance traits, and other known determinants of contest outcome (i.e. prior experience, residency, etc) when lizards meet under more natural conditions.

Chapter IV

The colour morphs of *P. muralis* are often thought to reflect an alternative set of underlying reproductive strategies involving differential socio-sexual behaviour and/or space use. Conducting behavioural observations both in a free-ranging population from eastern Pyrenees and in ten experimental mesocosm enclosures, we did not find evidence suggesting that the alternative colour morphs in *P. muralis* may obtain their reproductive success through differential social behaviour, space use, or reproductive investment strategies. These results contradict the commonly held hypothesis that *P. muralis* morphs reflect alternative behavioural strategies, and suggest that we should instead turn our attention to other potential functional explanations. We discuss the worth of investigating the existence of spatially (or temporally) varying correlations between polymorphic colour expression and other phenotypic differences in *Podarcis* lizards, as well as evaluating the relative importance of spatially-varying selection and genetic drift in shaping inter-populational differences in morph composition.

Chapter V

Correlational selection and heterosis are two of the mechanisms commonly thought to maintain colour polymorphism in nature. Both mechanisms have contrasting predictions regarding the effect of parental morph combination on offspring viability and fitness. We conducted controlled matings among pure colour morphs and found no overall effect of morph combination on clutch viability, offspring mass, short-term survival, and newborn foraging and antipredator behaviour. Our results refute the existence of alternative breeding investment strategies in female morphs, while also showing that inter-morph reproduction remains entirely viable among colour morphs. By keeping the juveniles in outdoor enclosures for a year, we found support for the hypothesis that orange and yellow colour expression depends on two recessive alleles located at two separate autosomal loci. This is in agreement with theoretical expectations that colour polymorphism should be governed by few loci with major phenotypic effects. Lastly, we show that juvenile ventral coloration reflects ultraviolet light and is likely perceived by conspecifics as a chromatically distinct colour different from any of the morph colours expressed by adult lizards.

Chapter VII

ROMÁN.- "Inventen, pues, ellos y nosotros nos aprovecharemos de sus invenciones. Pues confío y espero en que estarás convencido, como yo lo estoy, de que la luz eléctrica alumbra aquí tan bien como allí donde se inventó."

SABINO.- "Acaso mejor."

·Miguel de Unamuno, El pórtico del templo (1906)·

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Chapter VII:

Resumen en castellano

7.1. Introducción

7.1.1. El enigma del polimorfismo y su estabilidad a lo largo del tiempo

Uno de los principales objetivos de la biología evolutiva es entender los procesos evolutivos implicados en el origen y el mantenimiento de la variabilidad fenotípica. Muchas especies filogenéticamente alejadas presentan dos o más formas fenotípicas discretas (i.e. morfos), cuya expresión es genética (no varía a lo largo de la vida del animal adulto ni depende del ambiente o la condición física), y que parecen mantenerse en una misma población sin desaparecer aunque sus frecuencias relativas puedan oscilar a lo largo del tiempo (Ford 1945; Roulin 2004; Gray and McKinnon 2007). Cuando estas formas fenotípicas difieren en su coloración corporal reciben el nombre de morfos de color. Las especies polimórficas suponen un enigma particularmente interesante para la biología evolutiva. En el supuesto de que uno de los morfos tuviese una mayor eficacia biológica, esperaríamos que acabase fijándose en la población, conduciendo a la extinción del resto de morfos y a la desaparición del polimorfismo. Aunque los morfos no difiriesen en su eficacia biológica, el polimorfismo podría acabar perdiéndose debido a procesos estocásticos (i.e. deriva genética), particularmente en poblaciones pequeñas. Por último, el polimorfismo puede evolucionar en una especie si existe un régimen de selección disruptiva por el que ciertos fenotipos extremos obtienen una ventaja selectiva respecto a otras formas intermedias (Gray and McKinnon 2007; Wellenreuther et al. 2014; Svensson 2017). En este caso, sin embargo, es esperable que la selección en contra de estas formas intermedias resulte en un aislamiento reproductivo entre los morfos, y que el polimorfismo constituya un preludio más o menos breve a la especiación. Por tanto, el mantenimiento del polimorfismo a largo plazo requiere de mecanismos de selección estabilizadora (i.e. *balancing selection*) que contrarresten el efecto erosivo de la deriva génica, la selección y la posible aparición de barreras reproductivas entre morfos (Sinervo and Svensson 2002; Gray and McKinnon 2007; Mckinnon and Pierotti 2010).

7.1.2. Mecanismos para el mantenimiento del polimorfismo en la naturaleza

El valor adaptativo del polimorfismo está frecuentemente asociado al mecanismo de selección responsable de su mantenimiento en el tiempo (Roulin 2004). Los morfos suelen diferir en otros rasgos (i.e. comportamiento, fisiología) además de su coloración. Se considera que estas covariaciones han surgido mediante procesos de selección disruptiva que favorecen distintas combinaciones óptimas de rasgos fenotípicos (i.e. estrategias alternativas; Sinervo and Svensson 2002*b*; Mckinnon and Pierotti 2010). Un buen ejemplo de este proceso lo encontramos en el caso de la polilla *Biston betularia*, en la que podemos encontrar dos morfos de colores distintos: uno de color claro y otro de color oscuro. Durante la primera parte del último siglo, el oscurecimiento de los troncos de los árboles en ciertas áreas industrializadas confirió una ventaja selectiva a las polillas de morfo oscuro (que resultaban más difíciles de detectar por parte de los depredadores), lo que provocó un aumento en su frecuencia respecto al morfo claro (Cook et al. 2012). Este caso ilustra cómo la evolución puede favorecer correlaciones genéticas entre el polimorfismo de color y otros rasgos (e.g. comportamiento), ya que las polillas que elijan posarse en superficies sobre las que sean crípticas perecerán en menor medida que las que elijan posarse sobre superficies que contrasten con su propia coloración (Kettlewell 1955; Majerus 1998, 2005). Además de en un contexto de cripsis, las coloraciones polimórficas pueden participar en otros contextos relevantes para la selección, y desempeñar por tanto una función clave para la eficacia de distintos tipos de estrategias alternativas (e.g. mimetismo, aposematismo, trampas sensoriales, termorregulación, señalización; Galeotti et al. 2003; Roulin 2004; Mckinnon and Pierotti 2010). Sin embargo, la asociación entre las distintas estrategias alternativas y los morfos de color también podría producirse por la existencia de interacciones genéticas entre los genes responsables de la expresión del polimorfismo y de otros rasgos relevantes para la selección (e.g. ligamiento, pleiotropía). En este caso, los morfos constituirían un subproducto no adaptativo de la selección sobre estos otros rasgos, acompañando a las distintas estrategias pero sin cumplir ninguna función esencial para su eficacia (Roulin 2004; Mckinnon and Pierotti 2010; Svensson 2017). Este tipo de interacciones genéticas podrían estar detrás de la frecuente asociación entre polimorfismos de color y estrategias vitales caracterizadas por diferencias morfológicas, fisiológicas y de comportamiento. Aunque la mayor parte de los estudios se centran en las estrategias alternativas de reproducción, las ventajas conferidas por cada estrategia pueden manifestarse en otros aspectos de la vida de los organismos, como la evitación de los depredadores, el forrajeo o el estado inmunológico.

Para que un polimorfismo de color sea estable en el tiempo no basta con que cada morfo adopte una estrategia alternativa distinta. De hecho, la evolución de estrategias alternativas en cada morfo puede levantar barreras reproductivas entre estos al penalizar la producción de descendencia con rasgos intermedios, abriendo el camino a un proceso de diversificación por el que cada morfo daría lugar a una nueva especie. Numerosos ejemplos en la literatura demuestran cómo la selección sobre distintas combinaciones óptimas de rasgos (selección correlativa) favorece el apareamiento concordante y la aparición de incompatibilidades genéticas entre los morfos (Roulin 2004; Gray and McKinnon 2007; Pryke and Griffith 2009*a*; Lancaster et al. 2014). Por tanto, además de las estrategias, es necesario que exista un régimen de selección por el que las distintas estrategias obtengan el mismo éxito reproductivo en promedio (Shuster and Wade 2003). Existen cuatro mecanismos de selección distintos por los que esta situación puede alcanzarse: i) cuando la eficacia de cada estrategia depende de su frecuencia relativa en la población (selección dependiente de frecuencia), ii) cuando la eficacia de cada estrategia depende del número absoluto de individuos en la población (selección dependiente de densidad), iii) cuando las interacciones competitivas entre las distintas estrategias son no transitivas (es decir, semejantes a un juego de piedra, papel y tijera), y iv) cuando la heterogeneidad ambiental provoca que la selección sobre las distintas estrategias varíe en el espacio o en el tiempo (selección fluctuante) (Brockmann 2001; Galeotti et al. 2003; Roulin 2004; Roulin and Bize 2007; Taborsky et al. 2008; Taborsky and Brockmann 2010; Wellenreuther et al. 2014; Svensson 2017). Estos cuatro mecanismos a menudo actúan junto a otros procesos (e.g. dinámicas migratorias de fuente-sumidero, preferencias de apareamiento sesgadas por el morfo, incompatibilidades genéticas) haciendo que el mantenimiento del polimorfismo responda a escenarios evolutivos muy distintos en cada una de las distintas especies examinadas hasta la fecha.

Además de la existencia de estrategias alternativas, existen otros mecanismos capaces de explicar el mantenimiento del polimorfismo en poblaciones naturales. Por ejemplo, dos o más morfos alternativos pueden coexistir si los individuos genéticamente intermedios (i.e. heterocigotos) obtienen una ventaja en términos de eficacia biológica (Gratten et al. 2008, 2010; Johnston et al. 2013). Al tener un menor de nivel de endogamia que los individuos homocigotos, los heterocigotos evitan la expresión de un mayor número de rasgos deletéreos (i.e. "ventaja del heterocigoto"), lo que les capacita para lidiar con un mayor rango de ambientes. Además, si esta ventaja concierne exclusivamente a la probabilidad de que un individuo alcance la edad de reproducción, el polimorfismo podrá mantenerse en el tiempo aunque en la especie exista preferencia por el apareamiento concordante (Roulin 2004; Roulin and Bize 2007).

7.1.3. El polimorfismo de color en lagartos

Varias especies de lagartos alejadas tanto filogenética como geográficamente presentan polimorfismos de color y constituyen, por tanto, un grupo adecuado para poner a prueba hipótesis acerca de la evolución y función de los polimorfismos. En la mayor parte de las familias en las que se han descrito morfos de color, las coloraciones alternativas se expresan en la superficie ventral del animal, particularmente en la garganta (Stuart-Fox et al. 2020). Parece haber también una notable convergencia en cuanto a los colores exhibidos. La mayor parte de las especies muestran combinaciones de los mismos tres fenotipos: un morfo naranja o rojo, otro morfo amarillo y un morfo blanco o azulado. En algunas especies también se dan coloraciones intermedias que pueden combinar dos de estos tres colores (Huyghe et al. 2007; Corl et al. 2010; Paterson and Blouin-Demers 2018; Stuart-Fox et al. 2020). Las coloraciones naranjas y amarillas están basadas en la acumulación de pigmentos (pteridinas y carotenoides) en las capas superiores de la dermis, pero la importancia relativa de cada tipo de pigmento parece variar entre especies (San-Jose et al. 2013; Haisten et al. 2015; Andrade et al. 2019; Stuart-Fox et al. 2020). Las coloraciones blancas o azuladas son, probablemente, de naturaleza estructural, y sus propiedades espectrales dependen del ordenamiento de los cristales de guanina y su efecto sobre la dispersión de la luz incidente en la dermis (Bagnara et al. 2007; Umbers 2013). Aunque la arquitectura genética del polimorfismo se ha estudiado solo en pocas especies, la evidencia sugiere una base genética simple, con uno o dos loci autosómicos y tres o cuatro alelos involucrados en la expresión de las distintas coloraciones alternativas (Rankin et al. 2016; McLean et al. 2017; Stuart-Fox et al. 2020).

La investigación acerca del significado funcional del polimorfismo en lagartos se ha visto notablemente influida por los primeros trabajos realizados durante los años 80 y 90 en la lagartija de árbol norteña (*Urosaurus ornatus*; Hover 1985; Thompson and Moore 1991; Carpenter 1995) y la lagartija de mancha lateral norteña (*Uta stansburiana*; Sinervo and Lively 1996; Sinervo et al. 2000; Zamudio and Sinervo 2000; Calsbeek and Sinervo 2002). En ambas especies, la coloración ventral parece afectar de forma crítica a las interacciones sociales (además de estar asociada con otras diferencias morfológicas, fisiológicas y de comportamiento) lo que sugiere la existencia de estrategias alternativas de reproducción (Sinervo and Lively 1996; Sinervo et al. 2000*a*; Lattanzio and Miles 2016; Taylor and Lattanzio 2016; Paterson and Blouin-Demers 2018). En el caso de *U. stansburiana*, el seguimiento longitudinal de una población concreta en "Los Baños" (California, USA) permitió al equipo de investigación liderado por Barry Sinervo explicar el mantenimiento de tres morfos distintos en esta especie como un juego de piedra, papel y tijera entre tres estrategias alternativas de reproducción en machos, cada una asociadas genéticamente a un morfo distinto (Sinervo and Lively 1996). Según las observaciones de comportamiento publicadas por este grupo, los machos de coloración naranja son muy agresivos con otros machos y a menudo desplazan a los machos azules para usurpar sus territorios. Aunque esta estrategia les permite aparearse con un número elevado de hembras, los machos naranja acaban patrullando espacios domésticos demasiado grandes para ser defendidos de forma efectiva, y a menudo pierden paternidad en favor de los machos de morfo amarillo. Estos últimos supuestamente imitan tanto el comportamiento como la coloración de las hembras receptivas y obtienen su éxito reproductivo de manera oportunista, realizando incursiones en los territorios de otros machos para copular con las hembras disponibles (pero sin defender un territorio propio). A su vez, los machos azules compensan su desventaja competitiva frente a los machos naranjas jugando una estrategia de guarda de la pareja que les protege en gran medida de la estrategia oportunista de los machos amarillos (Sinervo and Lively 1996; Sinervo et al. 2000*a*, 2006*b*; Zamudio and Sinervo 2000, 2003). De esta forma el polimorfismo se mantiene mediante un juego cíclico de piedra, papel y tijera en la que cada estrategia, cuando domina en la población, es vulnerable a la invasión por parte de otra de las estrategias. En machos, esta dinámica da lugar a oscilaciones periódicas de unos 5-6 años en la frecuencia relativa de cada uno de los morfos (Sinervo and Lively 1996; Alonzo and Sinervo 2001; Sinervo et al. 2006*b*, 2007; Alonzo and Calsbeek 2010). Además, estos ciclos se combinan con oscilaciones periódicas (de unos dos años) en las frecuencias relativas de los morfos en hembras, donde la coloración ventral se asocia a dos estrategias distintas de inversión reproductiva (i.e. las hembras naranjas invierten en la calidad de la descendencia mientras que las amarillas priorizan la cantidad) (Sinervo et al. 2000*b*, 2010; Sinervo 2001; Sinervo and Zamudio 2001). A la luz de estos resultados, Zamudio y Sinervo indicaron que los lagartos presentaban una serie de características ecológicas que favorecían la evolución de estrategias alternativas de reproducción con base genética, y que era esperable que en futuros estudios se detectasen escenarios evolutivos semejantes a los descritos en *U. stansburiana* en otras especies de lagartos polimórficos (Zamudio and Sinervo 2003; Sinervo and Calsbeek 2006). Por varias razones, señalaron a las lagartijas del género *Podarcis* como un grupo particularmente prometedor (Sinervo et al. 2007; Calsbeek et al. 2010).

7.1.4. Especie de estudio: la lagartija roquera (*Podarcis muralis*, Laurenti 1768)

La especie del género *Podarcis* cuyo polimorfismo de color ha sido mejor estudiado es la lagartija roquera (*Podarcis muralis*). Esta especie destaca por ser la más ampliamente distribuida del género, con poblaciones desde el Norte de la Península Ibérica hasta el Mar Negro, y varios linajes en las islas y penínsulas mediterráneas (Arnold et al. 2002; Salvi et al. 2013). Esta especie presenta la coloración típica de muchos lacértidos: un dorso parduzco relativamente críptico, una coloración ventral polimórfica, y una línea de manchas azul-UV en la hilera de escamas ventrales externas (Pérez i de Lanuza et al. 2013*b*). Por su conspicuidad y su posición corporal (adecuada para controlar su visibilidad mediante la postura), la coloración ventral y las manchas azul-UV podrían funcionar como señales cromáticas (Font et al. 2009; Marshall and Stevens 2014; Pérez i de Lanuza et al. 2014; Martin et al. 2015*c*). En muchas poblaciones se distinguen dos coloraciones ventrales alternativas (morfos): blanco (w=white) o naranja (o=orange), y en algunas poblaciones aparece también un tercer morfo amarillo (y=yellow). Además, pueden encontrarse, normalmente en menor frecuencia, morfos de coloración intermedia, combinando manchas de dos colores: amarillo-naranja y blanco-naranja (Calsbeek et al. 2010; Pérez i de Lanuza et al. 2013*a*, 2019). Sin embargo, como en muchos otros lagartos polimórficos, la clasificación de los morfos se ha realizado de acuerdo a la visión humana, y aún no se ha validado de acuerdo al sistema visual de la especie (véase Teasdale et al. 2013). La coloración ventral de las lagartijas al nacer es blanquecina o grisácea para el ojo humano (aunque nunca se ha caracterizado con métodos objetivos de medición del color), y la coloración adulta se fija al alcanzar la madurez sexual (uno o dos años más tarde dependiendo de la población; Pérez i de Lanuza et al. 2013*a*). Aunque aún se sabe poco de la base genética de este polimorfismo, un estudio genómico reciente detectó relación entre la presencia de dos alelos recesivos en dos loci autosómicos distintos (implicados en el metabolismo de pteridinas y carotenoides), y la expresión de coloración naranja y amarilla (Andrade et al. 2019). Este mismo estudio confirmó que la coloración ventral es pigmentaria al revelar que los lagartos amarillos y naranjas difieren principalmente en la proporción de carotenoides y pteridinas integumentarias (y no en los pigmentos implicados). El cruce de animales de distinto morfo y su mantenimiento en cautividad hasta la expresión de la coloración adulta ayudaría a confirmar el mecanismo de herencia y a obtener más información acerca de la ontogenia del polimorfismo del color ventral en esta especie.

La hipótesis de que los distintos morfos de color de *P. muralis* reflejan estrategias alternativas de reproducción semejantes a las descritas en *U. stansburiana* ha dominado la literatura hasta hoy (Galeotti et al. 2007, 2013; Scali et al. 2013; Sacchi et al. 2017*b*; Coladonato et al. 2020; Mangiacotti et al. 2020). En este sentido, si bien es cierto que se han detectado diferencias entre los morfos en distintos rasgos (sobre todo fisiológicos), el patrón emergente es incoherente entre los distintos estudios, y dista mucho de aportar evidencia concluyente acerca de la existencia de estrategias alternativas bien definidas en los distintos morfos (Scali et al. 2013; Sacchi et al. 2017*b*, 2018). La realidad es que la existencia de estrategias alternativas en esta especie a menudo se ha asumido a partir de evidencias indirectas, mientras que aspectos clave para el éxito reproductivo de la especie (i.e. comportamiento social, sexual y espacial) han sido ignorados o estudiados de forma inapropiada (Calsbeek et al. 2010; Coladonato et al. 2020; Mangiacotti et al. 2020). En esta tesis tratamos de paliar estas carencias y descifrar el valor adaptativo del polimorfismo de color en la lagartija roquera mediante el uso de herramientas procedentes de la ecología sensorial, la teoría sobre selección sexual y la etología.

7.2. Objetivos

Esta tesis se centra en aspectos que han sido ignorados o inadecuadamente estudiados en anteriores trabajos que exploraban la relación entre comportamiento, eficacia biológica y polimorfismo de color en la lagartija roquera (*Podarcis muralis*), una especie de lacértido donde suele asumirse la existencia de estrategias alternativas de reproducción asociadas a cada morfo. En concreto, con esta tesis pretendemos:

a) Mejorar la consistencia con la que clasificamos los morfos explorando la discriminabilidad de la variación cromática para el sistema visual de la especie mediante modelos visuales y un experimento de comportamiento (**Capítulo II**).

b) Explorar la relación entre la coloración (incluyendo la coloración ventral polimórfica), el comportamiento agonístico y la habilidad competitiva en enfrentamientos por parejas entre machos (**Capítulo III**).

c) Examinar la existencia de estrategias alternativas de reproducción que impliquen diferencias en el comportamiento social, sexual o espacial de los machos, así como la existencia de estrategias alternativas de inversión reproductiva (r/K) en hembras (**Capítulos IV y V**).

d) Evaluar si la combinación parental de morfos afecta a la viabilidad de la descendencia, de acuerdo a lo esperado bajo selección correlativa o ventaja del heterocigoto (**Capítulo V**).

e) Incrementar nuestro conocimiento sobre la herencia y la ontogenia del polimorfismo de color (**Capítulo V**).

7.3. Capítulo II: Discriminación de los morfos de color en la lagartija roquera por el sistema visual de la especie

7.3.1. Introducción

Varios trabajos sobre el polimorfismo de color en *P. muralis* asumen una función comunicativa para la coloración ventral en el contexto de las interacciones sociales entre individuos de la misma especie (Sacchi et al. 2017*a*; Coladonato et al. 2020; Mangiacotti et al. 2020). Un requisito indispensable para la validación de esta hipótesis es la obtención de evidencia empírica de que las lagartijas perciben los distintos morfos de color como categóricamente distintos y pueden discriminarlos (Endler 1990; Maynard-Smith and Harper 2003; Tibbetts et al. 2017). Sin embargo, ningún estudio ha examinado esta cuestión en *P. muralis* de acuerdo al sistema visual de la especie (Teasdale et al. 2013).

7.3.2. Métodos y resultados

En este capítulo, utilizamos tanto modelos visuales como un experimento de comportamiento para evaluar la discriminabilidad de los tres colores básicos que conforman el polimorfismo de color en *P. muralis*. Por una parte, calculamos las distancias cromáticas en el espacio de color perceptual de *P. muralis* entre las distintas combinaciones de morfos, y encontramos que probablemente las lagartijas perciben los tres colores como cromáticamente distintos, y que la distancia perceptual es mayor entre la coloración naranja y la blanca que entre la naranja y la amarilla. Por otra parte, utilizamos un experimento de aprendizaje para dilucidar si *P. muralis* es capaz de discriminar estímulos de color diseñados para imitar los morfos de color de la especie. Entrenamos a 20 lagartijas adultas para que se alimentasen de un dispositivo de madera provisto de cuatro pocillos, cada uno de ellos cubierto con una tapa y marcados con una pegatina de un color distinto (naranja, amarillo, blanco y un control acromático con la misma luminancia que la del color de entrenamiento). Para la mitad de las lagartijas, la presa se introdujo siempre en el pocillo marcado con el color amarillo, mientras que para la otra mitad la presa siempre estaba en el pocillo marcado con la pegatina naranja. Tras un promedio de seis ensayos las lagartijas mostraron evidencias de aprendizaje, reduciendo significativamente el tiempo transcurrido hasta hallar el alimento (i.e. latencia) y explorando menos pocillos antes de dar con el correcto que los esperados si hubiesen elegido al azar.

7.3.3. Discusión

Nuestros resultados confirman que las lagartijas roqueras pueden discriminar entre estímulos que imiten los tres morfos de color puros presentes en la especie. La inclusión de un control acromático nos permite concluir que la discriminación se basa en las propiedades cromáticas de los estímulos, y no en diferencias de luminancia (Kelber et al. 2003). Además, el hecho de que la distancia cromática (en el espacio perceptual de la especie) varíe significativamente dependiendo de la combinación de morfos puede tener implicaciones adicionales sobre la detectabilidad y eficacia de la señal, y por tanto plantea cuestiones interesantes acerca del diseño de esta posible señal. Aunque estas observaciones constituyen un primer paso imprescindible para poner a prueba la hipótesis de que los morfos de color puedan actuar como señales visuales en un contexto de comunicación intra-específica, aún quedan varias cuestiones que deberían ser evaluadas en futuros trabajos. Por ejemplo, es posible que pese a ser capaces de discriminar los morfos de color, estos no tengan ningún efecto sobre el comportamiento de los conespecíficos. Además, este estudio se ha centrado en la coloración exhibida por los morfos puros, pero ignoramos si las lagartijas roqueras perciben a los morfos mixtos como una categoría aparte, o por el contrario los incluyen dentro de uno de los morfos puros.

7.4. Capítulo III: El papel de la coloración en combates entre machos de lagartija roquera

7.4.1. Introducción

Los machos adultos de lagartija roquera muestran un dorso críptico (que posiblemente evolucionó para eludir la detección por parte de los depredadores) y unas manchas de color bastante más conspicuas en su superficie ventral y ventrolateral (Pérez i de Lanuza and Font 2015). Además del polimorfismo de color ventral, en las escamas ventrales externas los machos muestran dos tipos de manchas: unas manchas oscuras basadas en melanina y otras que presentan su pico de reflectancia en el ultravioleta cercano (azules para el ojo humano; Pérez i de Lanuza et al. 2014). Durante las interacciones agonísticas, los machos exhiben una serie de posturas que favorecen la visibilidad de estas manchas de color, sugiriendo una posible función comunicativa en el contexto de la competencia intrasexual (Kitzler 1941; in Den Bosch and Zandee 2001; Baird 2013).

7.4.2. Métodos y resultados

Para evaluar la importancia relativa de cada una de estas manchas sobre la intensidad y el resultado de los combates intrasexuales, capturamos 60 machos de lagartija roquera en distintas poblaciones del Pirineo (20 de cada morfo) y diseñamos un torneo anidado en el que cada lagartija se enfrentó a rivales de tamaño semejante en una arena neutra. En cada combate, registramos el comportamiento de cada oponente y determinamos un vencedor solo si uno de los machos dejó de mostrar comportamientos agresivos y únicamente huía de su rival. Aunque no encontramos relación entre el tamaño y las propiedades espectrales de las manchas azul-UV y la habilidad competitiva (calculada mediante un modelo Bradley-Terry; Stuart-Fox et al. 2006; Firth and Turner 2012), las otras dos manchas de color examinadas sí que parecieron afectar al resultado de estos conflictos. Los machos de morfo naranja y aquellos cuyas manchas oscuras cubrían un área proporcionalmente menor que las de sus rivales mostraron una tendencia a resultar perdedores en enfrentamientos contra otros machos (u otros morfos).

7.4.3. Discusión

Entre las manchas de color examinadas, la coloración oscura destacó por presentar la relación más fuerte y estrecha con la habilidad competitiva de los machos y es, por tanto, la mejor posicionada para funcionar como una señal agonística. En particular, la observación de que la intensidad de los combates fue mayor cuando menor fue la diferencia en el tamaño de las manchas oscuras de los oponentes es especialmente notable, puesto que sugiere que los machos usan la información contenida en la señal para ajustar su inversión en el combate (Enquist and Leimar 1983; Arnott and Elwood 2009). La ausencia de relación entre las manchas azul-UV y la habilidad competitiva resulta, en cierta medida, sorprendente, dado que existen convincentes razones científicas para pensar que estas manchas podrían desempeñas un papel en el contexto de la competencia entre machos (Marshall and Stevens 2014; Pérez i de Lanuza et al. 2014; Martin et al. 2015*c*). Es posible que su efecto sea más importante en fases tempranas de los enfrentamientos, o que su presencia caracterice a los machos sexualmente maduros, pero que ni su tamaño ni sus propiedades espectrales informen acerca de la habilidad competitiva de su portador. La existencia de una desventaja competitiva en los machos de morfo naranja podría plantear interesantes cuestiones respecto a la existencia de una estrategia alternativa en este morfo (quizás una estrategia no-territorial que obtenga su éxito reproductivo de manera oportunista; Waltz 1982; Taborsky 2001; Shuster and Wade 2003). Sin embargo, puesto que los machos naranjas también mostraron manchas oscuras más pequeñas, nuestros datos no nos permiten dilucidar la importancia relativa de ambos tipos de manchas. Concluimos que para seguir investigando la posible existencia de estrategias alternativas en los morfos de color sería necesario examinar otros aspectos relacionados con la competencia intrasexual como la territorialidad, el comportamiento social o la competencia espermática (Healey et al. 2007; Olsson et al. 2009).

7.5. Capítulo IV: Comportamiento socio-sexual y uso del espacio en los morfos de color de la lagartija roquera

7.5.1. Introducción

La hipótesis de que el polimorfismo de color refleja estrategias alternativas de reproducción semejantes a las descritas en *U. stansburiana* subyace a la mayoría de los estudios sobre el valor adaptativo del polimorfismo en lagartos (Zamudio and Sinervo 2003; Olsson et al. 2013; Stuart-Fox et al. 2020). En este sentido, las lagartijas del género *Podarcis* (y especialmente la lagartija roquera, *Podarcis muralis*) se consideran un grupo particularmente prometedor. En primer lugar, como mínimo 13 de las 24 especies reconocidas en el género presentan polimorfismos de color en la superficie ventral del cuerpo, pudiendo distinguirse hasta tres morfos "puros" (naranja, blanco, amarillo) y uno o más morfos mixtos (Arnold et al. 2002; Huyghe et al. 2007; Sacchi et al. 2007; Runemark et al. 2010; Andrade et al. 2019; Pérez i de Lanuza et al. 2019; Brock et al. 2020*b*; Jamie and Meier 2020). En segundo lugar, muchas de estas especies son filopátricas y ocupan hábitats donde los recursos importantes para la reproducción (e.g. refugios, zonas de asoleamiento) son fácilmente monopolizables (Strijbosch et al. 1980; Barbault and Mou 1988; Edsman 1990, 2001; Carretero 2007; Sinervo et al. 2007; Calsbeek et al. 2010; Font et al. 2012*a*). Por lo general, las hembras parecen verse atraídas por la calidad o familiaridad del hábitat, y no por ninguna característica particular de los machos (Edsman 1990, 2001; Font et al. 2012*a*). Por tanto, los machos experimentan una intensa competencia intrasexual en el contexto de disputas territoriales por acceder y defender estos recursos (y a las hembras asociadas) de otros machos sexualmente maduros (Edsman 1990; Font et al. 2012*a*). A pesar de esto, las hembras suelen aparearse con más de un macho por ciclo reproductivo de forma que la mayoría de las puestas muestran paternidad múltiple, lo que extiende el campo de acción de la selección sexual para los machos más allá de la cópula en forma de competencia espermática (Oppliger et al. 2007; Uller and Olsson 2008; Heathcote et al. 2016). Todos estos factores aumentan la variabilidad en el éxito reproductivo de los machos y reducen la posibilidad de que un macho pueda experimentar distintos ambientes durante su vida, lo que favorece la aparición de estrategias alternativas de reproducción con base genética en este sexo (Brockmann 2001; Shuster and Wade 2003; Zamudio and Sinervo 2003). Dada la dificultad de realizar registros de comportamiento y determinar el éxito reproductivo individual en poblaciones naturales, la mayor parte de los estudios centrados en *P. muralis* han priorizado la detección de diferencias fisiológicas entre los morfos (e.g. estado inmune, respuesta al estrés, niveles hormonales; Galeotti et al. 2007; Calsbeek et al. 2010; Sacchi et al. 2017*b*) asumiendo de forma indirecta la existencia de diferencias clave en el comportamiento socio-sexual o espacial compatibles con la presencia de estrategias alternativas de reproducción en machos.

7.5.2. Métodos y resultados

Para estudiar la posible existencia de estrategias alternativas de reproducción en poblaciones polimórficas de *P. muralis*, en este capítulo analizamos el comportamiento social, el uso del espacio y el éxito reproductivo de los morfos de color puros en una población natural del Pirineo oriental y en diez cercados experimentales (mesocosmos). La población natural estudiada habita una serie de muros de piedra (hoy abandonados y parcialmente cubiertos de vegetación) situados en una pendiente orientada hacia el este en la localidad francesa de Angoustrine-Villeneuve-des-Escaldes. La abundante población de lagartija roquera presente en estos muros nos permitió reunir un considerable volumen de datos sobre el comportamiento espacial de los lagartos durante las primaveras de cinco años consecutivos (2006-2010). Para los cercados experimentales utilizamos las instalaciones de la *Station d'Ecologie Theorétique et Experiméntale* (Moulis, Francia). Cada cercado consistía en un área (48 m^2) de vegetación herbácea natural en la que instalamos seis pallets de madera, rocas, troncos y piedras para crear un hábitat atractivo para los lagartos. Colocamos los seis pallets en dos hileras y creamos dos tipos de hábitats (de alta y baja calidad) variando la cantidad de ladrillos, troncos y rocas apilados sobre cada tipo de pallet. Registramos el comportamiento de los lagartos durante las interacciones sociales, y cuando las hembras mostraron síntomas de gravidez, recapturamos a todas las lagartijas e incubamos las puestas para determinar la paternidad mediante análisis genéticos de microsatélites. En la población natural, no encontramos un efecto del morfo sobre la movilidad inter-anual, el tamaño del espacio doméstico o el solapamiento entre machos y hembras. De forma similar, el principal determinante del éxito reproductivo de los machos en los cercados experimentales fue la dominancia social, sin que el morfo jugase ningún papel relevante. Mientras que la mayor parte de las hembras se establecieron en los hábitats de alta calidad, solo un tercio de los machos (aquellos que mostraron mayor dominancia social) lograron establecer sus territorios alrededor de estos hábitats y desplazar al resto de machos a los pallets de baja calidad. Estos machos ganaron más enfrentamientos contra otros machos, presentaron espacios domésticos más pequeños, se aparearon y guardaron a un mayor número de hembras y experimentaron una competencia espermática menos intensa que los machos establecidos en pallets de baja calidad. Sin embargo, los morfos de color no difirieron en su habilidad para establecerse en pallets de alta calidad, imponerse en enfrentamientos agonísticos o mantener interacciones reproductivas con hembras. En consecuencia, los machos de distinto morfo se aparearon con un número similar de hembras, dejaron un número similar de descendientes y compartieron paternidad con un número similar de machos.

7.5.3. Discusión

Los resultados de este capítulo constituyen el análisis más detallado hasta la fecha de las posibles diferencias de comportamiento entre los morfos de *P. muralis* y aportan evidencia en contra de la hipótesis de que distintas estrategias alternativas de reproducción subyacen al polimorfismo de color en esta especie. En las especies con sistemas de apareamientos basadas en la defensa de recursos (como la lagartija roquera), es esperable que la existencia de estrategias alternativas de reproducción se manifieste en forma de diferencias en la actividad, dominancia, territorialidad o comportamiento social entre los morfos (Sinervo et al. 2000*a*; Zamudio and Sinervo 2000, 2003; Taborsky 2001; Taborsky and Brockmann 2010). Nosotros no encontramos evidencia de estas diferencias. Además, la correspondencia entre la dominancia espacial, el comportamiento social y el éxito reproductivo de los machos sugiere que ninguno de los morfos presenta adaptaciones fisiológicas que pudiesen otorgarle una ventaja en el contexto de la selección sexual post-cópula (e.g. mayor volumen de eyaculado). A la luz de estos resultados, y teniendo en cuenta el considerable desacuerdo en la literatura acerca del significado funcional del polimorfismo de color en lacértidos (Huyghe et al. 2007, 2009*a*; Calsbeek et al. 2010; Runemark et al. 2010; San-Jose et al. 2014; Brock et al. 2020*b*), proponemos revisar el papel central que tradicionalmente se ha otorgado a las estrategias alternativas de reproducción en el mantenimiento del polimorfismo de color en este grupo, y ampliar nuestra perspectiva para incorporar otros procesos evolutivos escasamente estudiados. Por ejemplo, sugerimos considerar la posibilidad de que la correlación entre el polimorfismo de color y otros rasgos fenotípicos haya ocurrido solo bajo ciertas condiciones o en ciertos ambientes (Roulin 2004; Mckinnon and Pierotti 2010), produciendo una discontinuidad que puede ayudarnos a explicar la pérdida del polimorfismo en varias especies de lagartijas del género *Podarcis,* así como la considerable variabilidad geográfica en el número y la frecuencia relativa de los distintos morfos (Runemark et al. 2010; Pérez i de Lanuza et al. 2018*b*; Brock et al. 2020*b*).

7.6. Capítulo V: Viabilidad, comportamiento y expresión del color en la descendencia de cruces entre los distintos morfos de color presentes en la lagartija roquera

7.6.1. Introducción

Aunque las estrategias alternativas de reproducción han sido descritas con mayor frecuencia en machos, este sesgo podría deberse simplemente a que entendemos mejor los factores que explican el éxito reproductivo en machos que en hembras (Shuster and Wade 2003; Neff and Svensson 2013; Lindsay et al. 2019). Sin embargo, las hembras también varían en su eficacia biológica, y varios estudios en lagartos sugieren la existencia de estrategias alternativas de inversión reproductiva en hembras por las que un morfo prioriza la calidad de los descendientes mientras otro prioriza la cantidad (Sinervo et al. 2000*b*; Sinervo and Zamudio 2001; Svensson et al. 2001*b*). La evolución de estrategias alternativas a menudo levanta barreras reproductivas entre los morfos (e.g. apareamiento concordante, incompatibilidades genéticas) con el fin de transmitir a la descendencia las combinaciones de rasgos óptimas y evitar la producción de descendencia con combinaciones de rasgos desventajosas (Sinervo and Svensson 2002; Pryke and Griffith 2009*a*; Mckinnon and Pierotti 2010). Por otra parte, la existencia de una "ventaja del heterocigoto" (uno de las mecanismos capaces de explicar la persistencia del polimorfismo de color en poblaciones naturales), debería tener el efecto opuesto sobre la viabilidad de los cruces entre morfos (resultando más viables los descendientes de cruces entre morfos; Roulin 2004; Gratten et al. 2008; Johnston et al. 2013; Wellenreuther et al. 2014).

7.6.2. Métodos y resultados

Para estudiar el efecto de la combinación parental de morfos sobre la viabilidad de la descendencia, realizamos una serie de cruces dirigidos en 44 recipientes circulares (170 cm de diámetro, 60 cm de altura) instalados al aire libre en la *Station d'Ecologie Theóretique et Experiméntale* (Moulis, Francia). En cada recipiente introdujimos tres hembras (una de cada morfo puro) y un macho (15 recipientes con macho blanco, 15 recipientes con macho amarillo y 14 recipientes con macho naranja). Registramos los apareamientos y las guardas de pareja durante un mes, y posteriormente incubamos las puestas y determinamos la paternidad. Para cada hembra registramos el tamaño de puesta, el número de huevos fértiles e infértiles y el peso de los juveniles al nacer. Además, para medir la calidad de la descendencia, sometimos a los juveniles a unas pruebas de comportamiento en las que evaluamos la habilidad para la obtención de alimento y la respuesta anti-depredadora. Después, instalamos a los juveniles en los mismos recipientes al aire libre y los visitamos al cabo de un año para evaluar el crecimiento y la supervivencia, y obtener datos acerca de la herencia y desarrollo de los morfos. Al caracterizar la coloración exhibida por los juveniles al nacer (blanca al ojo humano) de acuerdo al espacio perceptivo de la especie, encontramos que esta refleja en mayor medida el UV cercano y probablemente es percibida por los conespecíficos como un color crómaticamente distinto de cualquiera de los morfos expresados por los adultos. Además, la coloración expresada por los juveniles un año despúes coincidió con la base genética para el polimorfismo de color en *P. muralis* sugerida en un estudio genómico previo (Andrade et al. 2019). Es decir, que la expresión de coloración amarilla y naranja requiere de homocigosidad recesiva en dos locis autosómicos distintos. Respecto a la existencia de estrategias alternativas asociadas a los morfos en hembras, aunque por regla general el tamaño de puesta se relacionó negativamente con el peso de los juveniles al nacer, no encontramos diferencias en ninguna de estas variables entre los distintos morfos. Aunque encontramos un menor éxito de fertilización en uno de los cruces posibles (cuya causa es difícil de aclarar), en general no encontramos evidencias de que la combinación parental de morfos afectara a la viabilidad embrionaria, al desempeño en las pruebas de comportamiento o la supervivencia.

7.6.3. Discusión

Los resultados de este trabajo refutan la existencia de estrategias alternativas de inversión reproductiva asociadas al polimorfismo de color en hembras de *P. muralis*. Además, en contra de lo esperado si existiese selección correlativa o una ventaja del heterocigoto, no encontramos evidencia de que la combinación parental de morfos afecte de forma determinante a la viabilidad, comportamiento o supervivencia de la descendencia. El menor éxito de fertilización que encontramos en cruces heteromórficos se debe a un único tipo de cruce de los seis posibles (hembra amarilla con macho naranja). Además, no parece reflejarse en el cruce contrario (con los sexos cambiados) y no encaja bien con lo esperado bajo selección correlativa (que debería afectar más a la viabilidad embrionaria que a la fecundación; Gray and McKinnon 2007; Pryke and Griffith 2009; Lancaster et al. 2014). En resumen, los resultados sugieren que, pese a que muchos investigadores consideran el polimorfismo como la antesala evolutiva de la especiación, no existen barreras reproductivas importantes al apareamiento entre morfos en *P. muralis*. Para comprender las razones por las que nuestros resultados contradicen lo hallado en otros estudios previos (Galeotti et al. 2013), proponemos investigar si los distintos linajes de *P. muralis* pueden diferir en la correlación entre el polimorfismo de color y otros rasgos fenotípicos de interés para la selección, así como la posible dependencia ambiental de la eficacia biológica de los morfos (Roulin 2004; Svensson 2017). Nuestros resultados también incluyen importantes aportaciones al respecto de la herencia y la ontogenia de los morfos. Por una parte, la confirmación de que el polimorfismo de color en *P. muralis* presenta una base genética recesiva e implica a dos loci distintos coincide con otros trabajos recientes en sugerir que un número reducido de loci autosómicos gobiernan la expresión del polimorfismo en lagartos (Andrade et al. 2019; Stuart-Fox et al. 2020). A pesar de esta convergencia, los alelos responsables de la expresión de cada morfo parecen tener relaciones de dominancia distintas en cada especie estudiada, lo que puede tener implicaciones para su significado funcional y debería ser abordado en futuros trabajos. Por último, la caracterización del color ventral de los juveniles como un color cromáticamente diferenciado mejora nuestro entendimiento del desarrollo de los morfos, puesto que hasta ahora era común asumir que únicamente las lagartijas de morfo naranja o amarillo experimentaban un cambio ontogenético en la coloración, mientras que las lagartijas de morfo blanco retenían la coloración juvenil (Sacchi et al. 2007; Pérez i de Lanuza et al. 2013*a*; Pérez i de Lanuza and Font 2015).

7.7. Discusión general

A pesar del considerable interés que ha despertado la posible existencia de estrategias alternativas de reproducción en la lagartija roquera (y en otros lagartos polimórficos), varios aspectos clave de esta hipótesis han sido ignorados o se han estudiado de forma inapropiada. En esta tesis hemos tratado de paliar estas carencias adoptando un enfoque integrativo que incorporase nuestro conocimiento actual sobre la ecología sensorial de *P. muralis,* análisis cuantitativo del comportamiento, y métodos genéticos para determinar la eficacia biológica. En conjunto, nuestros resultados refutan la existencia de estrategias alternativas de reproducción que impliquen diferencias en el comportamiento social, sexual o reproductivo de los machos de distintos morfos, así como la existencia de estrategias alternativas de inversión reproductiva en hembras. Estos resultados contradicen las expectativas de muchos autores con respecto al significado funcional del polimorfismo de color en lagartos (Zamudio and Sinervo 2003; Sinervo et al. 2007; Calsbeek et al. 2010), y también están en aparente contradicción con algunos trabajos previos realizados en poblaciones polimórficas de lagartija roquera en el norte de la Península Itálica (Galeotti et al. 2013; Sacchi et al. 2017*b*, 2017*a*; Coladonato et al. 2020). La existencia de un sesgo de publicación a favor de los llamados "resultados positivos" (evidencia confirmatoria acerca de teorías conocidas) ha dificultado considerablemente la publicación de los trabajos contenidos en esta tesis (Csada et al. 1996; Scargle 2000; Jennions and Møller 2002). Consideramos necesario advertir de que nuestra comprensión del funcionamiento de la naturaleza depende de la acumulación imparcial de evidencia a favor y en contra de cada hipótesis, y que por tanto la existencia de sesgos que favorezcan la evidencia confirmatoria puede conducir a una sobreestimación ilusoria del apoyo empírico a ciertas hipótesis (Nickerson 1998; Scargle 2000; Thornton and Lee 2000). En este sentido, creemos que el proceso editorial de revisión debería juzgar la calidad de los trabajos en virtud de la validez de la metodología empleada para contestar a las preguntas científicas planteadas, en lugar de por los resultados obtenidos (Petticrew 1998; Rosenberg 2005; Pautasso 2010).

En lugar de ignorarlos, integrar los resultados aquí expuestos con la evidencia disponible podría mejorar considerablemente nuestra comprensión del significado funcional del polimorfismo de color en *P. muralis*. Tras dos décadas de trabajo, aún estamos lejos de comprender el valor adaptativo del polimorfismo de color en lacértidos, así como los mecanismos responsables de su persistencia en el tiempo. Por ahora, la principal conclusión que podemos extraer de la literatura (y de esta tesis) es que *U. stansburiana* no representa el modelo general en lagartos, en los que parece que la evolución del polimorfismo responde a escenarios evolutivos muy distintos en cada especie estudiada (Huyghe et al. 2007, 2009*a*; Sinervo et al. 2007; Calsbeek et al. 2010; Runemark et al. 2010; Runemark and Svensson 2012; Galeotti et al. 2013; San-Jose et al. 2014; Sacchi et al. 2017*b*; Brock et al. 2020*a*). A la luz de estos resultados, proponemos revisar el papel central que tradicionalmente se ha otorgado a las estrategias alternativas de reproducción en el mantenimiento del polimorfismo de color en este grupo, y ampliar nuestra perspectiva para incorporar otros procesos evolutivos escasamente estudiados.

En primer lugar, cabe recordar que existen otros contextos en los que el polimorfismo de color podría desempeñar una función relevante para la selección. A menudo se ha asumido que la coloración ventral podría estar influyendo en las interacciones sociales mediante la señalización de estrategias alternativas de comportamiento (Sinervo et al. 2007; Calsbeek et al. 2010; Mangiacotti et al. 2019; Brock et al. 2020*a*). Una hipótesis alternativa y plausible (que sin embargo ha recibido mucha menos atención) es la posibilidad de que la coloración ventral influya en las interacciones sociales al facilitar el reconocimiento individual entre conespecíficos (Taylor and Elwood 2003; Tibbetts and Dale 2007). La mayoría de los lagartos territoriales que han sido estudiados muestran el llamado efecto de "querido enemigo" (i.e. *dear enemy*): los machos (tras un período inicial de enfrentamientos con sus vecinos) modulan su agresividad en función de su familiaridad, reduciendo su agresividad en posteriores enfrentamientos con sus vecinos pero persiguiendo y escalando el conflicto fácilmente cuando se enfrentan a machos desconocidos (Tibbetts and Dale 2004; Stuart-Fox et al. 2006; Whiting et al. 2006; Ducrest et al. 2008; Tibbetts and Lindsay 2008; Tibbetts and Izzo 2010; Ligon and McGraw 2013; Briffa 2014). Sin embargo, algunos estudios en lagartijas del género *Podarcis* sugieren que los machos son capaces de discriminar entre rivales con los que están igualmente familiarizados en función de sus marcas de olor, recordando la posición de estas y dirigiendo mayor agresividad a aquellos individuos cuyas marcas solían encontrar en el centro de su terrario experimental (Carazo et al. 2008; Font et al. 2012*a*). A pesar de que tradicionalmente se ha considerado a los lacértidos como animales eminentemente quimiosensoriales (Mason and Parker 2010), la sofisticación de su sistema visual y la diversidad de vistosas coloraciones en este grupo hace razonable pensar que las señales visuales podrían también participar en el reconocimiento individual. El trabajo de Elizabeth Tibbetts y sus colaboradores en avispas del género *Polistes* demuestra que las señales visuales pueden jugar un papel tan importante como las señales químicas en el reconocimiento individual (Tibbetts 2002; Tibbetts et al. 2018, 2019, 2020). Estas avispas utilizan la variabilidad cromática en el rostro de sus conespecíficos para distinguir entre avispas conocidas y desconocidas. Además, la comparación de especies con distintos sistemas sociales, variabilidad cromática y habilidad para el reconocimiento individual sugiere que la menor agresión recibida en interacciones sociales favorece la aparición de fenotipos nuevos y distintos que faciliten el reconocimiento individual (Sheehan and Tibbetts 2010). En la lagartija roquera, a pesar de que los distintos morfos son diferenciados de forma discreta por el sistema visual de la especie, dentro de cada uno de los morfos existe una considerable variabilidad (tanto cromática como acromática; **Capítulo II**). Esta variabilidad (apreciable por el sistema visual de *P. muralis*) podría jugar un papel clave y hasta ahora inexplorado en la mediación de interacciones sociales al permitir a los individuos reconocer a sus conespecíficos y ajustar su comportamiento en consecuencia (Olsson 1994*b*; Whiting 1999; López and Martín 2002; Husak and Fox 2003; Font et al. 2012*a*). Futuros estudios podrían abordar esta cuestión, por ejemplo, examinando si la agresión dirigida a lagartijas con coloraciones experimentalmente alteradas disminuye a medida que sus oponentes se familiarizan con estas nuevas coloraciones (Tibbetts 2002; Tibbetts et al. 2008, 2017). Otra posibilidad sería evaluar si el rango de variabilidad cromática en distintas especies de *P. muralis* (con y sin polimorfismo ventral) correlaciona positivamente con su habilidad para el reconocimiento individual (Sheehan and Tibbetts 2010).

En segundo lugar, la evolución de estrategias alternativas de reproducción no es el único proceso capaz de generar selección dependiente de frecuencia y evitar la desaparición del polimorfismo en una especie. Algunas formas de selección apostática como serían la existencia de una ventaja para el depredador (o la presa) menos frecuente en cada momento (Lindström et al. 2001; Olendorf et al. 2006; Halpin et al. 2008; Lawrence et al. 2019), o una ventaja idéntica en un contexto de selección social (por ejemplo, en el resultado de los enfrentamientos intrasexuales o en la elección de pareja; Gosden and Svensson 2009; Dijkstra et al. 2010; Willink et al. 2019) también podrían asegurar la persistencia del polimorfismo en ausencia de diferencias comportamentales entre los morfos (Roulin 2004; Roulin and Bize 2007). Comprobar la existencia de selección dependiente de frecuencia es muy difícil en poblaciones naturales, pero creemos que el uso de diseños experimentales de mesocosmos (como el empleado en el **Capítulo IV**) podría constituir una aproximación eficaz para estudiar los cambios en la eficacia biológica de los morfos cuando se encuentran en menor frecuencia.

En tercer lugar, aunque la existencia de selección dependiente de frecuencia ha recibido mucho atención en lagartos, existen otros mecanismos capaces de mantener el polimorfismo (Roulin 2004; Mckinnon and Pierotti 2010; Svensson 2017). Un polimorfismo de color puede perpetuarse si la selección varía en el tiempo o en el espacio, y si la escala de los cambios y/o una dinámica balanceada de migración impide la desaparición de los morfo en desventaja (Roulin 2004; Mckinnon and Pierotti 2010; Svensson 2017). Un aspecto escasamente estudiado hasta ahora en *P. muralis* es la variabilidad geográfica en la composición del polimorfismo (i.e. el número de morfos presentes en una población y sus frecuencias relativas). Sin embargo, algunos trabajos realizados en poblaciones polimórficas de *P. muralis* en el Pirineo (incluyendo aquellas estudiadas en esta tesis), han descrito interesantes relaciones entre la composición poblacional del polimorfismo y factores ambientales, tanto bióticos (razón de sexos; Pérez i de Lanuza et al. 2017) como abióticos (clima; Pérez i de Lanuza et al. 2018). Estos estudios suponen una motivación para continuar indagando acerca de la posible dependencia ambiental de la eficacia biológica de los morfos, así como dilucidar la importancia relativa de la deriva genética, la selección fluctuante y el flujo génico sobre la variabilidad interpoblacional en el polimorfismo de la lagartija roquera.

En conclusión, con esta tesis esperamos haber aportado argumentos convincentes para ampliar nuestra perspectiva sobre el significado funcional del polimorfismo de color en lacértidos, además de sugerir algunas líneas de investigación prometedoras y contribuir de forma general a nuestro entendimiento de los procesos evolutivos responsables del mantenimiento de la variabilidad fenotípica en la naturaleza.

Chapter VIII:

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Appendix: Published articles

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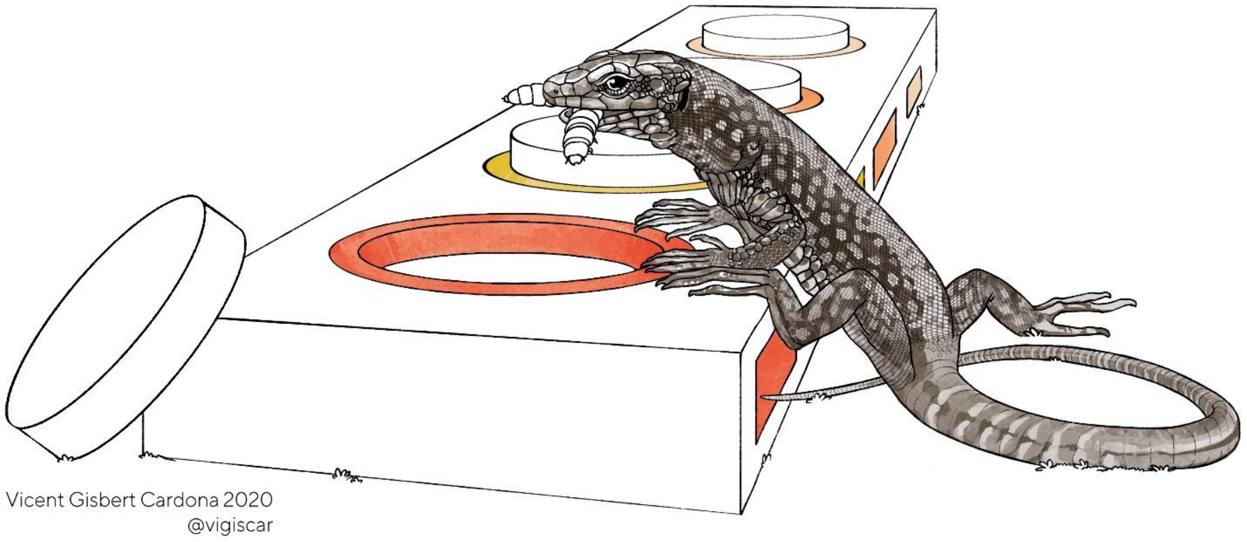
Chapter II

"The real thing is that there is no real world but as many worlds as species".

·Jakob von Uexk



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RESEARCH ARTICLE

Through the eye of a lizard: hue discrimination in a lizard with ventral polymorphic coloration

Guillem Pérez i de Lanuza[1,*,‡], Javier Ábalos[1,2,*], Alicia Bartolomé[1,2] and Enrique Font[2]

ABSTRACT

Colour polymorphisms are thought to be maintained by complex evolutionary processes, some of which require that the colours of the alternative morphs function as chromatic signals to conspecifics. Unfortunately, a key aspect of this hypothesis has rarely been studied: whether the study species perceives its own colour variation as discrete rather than continuous. The European common wall lizard (*Podarcis muralis*) presents a striking colour polymorphism: the ventral surface of adults of both sexes may be coloured orange, white, yellow or with a mosaic of scales combining two colours (orange–white, orange–yellow). Here, we used a discrimination learning paradigm to test whether *P. muralis* is capable of discriminating colour stimuli designed to match the ventral colours of conspecifics. We trained 20 lizards to eat from colour-coded wells bored in wooden blocks. Blocks had four colour-coded wells (orange, white, yellow and an achromatic control), but only one contained food (mealworm larvae). After six trials, the lizards performed significantly better than expected by chance, showing a decrease in both the number of wells explored and the latency to finding the food. Using visual modelling techniques, we found that, based on their spectral properties and the lizards' cone sensitivities, the ventral colours of *P. muralis* correspond to discrete rather than continuous colour categories, and that colour discriminability (i.e. distance in perceptual space) varies depending on the morphs compared, which may have implications for signal detection and discrimination. These results suggest that *P. muralis* can discriminate hue differences matching their own ventral colour variation.

KEY WORDS: Colour discrimination, Colour polymorphism, Colour vision, Learning experiment, Visual modelling

INTRODUCTION

Understanding the processes responsible for the evolution of population polymorphisms is one of the most exciting challenges facing evolutionary biology. Colour polymorphic species such as the peppered moth, *Biston betularia*, have been extensively used as models to test important evolutionary hypotheses about the origins and maintenance of phenotypic variation (Majerus, 1998; Gray and McKinnon, 2006; Oliveira et al., 2008; Svensson et al., 2009; McKinnon and Pierotti, 2010; Wellenreuther et al., 2014; Svensson, 2017). However, assessing colour variants and their functional significance in colour polymorphic species is not straightforward. Colour variation is often described from the perspective of a human viewer but should instead be judged from the perspective of the appropriate receivers (Bennett et al., 1994; Eaton, 2005), which requires the use of modern instrumentation and methods for objective colour characterization (e.g. reflectance spectrophotometry and visual modelling). Also, the widely held assumption that the colours of the different morphs act as chromatic signals and that conspecifics use colour variation to identify alternative phenotypes (e.g. related to age, sex, individual quality or reproductive strategies) and adjust their behaviour accordingly has rarely been tested.

Recent work with pollinating insects and birds and with cichlid fish underscores the importance of considering perceptual mechanisms in the study of colour polymorphisms (Chittka and Raine, 2006; Muchhala et al., 2014; Thairu and Brunet, 2015). Cichlids show striking and hypervariable interpopulation and intrapopulation colour polymorphisms, and abundant evidence has demonstrated that colour discrimination, visual ecology and sensory drive play a critical role in the evolution of this interesting polymorphic clade (e.g. Seehausen et al., 2008). However, there is no information on morph discrimination for most colour polymorphic species, including lizards (but see Teasdale et al., 2013; Merkling et al., 2016).

The European common wall lizard *Podarcis muralis* (Laurenti 1768) (family Lacertidae) has attracted much interest in studies of colour polymorphism (e.g. Calsbeek et al., 2010; Galeotti et al., 2013; Pérez i de Lanuza et al., 2013, 2017). To the human eye, this species may show up to five discrete ventral colour morphs that are fixed at sexual maturity: white, yellow and orange pure-colour morphs, as well as white–orange and yellow–orange mixed phenotypes that display a mosaic of scales of two different colours (sensu Pérez i de Lanuza et al., 2013; Pérez i de Lanuza and Font, 2015). These colours extend over the throat and the belly in males but, at least in some populations, are restricted to the throat in females (females in these populations have a white belly). Much effort has been devoted to the identification of consistent behavioural, morphological, physiological or ecological correlates of the colour variation, but the results are so far inconclusive (Sacchi et al., 2007; Calsbeek et al., 2010; Galeotti et al., 2013; Pérez i de Lanuza et al., 2014).

While it is often assumed that the ventral colours in *P. muralis* function as social signals, the evidence in this regard is very scant. It is possible that the ventral coloration, while correlated with other phenotypic traits, has no effect on receiver behaviour and is therefore not a chromatic signal to conspecifics. Rather, a link between polymorphic coloration and alternative phenotypes could result from pleiotropic effects of whatever genes are responsible for the polymorphism. However, there are some indications that lizards assess each other's ventral colours and adjust their behaviour based on their own colour relative to that of others with which they

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interact. For example, although morphs are not spatially segregated, males and females pair assortatively by ventral colour (Pérez i de Lanuza et al., 2013, 2016). Also, male ventral colour seems to be important in the resolution of lab-staged fights (Ábalos et al., 2016). Further, it has been suggested that females may adjust their breeding strategy according to their own and their mate's colour morph (Galeotti et al., 2013).

A necessary condition for the colour of alternative morphs to function as social signals is that the animals themselves can perceive them as different stimuli, which ultimately depends on their visual perception, not ours (Teasdale et al., 2013; Pérez i de Lanuza and Font, 2014). Research on colour polymorphic *P. muralis* rests on the reasonable assumption that lizards perceive their own chromatic variation as categorically distinct phenotypes (i.e. morphs), much as humans do. However, nobody has formally tested this assumption. Given the known differences between the visual systems of lizards and humans, establishing the existence of discrete colour morphs from the lizards' perspective is essential for many current hypotheses about the genetic underpinnings of the polymorphism and the evolutionary processes generating and maintaining it (e.g. Cote et al., 2008; Paterson and Blouin-Demers, 2017).

Although the human visual system has little trouble identifying discrete colour morphs in *P. muralis*, lizards could perceive their own colour variation in a different way. *Podarcis muralis* has, in common with other diurnal lizards, a sophisticated colour vision system with four types of single cones that are sensitive to light in the wavelength range between 320 and 700 nm (Pérez i de Lanuza and Font, 2014; Martin et al., 2015). Their retinas also contain large numbers of long-wavelength sensitive double cones that are thought to be responsible for luminance (i.e. brightness) perception (Loew et al., 2002; Olsson et al., 2013). As the ventral colours differ both in spectral shape and in luminance (Pérez i de Lanuza et al., 2013; Pérez i de Lanuza and Font, 2015), discrimination of the alternative morphs could be based on either of these variables.

Discrimination experiments are a useful tool to confirm animal colour vision and the perception of colour differences (Kelber et al., 2003; Kelber and Osorio, 2010). There is no shortage of papers testing the ability of lizards to visually discriminate between stimuli of different size, shape, pattern, luminance or colour (i.e. hue). In his comprehensive review of learning processes in reptiles, Burghardt (1977) listed 12 such studies, of which half involved some type of hue discrimination, and more have been published in the ensuing decades. These studies have shown that lizards can discriminate between stimuli differing only in hue (e.g. Wagner, 1933; Swiezawska, 1949; Rensch and Adrian-Hinsberg, 1963; Elinor and Benes, 1969; Dücker and Rensch, 1973) or in luminance (e.g. Vance et al., 1965; Garzanit and Richardson, 1974; Peterson, 1976; Hodgkinson and Still, 1980). Unfortunately, few studies have used standard colour stimuli with known reflectance properties (e.g. Ostwald, Munsell) and many do not include luminance controls (i.e. greys). Luminance controls may be of little relevance if the focus of the study is learning per se rather than colour discrimination (e.g. Leal and Powell, 2012). But if the goal is to establish that colour vision is present, luminance controls are essential to ensure that the animals respond differentially to hue-independent stimuli (Kelber et al., 2003). Also, most studies using colours as discriminanda make no attempt to use colours that resemble natural stimuli that the animals might encounter in the field, such as colours of prey or conspecifics (but see Hews and Dickhaut, 1989).

Here, we used a behavioural experiment adapted from previously used experimental designs (Leal and Powell, 2012; see also Clark et al., 2014) to test whether *P. muralis* can discriminate the ventral colour variation shown by this species. Additionally, we reanalysed spectral data collected in previous studies (Pérez i de Lanuza et al., 2013, 2014; Pérez i de Lanuza and Font, 2015) and used visual modelling techniques based on the receptor noise model (Vorobyev and Osorio, 1998) to assess the colour variation and quantify the degree of discriminability among colour morphs from a lizard's visual perspective.

MATERIALS AND METHODS

We captured 20 lizards (10 males and 10 females) by noosing (i.e. using a pole with a slipknot that tightens around the neck of the lizard) on 8 July 2015 in Angostrina (Eastern Pyrenees, France). The lizards were individually held in cloth bags and transferred by car to the Ethology lab at the University of Valencia (470 km) on the day following their capture. In the laboratory, lizards were housed in individual terraria (20×40 cm and 26 cm high) provided with water, a shelter, and a brick over which an incandescent reflector lamp (40 W; Parabolica RP50 Radium, Wipperfürth, Germany) was suspended. Terraria were housed in an animal room with temperature and light cycle mimicking average field conditions at the capture site (thermal gradient of 24–40°C inside the terraria during the day; 12.5 h light:11.5 h dark). In addition, ultraviolet (UV)-rich fluorescent tubes (Reptistar 5.0, Sylvania, Danvers, MA, USA; colour temperature 6500 K) suspended above the terraria were switched on for 1.5 h (12:00 h–13:30 h) three times per week. During the colour discrimination experiments (11 July to 27 August 2015), lizards had access to food only during the experimental trials, but individuals failing to eat in five consecutive trials were discarded from the experiment and fed 3–4 times per week. After the experiments were completed, all lizards were released back at their capture location on 31 August 2015. Lizards were captured under research permit number 2013095-0001 from the Préfecture des Pyrénées-Orientales (France). This research complied with the ASAB/ABS Guidelines for the Use of Animals in Research and all applicable local, national and European legislation.

Colour discrimination experiment

For the colour discrimination experiment, we trained lizards to eat mealworm larvae (*Tenebrio molitor*; ca. 2 cm long and 150 mg) dusted with vitamins (Exo Terra, Montreal, QC, Canada) from a well in a wooden block. The block had four evenly spaced circular wells (2 cm diameter, 1.5 cm deep) and each well was associated with a different colour by means of two coloured paper stickers: a ring-shaped sticker surrounding the well's entrance and a rectangular sticker marking its position on the lateral side of the block (Fig. 1). In total, we used 10 wooden blocks, each of which was used by only two lizards. Each block had three wells fitted with orange, white and yellow stickers resembling the ventral colours of *P. muralis*, and a fourth grey-coloured sticker having the same luminance as the training colour (see 'Experimental stimulus design', below). To prevent the lizards from locating prey using chemical cues, we placed two live mealworm larvae inside all wells for 2 weeks before the start of the experiment, and two nights per week during the experiment (Monday and Thursday; 19:30 h–09:30 h).

We trained half the animals (five males and five females) to eat from the orange well, and the other half to eat from the yellow well (Fig. S1). To begin a trial, we introduced two larvae into the orange or yellow well, and positioned the wooden block inside the terrarium of the animal to be tested (4 cm from the shelter's entrance; see Fig. 1). A trial ended when the lizard located and ate

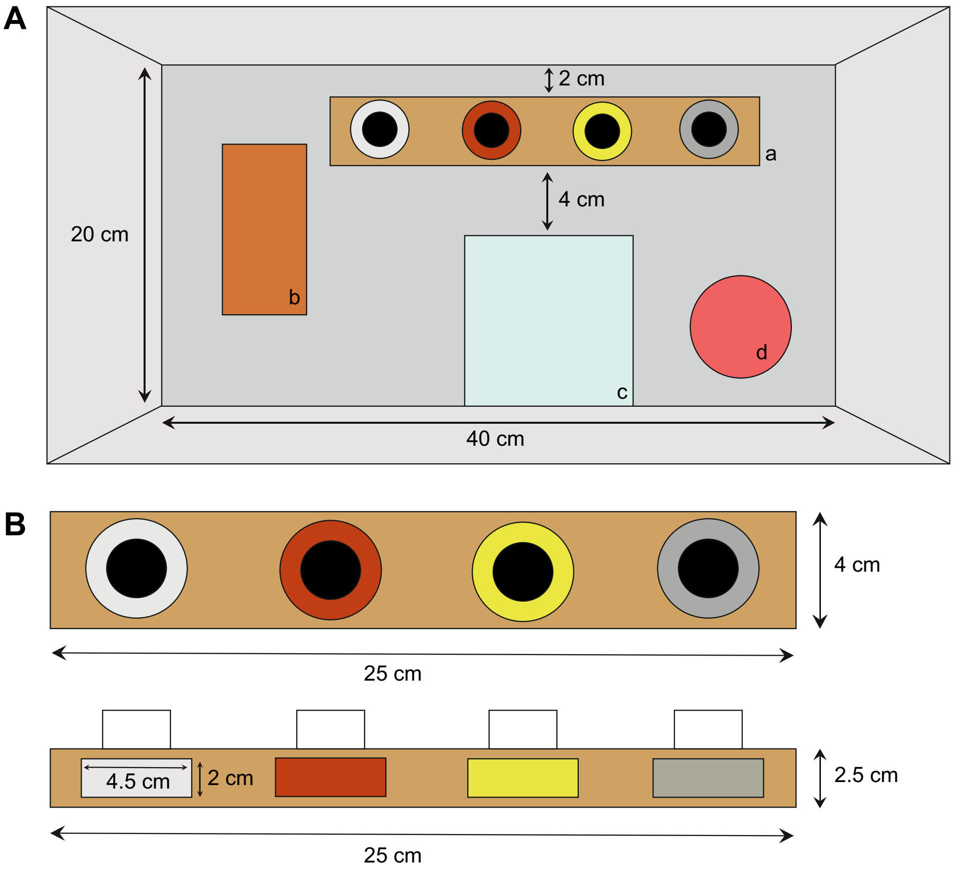

Fig. 1. Experimental terraria. (A) Location of elements within the individual terraria: (a) wooden block (only inside the terraria during experiments); (b) basking brick; (c) shelter; (d) water dish. (B) Schematic view of the experimental wooden block as seen from above (top, lids removed) and from the side facing the shelter (bottom, lids on wells).

the prey or after 25 min had elapsed. We tested each lizard on alternate days to ensure motivation toward the food reward (one trial per day). Prior to each trial, the position of the coloured stickers was determined using a random number generator, discarding combinations in which the larvae would remain in the same position as in the previous trial. During trials, both the UV-rich fluorescent tube and the incandescent lamp were on, providing a continuous light spectrum across the entire visual range of *P. muralis* (see irradiance spectrum in Fig. S2). We conducted trials during the lizards' natural daily period of activity (10:00 h–13:30 h and 16:00 h–19:00 h local time).

The experiment comprised a training phase and a testing phase. Training consisted of six trials (12 days) during which the mealworm larvae were in the orange or yellow well and we gradually reduced their visibility by partially covering the well with a white plastic lid weighing 8.5 g (i.e. covering 50% of the opening in trials 1 and 2, 75% in trials 3 and 4, 95% in trials 5 and 6). During the testing phase (18 trials, 36 days), we presented the wooden block with all four wells completely covered and videorecorded every trial using a photographic camera (Canon© EOS 60D, Tokyo, Japan) mounted on a tripod. We played back each filmed trial and one researcher (J.A.) recorded whether or not the animal found and consumed the prey, the number of incorrect lids lifted (errors), and the time elapsed from when it left the refuge until it lifted the correct lid (latency).

Experimental stimulus design

Experimental stimuli were designed to resemble the natural colour variation found in *P. muralis* ventral coloration. We prepared a palette of whites, yellows and oranges in Adobe Illustrator and printed them on five types of paper differing in whiteness, brightness and shade (resulting in 790 stimuli). These stimuli were measured with a portable spectrometer (see details below) and differences from natural lizard reflectance spectra (averaged over at least 164 spectra per morph; Fig. 2) were explored graphically, comparing colour variables (i.e. hue, chroma and brightness), and calculating chromatic and achromatic distances between any two colours using visual modelling (see details below). We chose the three chromatic stimuli that best matched natural colours (orange: CMYK=0%, 99%, 91%, 0%, Couché mate 130 g m^{-2}; white: CMYK=6%, 10%, 21%, 2%, Color copy 250 g m^{-2}; yellow: CMYK=0%, 23%, 86%, Couché mate 130 g m^{-2}; Fig. 2; Fig. S3).

By presenting an achromatic control with the same luminance as the training colour, we controlled the possibility that lizards base their discrimination on luminance differences among the colour stimuli. We designed two achromatic controls that were isoluminant with the chromatic yellow and orange experimental stimuli. Unfortunately, no evidence regarding how lizards judge luminance differences is available. Therefore, we conservatively designed the achromatic stimuli to show an absolute luminance (i.e. the integral of the spectral curve) similar to that of the chromatic stimuli (orange–achromatic: CMYK=0%, 0%, 0%, 82%, Couché mate 130 g m^{-2}; yellow–achromatic: CMYK=0%, 0%, 0%, 67%, Couché mate 130 g m^{-2}; Fig. 2; Fig. S3). However, as it has been hypothesized that luminance is perceived by a sensory channel involving the long-wavelength sensitive cones (as single cones: Fleishman et al., 1997; Fleishman and Persons, 2001; or as the main component of double cones: Osorio and Vorobyev, 2005), we also compared luminance between the chromatic stimuli and their corresponding achromatic stimuli using visual models assuming that luminance is processed by the long-wavelength sensitive cones (see methodological details in 'Visual modelling', below).

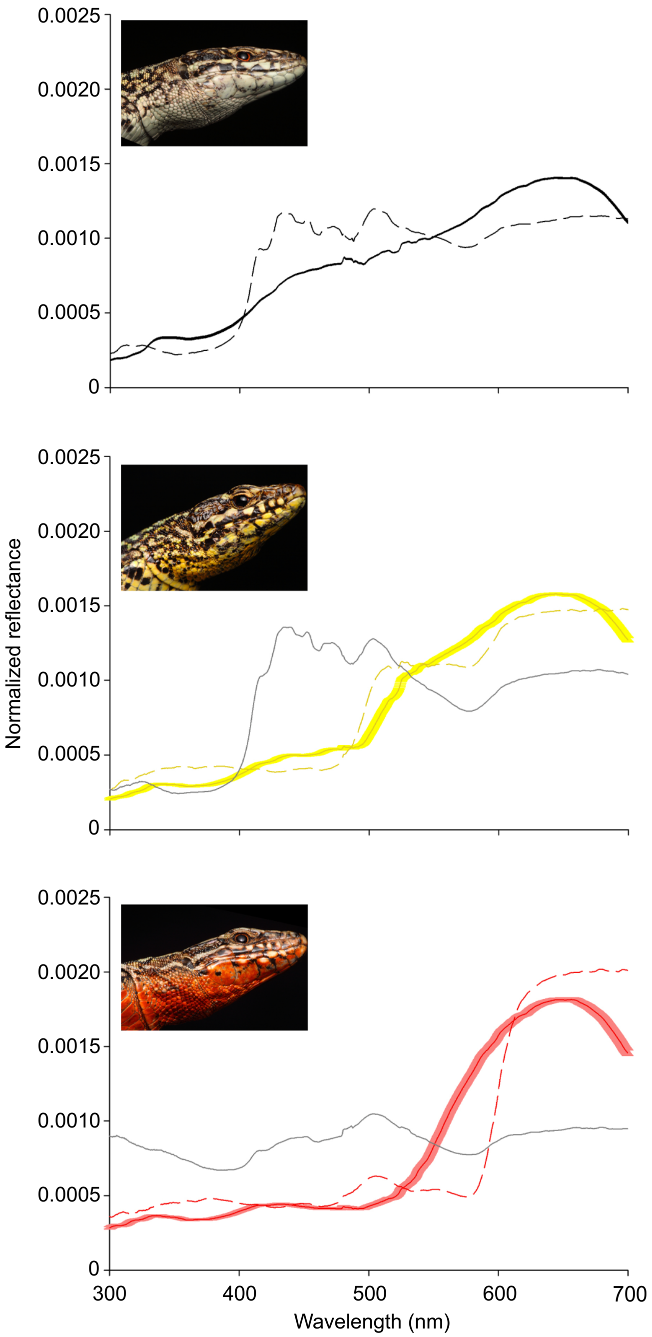

Fig. 2. Reflectance spectra. Lines represent spectra from natural *Podarcis muralis* throats (solid lines and shaded area represent means±1 s.e.m.) and the corresponding artificial stimuli (long-dashed lines represent the chromatic stimuli; grey lines for yellow and orange morphs represent the corresponding achromatic stimuli). For clarity, the spectra have been normalized by dividing the reflectance at each wavelength by the entire reflectance under the curve (i.e. luminance). See sample sizes for natural spectra in Materials and methods.

Statistical analyses

In order to account for inter- and intra-individual variability within trials, we grouped experimental trials in blocks of three, hence defining six blocks where the number of errors and mean latency were calculated. We then compared the mean number of errors per individual in each block with the average expected by chance (Baldwin, 1979; Margules and Gallistel, 1988; Brannon and Terrace, 1998). With four options to choose, the maximum number of errors is three. If choosing randomly, lizards are equally likely to make 0, 1, 2 or 3 errors. By multiplying each number of errors by its probability (1/4) we obtain the mean number of errors expected by chance ($\bar{X}_{\mathrm{errors}}=0\times1/4+1\times1/4+2\times1/4+3\times1/4=6/4=1.5$). We established better-than-chance performance in a block of trials as the criterion for successful learning.

To check for a decrease in both the mean number of errors and the mean latency with time, we ran two generalized linear mixed models (GLMMs) with errors (Poisson distribution) and latency (Gaussian distribution) as dependent variables, block and sex as fixed factors, and animal identity, training colour and (only in the model with number of errors) whether or not the animal located the prey as random factors. We checked graphically that both the number of errors and latency followed non-normal distributions (qqplots in R; http://www.R-project.org/). We power-transformed latency to follow a normal distribution by calculating the fourth root of each value (Shapiro–Wilk test for normality: W=0.99, P=0.14). We did not transform the number of errors as transforming count data is not recommended (O'Hara and Kotze, 2010), and adjusted a GLMM following a Poisson distribution after checking the mean and the variance of this variable had similar values ($\bar{X}$=1.12, S^2=0.98).

In addition, as a more conservative test of discrimination learning, we coded the performance of each individual lizard in each trial as either 1 (if the lizard's first choice was correct) or 0 (if the lizard made any number of errors). Then, we fitted a logistic mixed model with the lizard's performance as dependent variable, trial and sex as fixed effects, and animal identity, training colour and whether or not the animal located the prey as random factors. We plotted the fitted model with confidence intervals against time (i.e. trial) to check for a significant increase in the probability of correct first choice from chance levels (0.25).

Model fitting and model selection were conducted using backward single-term deletions ($P\leq0.05$) of the saturated model followed by model comparisons via likelihood ratio tests (lme4 package in R: Bates et al., 2015; http://www.R-project.org/). We did not find a problem of over-dispersion in the Poisson model (ϕ=1.002). We explored graphically that residuals from both models conformed to normality and homoscedasticity assumptions by plotting them against the logarithm of the fitted values.

Visual modelling

To determine quantitatively the discriminability between pairs of ventral colours (i.e. white against yellow, white against orange, and yellow against orange) we used Vorobyev and Osorio's (1998) receptor noise model. Calculations were performed in R 3.3.2 (http://www.R-project.org/) using the package PAVO (Maia et al., 2013). We assumed a cone abundance ratio of 1:1:1:4 (corresponding to the UV-, short-, middle- and long-wavelength sensitive cones; J. F. Le Galliard, personal communication based on results from Martin et al., 2015), and a Weber fraction of 0.05 for the long-wavelength sensitive cone (Siddiqi et al., 2004; previously used in other studies with lacertids: Marshall and Stevens, 2014; Martin et al., 2015). Cone absorbance spectra of *P. muralis* were obtained from the literature (Martin et al., 2015). As the illuminant, we used the irradiance spectrum inside the experimental terraria resulting from the combination of the light provided by the incandescent lamp and the UV-rich fluorescent tube (Fig. S2). The discriminability between morphs was expressed as just noticeable differences (JND). A value of 1 JND is traditionally assumed as the threshold of discrimination between two colour patches, i.e. pairs of colours giving values <1 JND are not discriminable, values between

1 and 3 JND represent colours that are discriminable under good illumination conditions, and values above 3 JND represent easily discriminable colours (Siddiqi et al., 2004; Marshall and Stevens, 2014). However, as behavioural data to confirm this assumption in *P. muralis* are completely lacking, these thresholds have to be interpreted with caution. Therefore, we conservatively adopted a threshold of 3 JND to declare that two colours were discriminable to the lizards.

To determine quantitatively whether our experimental stimuli resembled the lizards' ventral coloration, we also used Vorobyev and Osorio's (1998) receptor noise model to calculate chromatic and achromatic distances between natural spectra and the spectra obtained from the artificial stimuli. Based on these analyses, we chose for behavioural experiments those artificial chromatic stimuli that minimize the chromatic distance when compared with natural spectra (see below), and those artificial achromatic stimuli that minimize the achromatic distance when compared with artificial chromatic stimuli.

For visual modelling, we used reflectance spectra of throat coloration in the same Pyrenean population of *P. muralis* from which experimental animals were obtained (Angostrina, Eastern Pyrenees, France). Spectra were compiled from previously published studies (Pérez i de Lanuza et al., 2013, 2014; Pérez i de Lanuza and Font, 2015; raw data are available from the corresponding author on request), resulting in a dataset of 643 adult lizards showing pure morphs (199 white males, 88 white females, 135 yellow males, 57 yellow females, 131 orange males and 33 orange females). Reflectance spectra were obtained with a USB-2000 portable spectrometer and a PX-2 xenon strobe light source (Ocean Optics Inc., Dunedin, FL, USA), calibrated with a Spectralon white diffuse reflectance standard (Labsphere) (for more details, see Font et al., 2009; Pérez i de Lanuza et al., 2013, 2014; Badiane et al., 2017). Irradiance inside the experimental terraria was measured with a second USB-2000 spectrometer calibrated by means of a LS1-CAL calibration light source (Ocean Optics), using a cosine-corrected irradiance probe (Ocean Optics CC-3-UV). To assess differences in discriminability (i.e. chromatic distances) between the different pairs of ventral colours, we used a generalized linear model including the paired colour combinations (i.e. white–yellow, white–orange and yellow–orange) and sex as factors, as well as their interaction.

As parameters used to run visual models (i.e. cone proportion: 1:1:1:4; Weber fraction=0.05) are not supported by any empirical data in *P. muralis*, and because small variations in visual parameters can affect the results of visual modelling (Lind and Kelber, 2009; Bitton et al., 2017; Olsson et al., 2017), we repeated the analyses to assess whether our results are robust to variations in these parameters. Therefore, we also ran models with cone proportions of 1:1:1:1, 1:1:1:8 and 2:3:3:11 (i.e. the original count presented by Martin et al., 2015), and Weber fraction values of 0.03 and 0.07.

RESULTS

Colour discrimination experiment

Twelve lizards (seven males: three trained to eat from the orange well and four from the yellow well; and five females: three trained to eat from the orange well and two from the yellow well) completed the experiment. The remaining eight individuals failed to uncover a well in five consecutive trials and were discarded from the experiment.

The mean number of errors per block was significantly smaller than expected by chance (1.5) in all but the first two blocks of trials (Table 1, Fig. 3). Both the number of errors (Fig. 3) and latency (Fig. S4; Table 2) showed a significant reduction with time (Errors~Stage, Z=−2.40, standard coefficient±s.e.=−0.30±0.13, P=0.016; see Table S2; Latency~Stage, t=−3.49, standard coefficient±s.e.=−0.55±0.16, P<0.0001; see Table S1). Also, we found an effect of sex on the number of errors (Errors~Sex, Z=2.58, standard coefficient±s.e.=0.34±0.13, P=0.001), with females committing fewer errors than males on average across blocks (mean±s.e.m., males: 1.24±0.09, females: 0.94±0.10; Fig. 2). After finding violations of normality in the model with the number of errors, we ran a bootstrap analysis based on 10,000 simulations and compared the mean coefficients, their standard errors and P-values with those obtained before. This analysis confirmed our GLMM results showing similar coefficients but smaller standard errors and P-values (see details in Table S1).

Table 1. Number of errors in colour discrimination experiment

		No. of errors		
Block	N	Mean	CI	P
1	32	1.34	0.35	0.158
2	34	1.29	0.41	0.153
3	32	1.19	0.36	0.049
4	36	1.08	0.36	0.013
5	35	1.09	0.30	0.008
6	33	0.73	0.27	$1.92e^{-5}$

Mean number of errors and 95% confidence interval (CI) in each of six consecutive blocks of trials (three trials per block) comprising the training phase and the experiment, and P-values for one-way Wilcoxon tests (μ<1.5). N, number of trials per block (out of 36) in which all 12 trained lizards uncovered at least one well.

Plotting the logistic mixed model fitted with confidence intervals against time showed that the probability of the lizards' first choice being correct nearly doubled during the experiment, from chance levels in the first trial (P±CI=0.23±0.08) to even odds in the last trial (P±CI=0.41±0.04; Fig. 4).

Throughout the experiment, no colour was overrepresented in the total record of errors (trained to orange, χ^2=3.15, d.f.=2, P=0.207; trained to yellow, χ^2=0.703, d.f.=2, P=0.704; Fig. 5). However, wrong first choices were biased towards yellow in lizards trained to orange more frequently than to white or to the achromatic grey control (χ^2=8.41, d.f.=2, P=0.015), while lizards trained to yellow showed a marginally non-significant bias toward orange (χ^2=5, d.f.=2, P=0.08; Fig. 5).

Visual modelling

The natural spectra from the three morphs are relatively segregated in colour space (Fig. 6). Assuming a discrimination threshold of 3 JND and the proposed cone proportions (i.e. 1:1:1:4) and Weber fraction (i.e. 0.05), the three colours are chromatically discriminable considering all paired combinations (Fig. 7). Similar results were found using models with alternative cone proportions and/or Weber fraction (see Materials and methods). The effect of considering other visual parameters is graphically illustrated in Fig. S6.

Discriminability varied with morph combination (t=−5.66, standard coefficient±s.e.=−0.13±0.02, P<0.00001), the white–orange combination being more discriminable than the white–yellow and the yellow–orange combinations (P<0.00001), and the white–yellow combination being more discriminable than the orange–yellow combination (P<0.00001). Male morphs were more discriminable than female morphs (t=−4.27, standard coefficient±s.e.=−0.07±0.02 P=0.00002), and the interaction between morph combination and sex was significant (t=4.10, standard coefficient±s.e.=0.05±0.01, P=0.00005). The results remained significant using visual models with alternative visual parameters (Table S3).

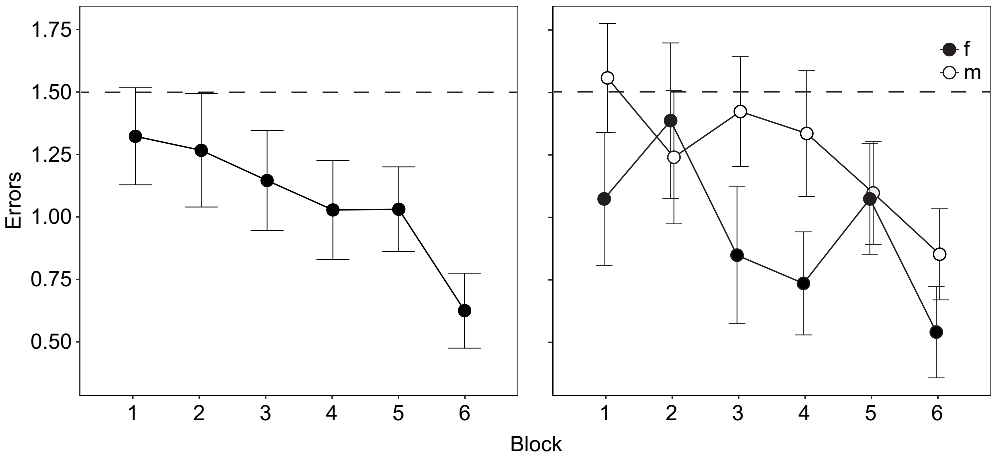

Fig. 3. Mean number of errors per block of trials. Left, mean number of errors, pooled from males and females (12 lizards, three trials per block). Right, mean number of errors per block of trials, grouped by sex (f, females; m, males). Each well of the wrong colour uncovered by a lizard in the allotted time (25 min) was considered an error (maximum of three errors). Error bars represent s.e.m. After two blocks of trials, the lizards responded to training by showing a significantly smaller number of errors than expected by chance (dashed line at 1.5).

DISCUSSION

Our results show that adult *P. muralis* learn to perform a procedural task (lifting the one lid among four giving access to prey in a colour-coded well), as shown by a significant reduction in both the number of errors and latency throughout the experiment. The fraction of lizards that successfully learned to perform the discrimination task (12 out of 20) is similar to that observed in experiments with other lizard species (e.g. Leal and Powell, 2012). The number of wrong choices was smaller than expected by chance after six trials (a week of testing) and the probability of correct first choice after 18 trials nearly doubled from initial chance levels. These results demonstrate that *P. muralis* is capable of discriminating experimental colour stimuli designed to match natural colours on the ventral surface of this species. Although the achromatic controls did not match perfectly the luminance of chromatic stimuli, as lizards did not choose the achromatic (grey) control incorrectly more often than the other available incorrect stimuli, we can reasonably assume that the discrimination was based on wavelength (i.e. hue) rather than luminance differences among stimuli. This evidence provides support for the idea that *P. muralis* is capable of discriminating its own ventral colour variation based on hue. Results of reflectance spectrophotometry and visual modelling reinforce this conclusion, showing that ventral colours of *P. muralis* correspond to discrete rather than continuous colour categories based on their spectral properties (independent of the human visual system) and lizard cone sensitivities. Interestingly, lizards were able to discriminate the two artificial stimuli showing the smallest perceptual distance (i.e. yellow versus orange), which is in the lower range of perceptual distances generated by the natural yellow and orange morphs (Fig. 7).

Our results bear out the assumption that the ventral polymorphism of *P. muralis* is discrete, encompassing several chromatically distinct morphs. On a practical note, our results also provide support for the use of a categorical classification of ventral colours in *P. muralis*, although perhaps not in other lacertid species. For example, in *Zootoca vivipara*, the assumption that the polymorphism is represented by categorically distinct colour morphs unleashed a heated controversy (Vercken et al., 2007, 2008; Cote et al., 2008). Unfortunately, although mate choice is involved in the maintenance of colour polymorphism in *Z. vivipara* (Sinervo et al., 2007; Fitze et al., 2014; San-José et al., 2014), colour discrimination among morphs was not tested and thus there is no conclusive evidence that lizards discriminate morphs visually, or that ventral colours in this species act as social signals.

Previous studies of the visual system of *P. muralis* demonstrated that ventral colours differ in conspicuousness when viewed against other body patches or against natural backgrounds (i.e. rocks, vegetation), which raises interesting questions regarding their potential role as social signals (Pérez i de Lanuza and Font, 2015). The results presented here indicate that colour discriminability varies according to the morphs being compared, white and orange being more discriminable than white and yellow, and yellow and orange ventral colours, and white and yellow being

Table 2. Latency in colour discrimination experiment

		Latency (s)		
Block	*N*	Mean	CI	s.e.m.
1	27	284.67	119.75	58.26
2	30	325.60	116.91	57.16
3	29	310.07	127.80	62.39
4	35	243.09	99.48	48.95
5	30	217.03	125.91	61.56
6	31	152.13	31.65	64.64

Mean latency, s.e.m. and 95% CI in each of six consecutive blocks of trials (three trials per block) comprising the training phase of the experiment. *N*, number of trials per block (out of 36) in which the trained lizards found the prey.

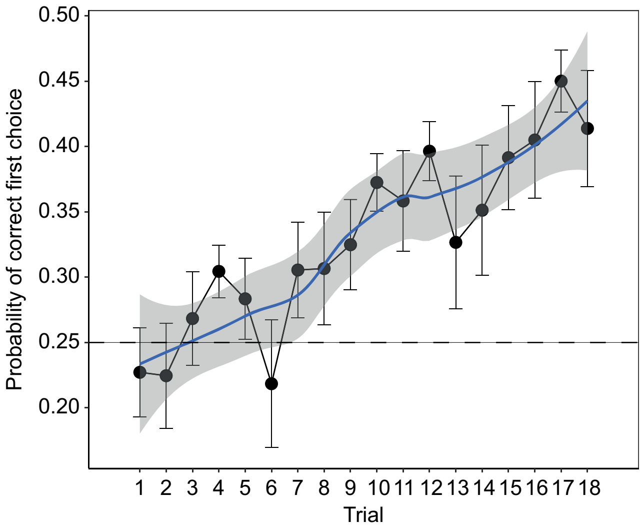

Fig. 4. Fitted logistic mixed model of the probability of correct first choice against time (i.e. trial). Circles and error bars represent mean probability with 95% confidence intervals in each trial. Blue line and shaded area are the smoothed tendency line with its confidence interval. The learning curve shows that the probability of correct first choice nearly doubled during the experiment.

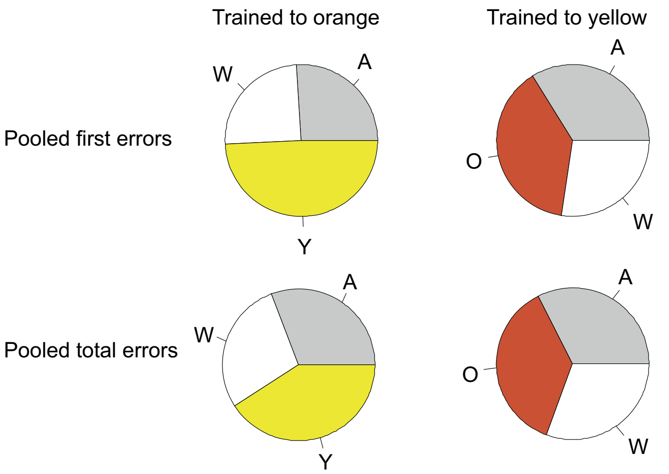

Fig. 5. Pie charts representing the relative frequency of each colour among the pooled errors performed by the lizards. Colour stimuli: O, orange; W, white; Y, yellow; A, achromatic stimulus matching the luminance of either orange or yellow.

more discriminable than yellow and orange combinations. Although behavioural results supporting this conclusion are lacking, chromatic distances obtained with visual modelling techniques may be good predictors of colour discriminability, even when distant colours are compared (Fleishman et al., 2016). Therefore, discriminability differences among pairs of ventral colours of *P. muralis* may have implications for signal detection and discrimination. This may be especially relevant for intrasexual and intersexual interactions that can be modulated by ventral colour, such as mate choice or male–male contests (Pérez i de Lanuza et al., 2013, 2016; Ábalos et al., 2016).

We found that sexes differ in colour morph discriminability, a finding that could have biological relevance (Zhou et al., 2015).

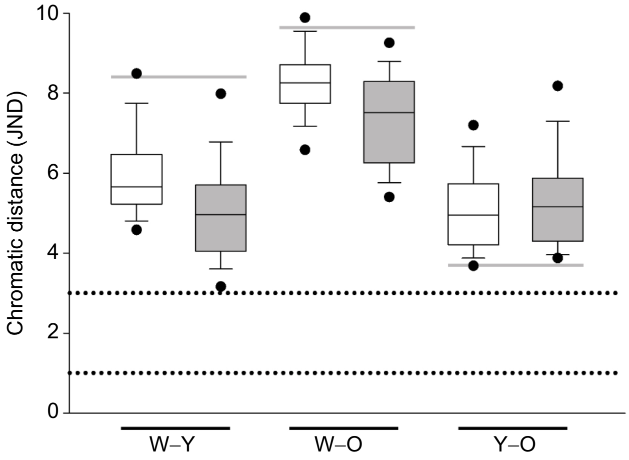

Fig. 7. Box-plots showing chromatic distances generated by pairs of colour morphs. Morphs: W, white; Y, yellow; O, orange. White box-plots correspond to males and grey box-plots correspond to females. In each case, horizontal lines, boxes, error bars and points indicate, respectively, the median, the 25–75% range, the 10th and 90th percentiles, and the 5th and 95th percentiles. Horizontal dotted lines indicate the discriminability thresholds of 1 just noticeable difference (JND; values above 1 JND indicate pairs of colours that are discriminable under good illumination conditions) and 3 JND (values above 3 JND indicate easily discriminable pairs of colours). Results considering other cone proportions and other Weber fraction values are detailed in Fig. S5. The impact of these variations is graphically reported in Fig. S6. Horizontal grey lines indicate chromatic distances of the experimental stimuli for each pair of colour morphs.

However, this result may be a consequence of sexual dichromatism (i.e. slight differences in spectra), resulting in small (but significant) differences in colour distances between males and females (see, for example, the chromaticity diagram in Fig. 6). We also found that males and females differ in the number of errors, but this difference may be caused by the small sample size used in the

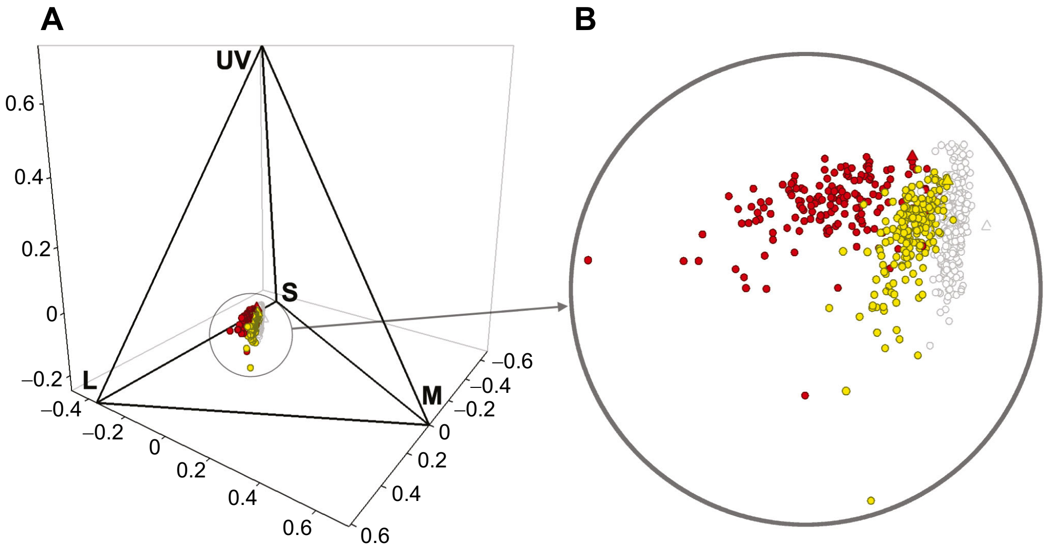

Fig. 6. Chromaticity diagram showing the location of chromatic points. (A) The entire receptor space; (B) detail of the volume occupied by the chromatic points. Circles correspond to chromatic points from the natural ventral colours (pooling males and females). Triangles correspond to the artificial stimuli. The colours of symbols indicate the morph. Sample sizes are given in Materials and methods. The overlap between the white and the yellow volumes amounts to 27.8% of the white volume and 4.9% of the yellow volume; the overlap between the white and the orange volumes represents 5.1% of the white volume and 0.7% of the orange volume; the overlap between the yellow and the orange volumes is 12.2% of the yellow volume and 9.1% of the orange volume. The white artificial stimulus is located outside (but close to) the cloud of white natural colours. The other two artificial stimuli fall within their respective cloud of natural colours, but are located at the periphery. However, note that the perceptual distance between the artificial stimuli is smaller than that of many chromatic points of different morphs. Wavelengths: S, short; M, middle; L, long; UV, ultraviolet.

experiment, which allows for extremely good performers to bias our results.

Although colour polymorphisms provide invaluable models to study the evolution and maintenance of polymorphisms generally (e.g. Roulin, 2004; Sinervo and Calsbeek, 2006; Chunco et al., 2007; Pryke and Griffith, 2009; McKinnon and Pierotti, 2010; McLean and Stuart-Fox, 2014; Wellenreuther et al., 2014; Svensson, 2017), insufficient attention has been paid to colour (morph) discrimination and its implications for understanding the significance of polymorphic coloration. It is often assumed that colour polymorphic animals perceive their own colour variation as different morphs, and that colour variants represent qualitatively different signals. However, this assumption has an unstable foundation if no evidence of colour discrimination is provided. We encourage other researchers to obtain evidence that their study animals are capable of chromatically discriminating their colour variation as independent colour morphs and show behavioural evidence of doing so.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: G.P.L.; Methodology: G.P.L., J.A., A.B.; Validation: G.P.L., E.F.; Formal analysis: G.P.L., J.A.; Investigation: G.P.L., J.A., A.B.; Writing - original draft: G.P.L., J.A.; Writing - review & editing: G.P.L., A.B., E.F.; Visualization: G.P.L., J.A., A.B.; Supervision: E.F.; Project administration: G.P.L., E.F.

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Supplementary information

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Chapter III

"The Universe is a dark forest. In this forest, hell is other people. An eternal threat that any life that exposes its own existence will be swiftly wiped out. This is the picture of cosmic civilization. It's the explanation for the Fermi Paradox".

·Cixin Liu, The Dark Forest·

"I come in peace… take me to your lizard".

·Douglas Adams, The Hitchhiker's Guide to the Galaxy·

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The role of male coloration in the outcome of staged contests in the European common wall lizard (*Podarcis muralis*)

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Abstract

Colour signals play a key role in regulating the intensity and outcome of animal contests. Males of the common wall lizard (*Podarcis muralis*) show conspicuous ventrolateral ultraviolet (UV)-blue and black patches. In addition, some populations express a striking ventral colour polymorphism (i.e., discrete orange, white and yellow morphs). In this study, we set out to evaluate the potential signalling function of these colour patches by staging pairwise combats between 60 size-matched adult lizards (20 per morph). Combats were held in a neutral arena, with each lizard facing rivals from the three morphs in a tournament with a balanced design. We then calculated a fighting ability ranking using the Bradley–Terry model, and used it to explore whether ventral colour morph, the size of UV-blue and black patches or the spectral characteristics of UV-blue patches (i.e., brightness, hue, chroma) are good predictors of fighting ability. We did not find an effect of the UV-blue patches on contest outcome, but the size of black patches emerged as a good predictor of fighting ability. We also found that winners were more aggressive when facing rivals with black patches of similar size, suggesting that black patches play a role in rival assessment and fighting rules. Finally, we found that orange males lost fights against heteromorphic males more often than yellow or white males. In light of these results, we discuss the potential signalling function of ventrolateral and ventral colour patches in mediating agonistic encounters in this species.

Keywords

fighting ability, intrasexual competition, colour polymorphism, UV signals, melanin, *Podarcis muralis*.

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1. Introduction

In a world of limited resources, males of many vertebrate systems often engage in contests with members of their own species, either over access to females or resources that are necessary to attract potential mates. Chromatic signals mediate the intensity of these confrontations by allowing animals to assess rivals prior to physically interacting, thereby reducing the costs of aggressive conflicts (Briffa, 2014). For example, in male widow birds (*Euplectes ardens*) the size and spectral properties of the red collar predict territorial behaviour and the intensity of aggressive responses against simulated intruders (Pryke et al., 2001). Similar colour ornaments acting as agonistic signals (i.e., armaments) have been described in numerous vertebrates and invertebrates (e.g., Pryke & Griffith, 2006; Miyai et al., 2011; Todd et al., 2011). In lizards, several colour signals have been shown to convey information about dominance and male fighting ability in intrasexual confrontations (Stapley & Whiting, 2006; Baird, 2013; Olsson et al., 2013). For instance, in the Australian frillneck lizard (*Chlamydosaurus kingii*), the carotenoid-based colour of the frill acts as a reliable signal of fighting ability, and males exhibiting more colourful frills are dominant in size-matched dyadic contests (Hamilton et al., 2013). Similarly, research on *Anolis* has demonstrated that the display of brightly coloured dewlaps plays an important communicative role in several contexts, including male–male contests (Jenssen et al., 2000; Tokarz et al., 2003; Simon, 2011), its role in determining contest outcome being dependent on the degree of territoriality of the species (Lailvaux & Irschick, 2007).

Lacertid lizards comprise 42 different genera and more than 300 species inhabiting a wide variety of habitats across the Old World (Uetz & Hosek, 2015). Although their mating systems are insufficiently documented, they seem to be characterized by strong male–male competition over resources (e.g., territoriality in the genus *Podarcis*; Edsman, 1990; Font et al., 2012) or females (e.g., mate-guarding in *Lacerta agilis*; Olsson, 1994; and *Lacerta schreiberi*; Marco & Pérez-Mellado, 1999). The role of colour signals in this group has been relatively neglected, possibly because lacertids have been traditionally considered to be mainly chemosensory (Mason & Parker, 2010). Despite this historical bias, lacertids have a colour vision system as sophisticated as that of other lizards that rely heavily on vision for many aspects of their biology (Pérez i de Lanuza & Font, 2014; Martin et al., 2015a), and very often exhibit complex colour patterns that at least in some

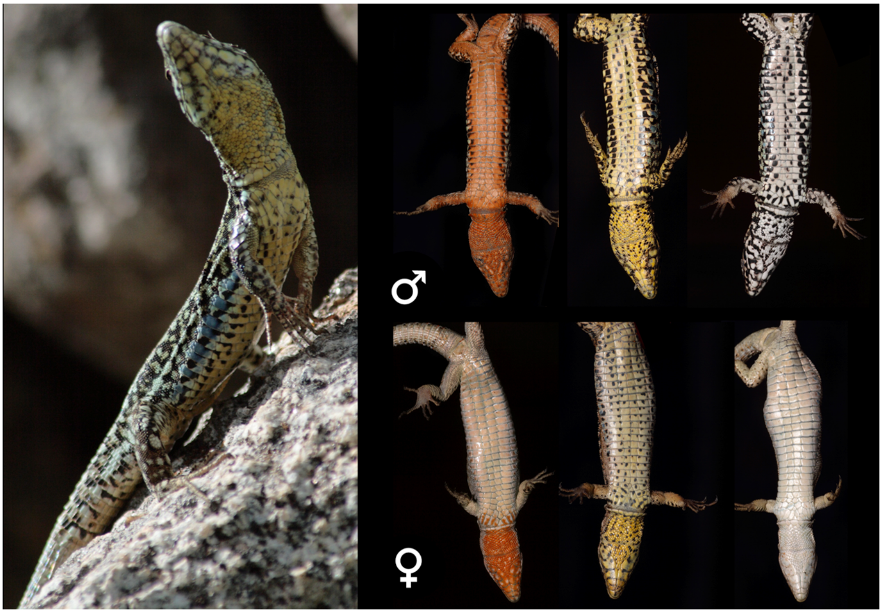

Figure 1. Left: male common wall lizard *Podarcis muralis*. This species has a relatively cryptic dorsum and conspicuous ventrolateral coloration. The ventrolateral area usually presents a complex pattern with black and UV-blue patches. Right: ventral view of both sexes to illustrate the colour polymorphism. Individuals from the three main morphs present orange, yellow or white ventral coloration. In our study population, the ventral colour is restricted to the throat in females, but extends to the belly in males. This figure is published in colour in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/journals/1568539x.

cases seem to have evolved under strong intrasexual competition in males (Bajer et al., 2011; Olsson et al., 2011; Pérez i de Lanuza et al., 2013a).

The common wall lizard (*Podarcis muralis*) is a suitable lacertid model in which to test ideas about the role of colour signals in animal contests. Males of *P. muralis* exhibit a complex ventrolateral pattern on their outer ventral scales (OVS) combining black melanin-based patches (Bowker et al., 1987) and conspicuous ultraviolet (UV)-blue structurally based patches (Figures 1, 2b; Pérez i de Lanuza & Font, 2015). Chromatic variables of male UV-blue patches have been found to correlate with bite force and body condition (Pérez i de Lanuza et al., 2014a), while a recent study showed that the overall area covered by black patches correlates positively with male dominance status (While et al., 2015). In addition to ventrolateral colour patches, some populations of *P. muralis* express a pigment-based ventral colour polymor-

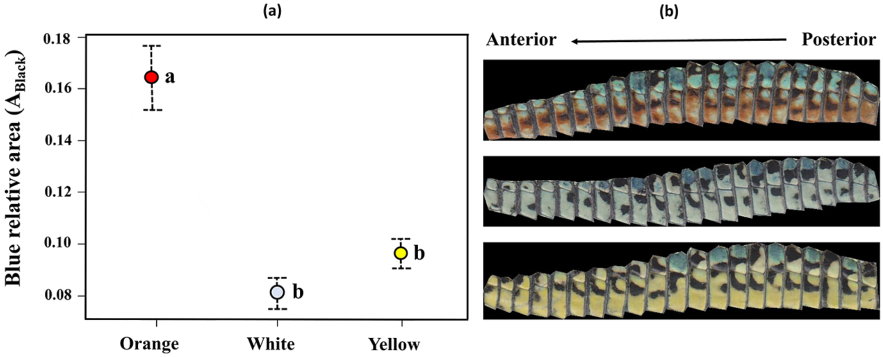

Figure 2. (a) Means of the relative blue area (A_{Blue}) in the ventrolateral patches, for each morph. Error bars represent the standard error of the mean. (b) Representative pictures of the ventrolateral pattern in males of the three pure morphs. Blue coloration sometimes extends to the second row of ventral scales in orange males, while this is rare in white or yellow morph males. This figure is published in colour in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/journals/1568539x.

phism with discrete white (w), orange (o) or yellow (y) pure morphs, as well as intermediate orange-yellow and white-orange morphs (Figure 1; Pérez i de Lanuza et al., 2013b). Although recent studies suggest that different phenotypic optima and breeding strategies may be favoured in each morph, the evolutionary origins and functional significance underlying colour polymorphism in *P. muralis* are still far from resolved (Calsbeek et al., 2010; Galeotti et al., 2013; Pérez i de Lanuza et al., 2013b; but see Sacchi et al., 2015). As in many *Podarcis* species, *P. muralis* shows a resource-based polygynous mating system in which males set out territories that overlap with the home ranges of several females, engaging in frequent contests with intruding males (Edsman, 1990). During these male–male interactions, lizards compress their body laterally (Kitzler, 1941; Olsson, 1992, 1994) in a way that exposes the ventrolateral colour patches, but also the ventral coloration, which could hence also be involved in signalling during agonistic encounters (see Huyghe et al., 2012). These territorial contests are pivotal for the reproductive success of males, since females seem to be attracted to good quality territories rather than to males of certain phenotypic characteristics (Font et al., 2012; Olsson et al., 2013), and frequently remain within the same territory even when the owner is removed and another male takes his place (Edsman, 2001).

In this study we explored the effect of ventral and ventrolateral colour patches during pairwise agonistic interactions between size-matched males with no previous experience that confronted each other in a neutral arena (i.e., no residency effect). We designed our dyadic encounters as a tournament in order to calculate an overall fighting ability for each individual that we could relate to chromatic variables of ventral and ventrolateral colour patches and to levels of winner/loser aggression. We calculated individual fighting ability using a Bradley–Terry model (B-T; Bradley & Terry, 1952; Firth & Turner, 2012), which allows for simultaneous analysis of the relative influence of multiple individual traits on fighting ability, can accommodate an incomplete matrix of confrontations, and takes proper account of dependency among contests involving the same individual (Stuart-Fox et al., 2006).

2. Materials and methods

2.1. Study species

Animals participating in this study came from a colour polymorphic population of *P. muralis* from the Cerdanya valley in the eastern Pyrenees (France). To minimize potential previous fighting experience between opponents, lizards were captured in eight different areas at least 300 m apart, or separated by a geographic barrier (e.g., river). Between 17 and 19 May 2014, we captured 20 adult males of each pure morph (i.e., w, white; y, yellow; o, orange) by noosing, and transported them to the laboratory at the University of Valencia (Valencia, Spain). We only captured lizards with a snout-to-vent length (SVL) larger than 65 mm to restrict the sample to large adult males, ensuring that all individuals participating in the tournament had fully developed colour patches (Pérez i de Lanuza et al., 2013b, 2014a) and were large enough to exhibit full-blown agonistic behaviour (Edsman, 1990), while minimising the range of possible size differences between potential contestants (Sacchi et al., 2009). For each lizard, we used SVL (± 1 mm) and body mass (± 0.1 g) measures at the time of capture to calculate a body condition index (BCI) as the residual from a least-squares linear regression of log(body mass) against log(SVL) (Green, 2001).

2.2. Animal housing and maintenance

In the laboratory, we housed lizards individually in glass terraria (20 × 40 and 25 cm high) with an artificial grass substrate, a water dish, a shelter, and a small brick for basking over which we suspended a 40 W incandescent lamp. Shelters consisted of two tiles (one serving as base and one serving as a removable lid) separated with felt so as to leave a 90 × 90 × 12 mm cavity for the lizard to take refuge. We covered all but the front wall of every terrarium with brown cardboard paper to prevent visual contact between animals from adjoining terraria. We set the temperature and light cycle to mimic average field conditions during the reproductive season at the capture site (15°C at night, 26°C during the day; 12.5L:11.5D), and additionally provided all lizards with full spectrum light (Reptistar 5.0: Sylvania, Danvers, MA, USA) for 1 h three times weekly (12.00–13.00 h) to ensure an effective calcium metabolism, preventing avitaminosis and metabolic bone disease (Adkins et al., 2003). We fed lizards *Tenebrio molitor* larvae dusted with vitamins (Exo Terra, Montreal, QC, Canada) three times weekly and allowed lizards to acclimate to laboratory conditions for 14 days before the experiments. Overall, animals stayed in the laboratory less than two months (19 May–13 July) before being released back to their capture location in the field.

2.3. *Experimental design*

In order to examine the effect of colour patches on male–male contest outcome, we used a tournament design in which every male faced three different rivals (one of each morph) with a minimum of three days between consecutive contests. The order of contests was randomized for each morph combination to control for a potential order effect. At the conclusion of the 90 initially planned contests, we staged nine additional heteromorphic fights between the three more successful individuals of each morph in order to increase the nestedness of the final tournament network and give additional resolving power to the B-T model (Stuart-Fox et al., 2006); note these fights were pooled with the rest in our analyses. To reduce the potential noise introduced by size asymmetry and prior experience (Baird, 2013), we allowed a maximum size difference between contestants of 10% in SVL, and only confronted males from different capture areas (see above). Finally, to eliminate the effect of residency, we staged the encounters in a neutral experimental arena consisting of a glass terrarium (70 × 30 and 40 cm high) divided into two equal compartments by an opaque partition. Each compartment was illuminated by a 40 W lamp and a full spectrum lamp (Reptistar 5.0) equipped with a high frequency ballast (Quicktronic, Osram, Munich, Germany) (Evans et al., 2006). We performed five combats daily between

Table 1.
Behaviours used to calculate the individual Aggression Score (AS) of lizards participating in the tournament (see text for details).

Behaviour	Description	Score
No response		0
Stare	Looks toward rival	1
Approach	Reduces distance with rival	2
Chase	Quickly follows fleeing rival	3
Display	Throat extension, trunk compression and back arching	4
Mouth gaping	Opens mouth	5
Lunge	Hits rival with closed mouth	6
Bite	Holds rival for <2 s	7
Bite-hold	Holds rival for >2 s	8

4 June and 1 July 2014 at the natural peak activity hours for this population (10.00–13.30 and 16.00–19.00 local time). Immediately before each trial, we randomly determined the initial side of the arena for each opponent, chased the lizards into their shelter, and transferred them into the experimental arena along with their own basking stone. We then disassembled the shelter leaving the base tile in the arena and allowed a 30 min acclimation period before withdrawing the opaque partition and allowing the opponents to interact. All contests were filmed with a digital video camera (XM2, Canon®, Tokyo, Japan). The experimental arena was thoroughly cleaned with water, then alcohol, and again with water after every combat.

In each contest, we designated a winner only if one of the males ceased agonistic behaviours (Table 1) and repeatedly fled if approached by its opponent. In addition to this categorical measure, we also calculated a quantitative variable based on the level of aggression shown by each lizard in each staged contest. In order to do this, we played back filmed contests and recorded each time a lizard performed any of the behaviours described in Table 1. We assigned a score to each of these behaviours based on Carazo et al. (2008), which allowed us to calculate an individual aggression score as the sum of every agonistic behaviour performed by each lizard in each contest (i.e., one aggression score per lizard and contest). We also calculated a measure of aggression ratio for each contest as the quotient between the winner aggression score and the sum of both winner and loser aggression scores (i.e., one aggression ratio per contest). We interrupted combats if they escalated to the point of risking injuries (bite-holds in head or limbs lasting more than

1 min), which was only necessary in one combat. None of the contests resulted in observable injuries.

2.4. Colour measurements

We determined morph visually at the time of capture in the field (Pérez i de Lanuza et al., 2013b). We recorded spectrophotometric measurements in a single session at the conclusion of all contests to minimize the stress induced by manipulation prior to the experiments. We recorded reflectance spectra of the UV-blue patches with a portable USB-2000 spectrometer equipped with a PX-2 Xenon strobe light (Ocean Optics, Dunedin, FL, USA; for further details see Font et al., 2009). Measurements encompassed the 300–700 nm range to cover the entire visual spectrum of *P. muralis* (Pérez i de Lanuza & Font, 2014; Martin et al., 2015a). We recorded spectra from the second, third and fourth UV-blue patches in both sides and averaged them to provide an individual mean spectrum for each lizard. We then extracted data for the standard variables describing colour: brightness (spectral intensity), chroma, and hue (Endler, 1990; Bradbury & Vehremcamp, 2011). We calculated brightness of the UV-blue patches (OVS-Qt) as the total area under the 300–700 nm reflectance curve, hue (OVS-hue) as the wavelength where peak reflectance is located, and UV-chroma (OVS-C_{UV}) as the area under the 300–400 nm reflectance curve divided by the area under the entire spectral curve (i.e., 300–700 nm) (Endler, 1990; Molina-Borja et al., 2006; Pérez i de Lanuza et al., 2014a).

In addition, we measured the relative ventrolateral area covered by black and blue patches in each lizard. For this purpose, we obtained an image of both sides of every lizard using a portable digital scanner (Lide 700F, Canon®), and then calculated the proportion of black and blue coloured area out of the total ventrolateral surface (Figure 2b) using ImageTool Version 3.0 (Wilcox et al., 2002). We defined total ventrolateral surface as the area covered by the first two rows of OVS, between the insertions of the fore and hind limbs. We determined the proportion of coloured area by dividing the blue or black area by total ventrolateral surface; the same researcher (J.A.) measured all areas following a blind protocol. We confirmed the reliability of these measurements by calculating repeatability (r) as defined by Lessels & Boag (1987) in a subsample of lizards ($N = 15$; $r_{\text{blue}} = 0.97$; $r_{\text{black}} = 0.97$). Due to an endoparasitic infection unnoticed at the time of capture, one lizard died in the laboratory before its colour patches could be measured, reducing the final sample for colour variables to 59 individuals.

2.5. Statistical analyses

We used the Bradley–Terry model (B-T model) for planned comparisons to extract a global index of fighting ability for each contestant (Bradley & Terry, 1952). Staged contests can be considered as a subset of all the possible interactions between rivals from which relative fighting ability can be estimated. The B-T model is a type of generalized linear model that takes proper account of dependencies within and between contests while accommodating an incomplete matrix of all possible interactions (Firth, 2005). This model assumes every contestant has a positive value of fighting ability, such that the most likely contest outcome is given by the ratio of the abilities of both contestants (Whiting et al., 2006), and estimates a hierarchy based on transitivity (Stuart-Fox et al., 2006). Using the R package BradleyTerry2 (Firth & Turner, 2012) in R 2.1.5.1 (R Core Team, 2014) we fitted B-T models to 99 fights involving 60 lizards, obtaining a ranking based on individual fighting ability estimates. The model was calculated with 'bias reduction', as is recommended when players face each other only once, and coding ties as half a win for each rival (Firth & Turner, 2012).

We used a generalized linear model (GLM) to explore the relationship between ventral colour morph (considered as a fixed factor), the three spectral variables of the UV-blue patches (i.e., OVS-Qt, OVS-Hue, OVS-C_{UV}), the relative area of blue (A_{Blue}) and black (A_{Black}) colour patches, body size (SVL), and fighting ability estimates as the dependent variable. We checked that all variables conformed to heteroscedasticity and normality assumptions, and assumed a Gaussian distribution for fitting the GLM. We started with the following full model representing our main hypothesis: Fighting ability $\sim$ Morph $+$ SVL $+$ OVS-Qt $+$ OVS-C_{UV} $+$ OVS-Hue $+$ A_{Blue} $+$ A_{Black}. Model fitting was done manually in R 2.1.5.1 (R Core Team, 2014) and model selection was conducted using backward single term deletions ($p \leqslant 0.05$) of the saturated model followed by model comparisons via likelihood ratio tests.

We run linear mixed effects models to explore the relationship between individual aggression score and colour variables. We also explored potential receptor-dependent costs of coloration (i.e., variation in aggression score caused by rival coloration) by running linear mixed models with SVL, OVS-Hue, OVS-C_{UV}, OVS-Qt, Morph, A_{Blue}, A_{Black} and rival aggressive score as fixed factors, and intercepts for winner and loser identity as random effects. Finally, in order to assess the relationship between aggression ratio, body size, morph, and the size of black colour patches, we run the following post hoc linear mixed effects model: Aggression ratio $\sim$ Winner $A_{\text{Black}} \times$ Loser A_{Black} + Winner Morph × Loser Morph + Winner SVL × Loser SVL + (1|Winner) + (1|Loser). p-values were obtained via likelihood ratio tests of the full model after single-term deletions.

3. Results

We could identify a winner in 76 out of 99 contests (Figure A1 in the Appendix). Overall, the mean aggression score of each individual male across contests was significantly correlated with the estimate of fighting ability calculated with the B-T model from the global tournament network (Mean aggression score $\sim$ Fighting ability, $R = 0.59$, $t_{56} = 5.515$, $p < 0.0001$, Figure A2 in the Appendix).

3.1. *Inter-morph differences*

Yellow males won 66% of heteromorphic combats ($N = 36$), while white males won 58% ($N = 39$) and orange males only 24% ($N = 37$). Yellow and white males defeated orange males in the majority of contests (W > O: 76%, $N = 17$; Y > O: 75%, $N = 20$), while results for contests between white and yellow males were more balanced (Y > W: 58%, $N = 19$). However, males with different ventral coloration did not differ in their mean aggression score across contests (Mean AS, $F_{2,58} = 1.73$, $p = 0.187$). We found no significant differences in body size (SVL, $F_{2,60} = 1.84$, $p = 0.168$) or body condition (BCI, $F_{2,60} = 2.858$, $p = 0.0658$) among morphs, despite a trend for yellow males in our sample to show lower BCI than males of the other two morphs (Figure A3 in the Appendix). UV-blue spectral variables did not differ between morphs (OVS-Qt, $F_{2,59} = 2.807$, $p = 0.0691$; OVS-Hue, $F_{2,59} = 0.088$, $p = 0.916$; OVS-C_{UV}, $F_{2,59} = 1.349$, $p = 0.268$). A_{Black} showed marginally non-significant differences between morphs (A_{Black}, $F_{2,58} = 3.038$, $p = 0.0561$), likely due to males of the white morph having slightly larger A_{Black} than males from the orange morph (Tukey's post hoc, W-O ± SEM $= 0.058 \pm 0.024$, $t_{2,58} = 2.39$, $p = 0.052$; Y-O ± SEM $= 0.042 \pm 0.025$, $T_{2,58} = 1.69$, $p = 0.218$; Y-W ± SEM $= -0.016 \pm 0.025$, $t_{2,58} = -0.64$, $p = 0.801$). Orange morph males had higher A_{Blue} than white and yellow males (Figure 2; A_{Blue}, $F_{2,58} = 25.49$, $p < 0.0001$; Tukey's post hoc, W-O ± SEM $= -0.083 \pm 0.012$, $t_{2,58} = -6.75$,

$p < 0.0001$; Y-O ± SEM $= -0.067 \pm 0.013$, $t_{2,58} = -5.33$, $p < 0.0001$), while the white and yellow morphs did not differ (Tukey's post hoc, Y-W ± SEM $= 0.016 \pm 0.013$, $t_{2,58} = 1.24$, $p = 0.434$).

3.2. *Predictors of fighting ability*

In accordance with the observed differences in contest outcome, we detected a marginally significant difference in the fighting ability of different morphs (one way-ANOVA: Fighting ability ∼ Morph, $T_{2,59} = 3.337$, $p = 0.043$; Figure 3). Orange males seem to have slightly lower fighting ability than yellow males (Tukey's post hoc, W-O ± SEM $= 0.007 \pm 0.003$, $t_{2,58} = 2.08$, $p = 0.104$; Y-O ± SEM $= 0.008 \pm 0.003$, $t_{2,58} = 2.37$, $p = 0.054$; Y-W ± SEM $= -0.001 \pm 0.003$, $t_{2,58} = 0.35$, $p = 0.93$).

However, after model selection, only A_{Black} (mean ± SEM $= 0.249 \pm 0.128$) remained as a significant predictor of individual fighting ability (Fighting ability $\sim A_{\text{Black}}$, $t_{56} = 2.453$, Std. Coef. $= 0.31$, $p = 0.017$, Figure 4). A_{Black} was not related to mean aggression score (Spearman correlation: $S_{56} = 26808$, $\rho = 0.175$, $p = 0.188$), BCI ($T_{56} = 0.032$, $p = 0.974$) or A_{Blue} ($t_{56} = -1.23$, $p = 0.223$). We found no relationship between the spectral variables of UV-blue patches and fighting ability ($R < 0.1$, $p > 0.2$ in all cases).

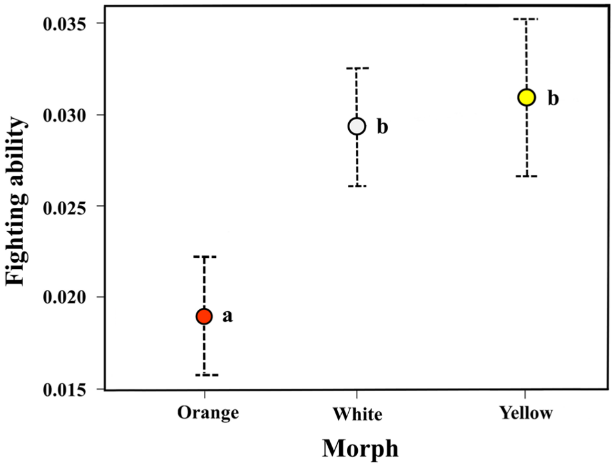

Figure 3. Within-morph means of fighting ability estimates obtained with the Bradley–Terry model. Error bars represent the standard error of the mean. This figure is published in colour in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/journals/1568539x.

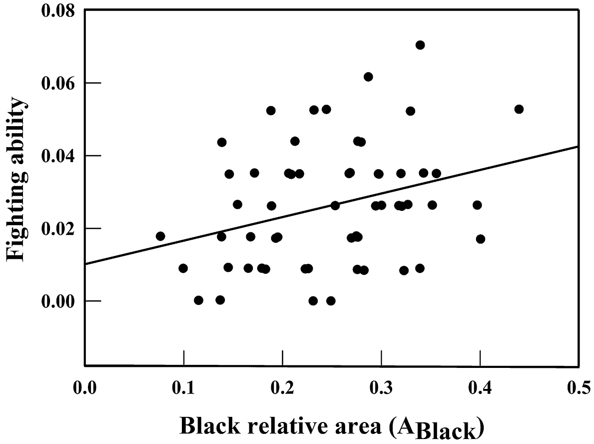

Figure 4. Scatterplot showing the relationship between fighting ability and black relative area in the ventrolateral scales (A_{Black}) for each individual participating in the tournament.

3.3. Predictors of aggression score and aggression ratio

Winner or loser aggression scores were unrelated to colour variables from any of the contestants. In every mixed model, the aggression score of one rival was best predicted only by the aggression score of its opponent (Winner aggression score $\sim$ Loser aggression score, $t_{5,76} = 5.6$, $p < 0.0001$). In contrast, aggression ratio showed a significant relationship with the A_{Black} of both contestants. Specifically, combats between males with similar A_{Black} resulted in higher aggression ratios. After model selection, aggression ratio was found to be significantly related to the interaction between the A_{Black} of each contestant (Winner $A_{\mathrm{Black}} \times$ Loser A_{Black}; $\chi^2 = 4.74$, df $= 1$, $p = 0.029$) (Table 2), but not with their morph (Winner Morph × Loser Morph; $\chi^2 = 6.63$, df $= 1$, $p = 0.16$), nor their SVL (Winner SVL × Loser SVL; $\chi^2 = 0.0003$, df $= 1$, $p = 0.99$). To examine the significance of this interaction, we used a 3D plot, which suggests that aggression ratio increases as the asymmetry between winner and loser A_{Black} decreases (Figure 5).

4. Discussion

In this study, we set out to assess the potential role of *P. muralis* ventral (i.e., morph; orange, yellow or white) and ventrolateral (i.e., UV-blue and black) colour patches in determining contest outcome in male–male conflicts. Neither the size nor the spectral variables of UV-blue patches affected contest

Table 2.
Mixed effects linear model used to explore the relationship between body size, morph, black coloration and aggression ratio in staged contests between *Podarcis muralis* male lizards.

Term	Coefficient	SE	Variance	SD	χ^2	df	p
Fixed factors							
Winner A_{Black} × Loser A_{Black}	0.19	0.09			4.74	1	0.029
Winner A_{Black}	−0.09	0.12			0.53	1	0.47
Loser A_{Black}	−0.02	0.13			0.03	1	0.87
Random factors							
Loser identity			0.41	0.64			
Winner identity			0.33	0.57			

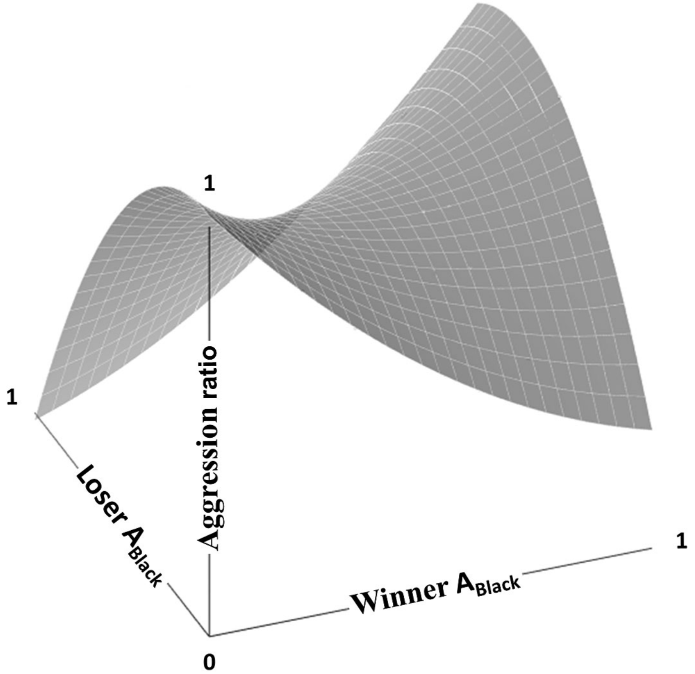

Figure 5. 3D plot exploring the relationship between aggression ratio and the interaction between the black relative area (A_{Black}) of both opponents.

outcome, but both A_{Black} and ventral colour morph did. A_{Black} was a particularly good predictor of fighting ability that also affected the difference in aggression score between winners and losers, in a way that suggests it may be functional as an agonistic signal (see below).

4.1. Black ventrolateral patches as chromatic signals of fighting ability

As stated, A_{Black} was the best predictor of fighting ability in our models, and we also found that the interaction between winner and loser A_{Black} was a significant predictor of the aggression ratio of a contest. Namely, winners behaved relatively more aggressively towards losers as the difference in winner-loser A_{Black} decreased, and less so as it increased. This effect was strong and persisted despite controlling for the interaction between both rivals' SVL in our post hoc model. Hence black coloration does not appear to act as a simple surrogate of size (Gosá, 1987) but rather as an agonistic signal used by males to assess their rivals' fighting ability, and adjust their investment in the fight accordingly. This is in agreement with a recent study reporting that the amount of ventral black coloration strongly correlates with dominance status in two different lineages of *P. muralis* (i.e., Tuscany and Western Europe lineages; While et al., 2015). Furthermore, results from this study strongly suggest that the introgression of the Italian lineage (with exaggerated black coloration) onto Western France is likely driven by male–male competition favouring this character (While et al., 2015).

Several studies across different taxa have also documented the influence of black (melanin-based) coloration in the formation of hierarchies, dominance status, and in the resolution of contests (insects: Tibbetts et al., 2010; fish: Horth, 2003; Johnson & Fuller, 2014; birds: González et al., 2002; Chaine et al., 2011; reptiles: Lebas & Marshall, 2001; Osborne, 2005; Mafli et al., 2011; Qi et al., 2011). Interestingly, and in agreement with predictions from the sequential assessment game model (Enquist & Leimar, 1983), we found that the most aggressive contests were those involving opponents with similarly-sized melanin patches, which has also been reported in organisms as phylogenetically distant as the paper wasp (*Polistes dominulus*; Tibbetts et al., 2010; Tibbets & Sheehan, 2011), or the bluefin killifish (*Lucania goodie*; Johnson & Fuller, 2014). Thus, melanin-based signals seem to convey valuable information about opponents' fighting abilities in a broad diversity of taxa.

Melanin-based signals are usually considered to have low production costs in vertebrates (Hill & Brawner, 1998; Badyaev & Young, 2004; Stoehr, 2006; but see Roulin, 2016), but there is evidence in different species suggesting they frequently act as badges of status with socially-enforced costs (e.g., Møller, 1987; Senar & Camerino, 1998; Tibbets & Dale, 2004; Diep & Westneat, 2013; Roulin, 2016). In addition, activity in the melanocortin system — which is responsible for the production of melanin-based coloration — covaries with several behavioural and physiological traits in vertebrates, such that darker individuals are often more aggressive, sexually more active and more resistant to various sources of stress than lighter animals (Ducrest et al., 2008). We suggest future studies should examine the mechanisms underlying honest signalling by means of melanin-based colour patches in *P. muralis*.

4.2. Size and reflectance of UV-blue patches are not related to contest outcome

We did not find a relationship between fighting ability or aggression score and the size or any of the spectral characteristics of UV-blue patches. Taken at face value, these results seem to argue against the possibility that conspicuous UV-blue patches convey information about male competitive skills. However, there is now compelling evidence suggesting that UV-blue patches may play an important role in intra-sexual competition in several *Podarcis* species (Marshall & Stevens, 2014; Pérez i de Lanuza et al., 2014a; Martin et al., 2015b), including *Podarcis muralis* (McGregor et al., 2016, unpublished data). It is possible that UV-blue patches play a significant role in more natural contexts than the short-range encounters artificially enforced by our experimental design (see also Martin et al., 2015b). It is also possible that, due to potential within-season changes in coloration, our spectrophotometric measurements, taken after the experiment was completed and towards the end of the reproductive season in this population, failed to reflect the true characteristics of UV-blue patches during combats (Martin et al., 2015b). Finally, our negative results may be due to UV-blue patches playing an important role only in the early stages of contests (when assessment takes place), but failing to predict contest outcome if the conflict escalates into physical aggression (Baird et al., 2013). We suggest future studies should aim to experimentally manipulate the area and spectral variables of the UV-blue patches, and set up contests in larger enclosures.

4.3. Morph-specific bias in fighting ability

Our finding that orange males have a propensity to lose heteromorphic confrontations seems to contradict a previous study that did not report any inter-morph differences in fighting ability in Italian polychromatic populations of *P. muralis* (Sacchi et al., 2009).

Although this discrepancy might arise from geographical/phylogenetic differences between the Italian and French lineages, it could also have originated from crucial differences in the experimental design and data analysis. Sacchi et al. (2009) found no differences among morphs in the aggression exhibited during contests, and no effect of ventral coloration over contest score (calculated as the difference in aggression score between contestants). In agreement with these results, in the present study we found no differences in levels of mean aggression score among morphs, nor of an effect of the opponents' ventral coloration on aggression ratio. However, the analysis of fighting ability estimates revealed significant inter-morph differences in the ability to win intrasexual confrontations. Estimating fighting ability directly from contest outcome in a nested tournament experimental design (rather than extrapolating it from aggressive scores obtained from independent subsets of contests; Sacchi et al., 2009), may have allowed for a more powerful analysis of the role played by ventral coloration in the agonistic context. Additionally, as Sacchi et al. (2009) performed combats in a resident-intruder scenario, residency — a strong determinant of contest outcome in lizard encounters (Whiting, 1999; Olsson & Shine, 2000) — may have easily masked the relatively weaker effect of ventral coloration (Stuart-Fox & Johnston, 2005). In *Lacerta agilis*, for example, resident males defeated intruder males in all staged combats regardless of their coloration (Olsson, 1993), while males with larger colour patches were more likely to win fights when confrontations took place in a neutral arena (Olsson, 1994). Other previous studies with lizards have also linked colour polymorphisms to differences in fighting ability using experimental designs that control for a residency effect. For example, in the agamid *Ctenophorus decresii* orange males consistently showed the highest levels of aggression when confronted to the other morphs (Yewers et al., 2016), while in *Ctenophorus pictus* red-headed males outcompeted yellow-headed males in dyadic contests (Healey et al., 2007). In the phrynosomatid *Urosaurus ornatus* blue–green throated males were more likely to defeat orange throated males (Carpenter, 1995). In contrast to our results, in populations of *Podarcis melisellensis* with the same type of orange–yellow–white ventral polymorphism as *P. muralis*, orange males show greater fighting ability than yellow or white males (Huyghe et al., 2012).

Sexual selection often plays a role in the origin and maintenance of population polymorphisms as strong intra-sexual competition might promote the evolution and maintenance of alternative mating strategies (Taborsky, 2008), which frequently correlate with discrete phenotypic traits (e.g., colour morphs; Wellenreuther et al., 2014). For example, in *Uta stansburiana* periodic oscillations in the relative frequencies of three discrete morphs reflect a cyclical 'rock–paper–scissors' game that is driven by frequency-dependent selection on three alternative reproductive strategies (Sinervo & Lively, 1996). Following these results, much attention has been paid to the possibility that other lizard colour polymorphisms might similarly reflect the existence of alternative reproductive tactics maintained by 'rock–paper–scissors' dynamics of selection. For instance, San-José et al. (2014) found that the lacertid *Zootoca vivipara* experiences similar fluctuations in the relative frequencies of morphs that appear to be consistent with frequency-dependent cycles of cumulative selection. However, the reason why alternative mating strategies should be associated with different colorations remains an intriguing question far from being resolved (Pérez i de Lanuza et al., 2013b; but see Sinervo et al., 2006), and future experimental studies in polymorphic systems should test the presumed signalling role of the alternative ventral colours.

Until now, research on colour polymorphism in *P. muralis* has revealed several between-morph differences in morphological and physiological traits (Sacchi et al., 2007a, b; Calsbeek et al., 2010; Galeotti et al., 2010, 2013) that might suggest that different phenotypic optima are being favoured in each morph (i.e., increased body size and susceptibility to infection in the orange morph; Calsbeek et al., 2010), but it is still unclear whether these differences correlate with morph-specific behavioural syndromes and/or mating strategies (but see Sacchi et al., 2009; Pérez i de Lanuza et al., 2013b; Sacchi et al., 2015). The lower fighting ability of orange males in our experiments could be explained by a number of causes, such as by inter-morph behavioural differences in territory acquisition and defence or in inherent fighting ability. The relative impact of colour signals on contest outcome has been found to vary in species of *Anolis* with different levels of territoriality (Lailvaux

& Irschick, 2007). It would be interesting to investigate whether the differences we observed in the fighting ability and black coloration of orange males might be explained by differences in territorial behaviour, and hence in the relative importance of black coloration, across morphs. We suggest future studies should take a closer look at inter-morph differences in fighting ability and other behavioural and physiological aspects relevant to male–male competition (e.g., territoriality and sperm competition).

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Appendix

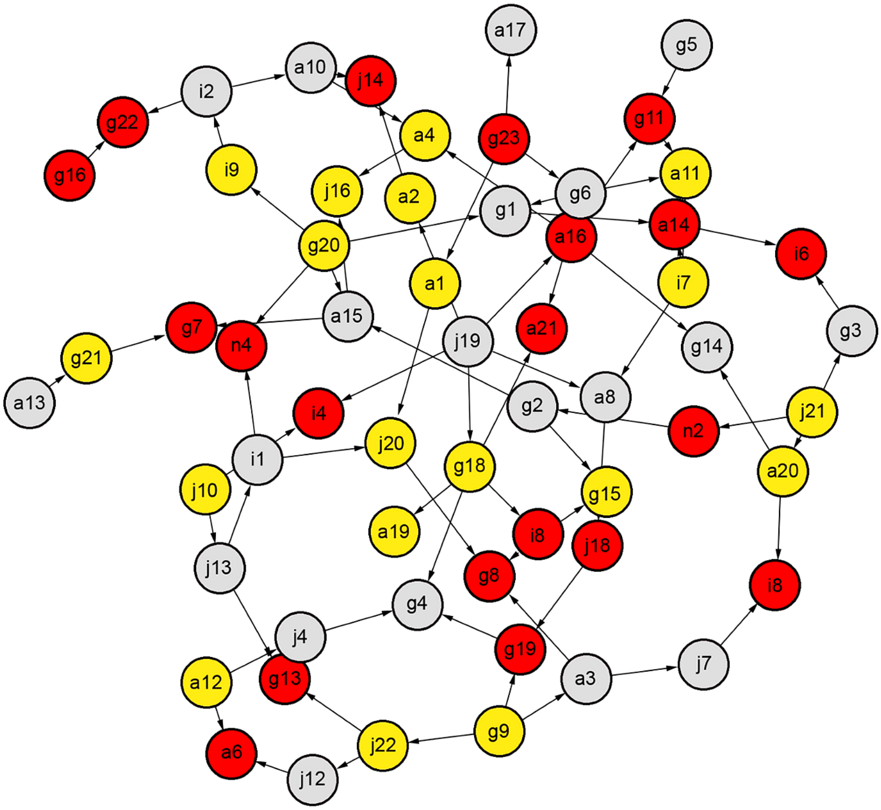


Figure A.1. Tournament network including all the 76 contests (out of 99 staged) in which a winner could be determined. Numbers inside circles denote individuals and the colour represents their morph (o, red; w, grey; y, yellow). Arrows connect opponents that were confronted, pointing toward the loser. The Bradley–Terry model calculates individual fighting ability estimates from nested tournament networks such as this one. This figure is published in colour in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/journals/1568539x.

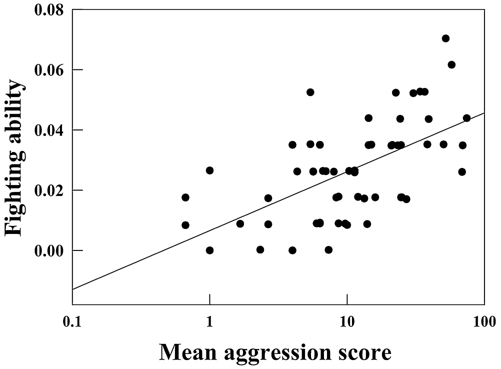


Figure A.2. Scatterplot showing the relationship between mean aggression score (AS) and fighting ability for each individual participating in the tournament.

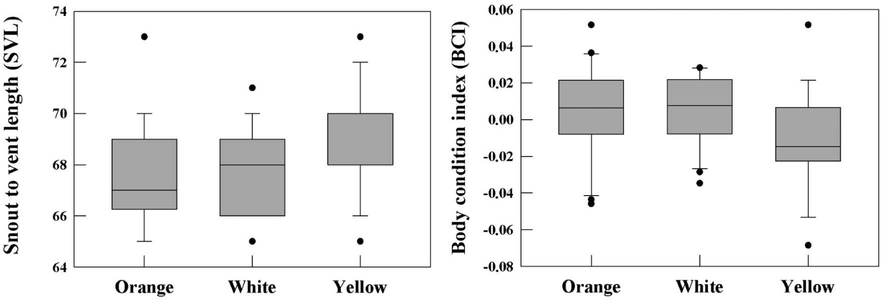
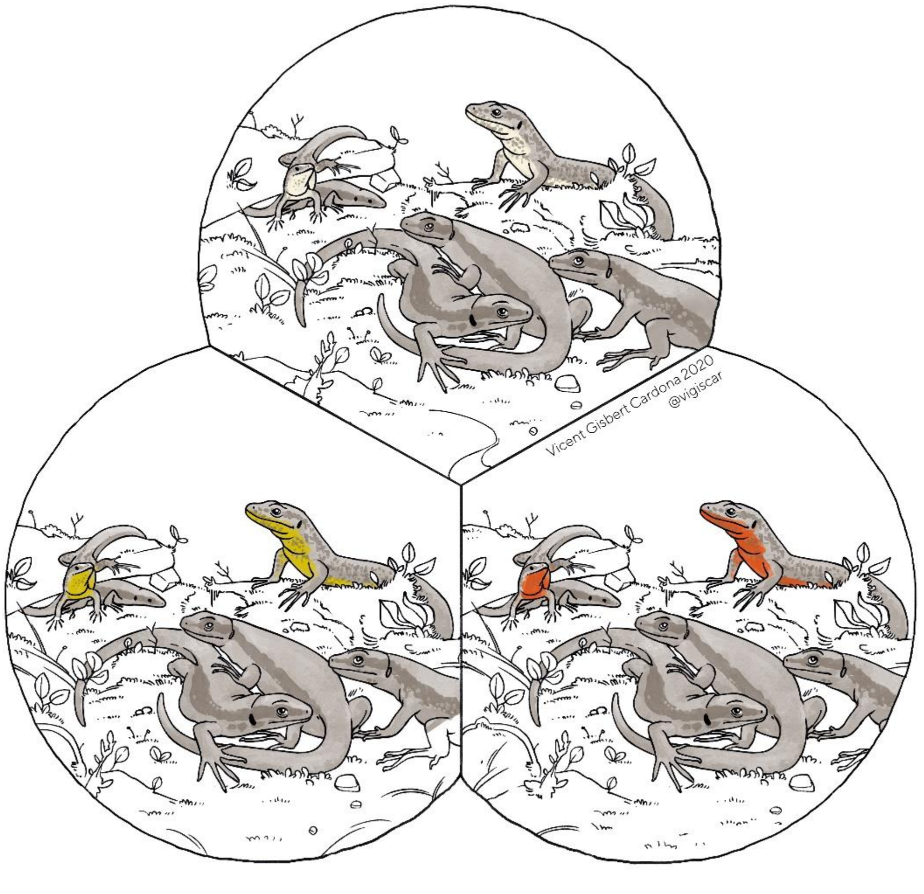


Figure A.3. Box plots showing the values of BCI and SVL separated by colour morph.

Chapter IV

"Everyone knows that dragons don't exist. But while this simplistic formulation may satisfy the layman, it does not suffice for the scientific mind. The brilliant Cerebron, attacking the problem analytically, discovered three distinct kinds of dragons: the mythical, the chimerical, and the purely hypothetical. They were all, one might say, non-existent, but each non-existed in an entirely different way".

·Stanislaw Lem, The Cyberiad·



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No evidence for differential sociosexual behavior and space use in the color morphs of the European common wall lizard (*Podarcis muralis*)

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Abstract

Explaining the evolutionary origin and maintenance of color polymorphisms is a major challenge in evolutionary biology. Such polymorphisms are commonly thought to reflect the existence of alternative behavioral or life-history strategies under negative frequency-dependent selection. The European common wall lizard *Podarcis muralis* exhibits a striking ventral color polymorphism that has been intensely studied and is often assumed to reflect alternative reproductive strategies, similar to the iconic "rock–paper–scissors" system described in the North American lizard *Uta stansburiana*. However, available studies so far have ignored central aspects in the behavioral ecology of this species that are crucial to assess the existence of alternative reproductive strategies. Here, we try to fill this gap by studying the social behavior, space use, and reproductive performance of lizards showing different color morphs, both in a free-ranging population from the eastern Pyrenees and in ten experimental mesocosm enclosures. In the natural population, we found no differences between morphs in site fidelity, space use, or male–female spatial overlap. Likewise, color morph was irrelevant to sociosexual behavior, space use, and reproductive success within experimental enclosures. Our results contradict the commonly held hypothesis that *P. muralis* morphs reflect alternative behavioral strategies, and suggest that we should instead turn our attention to alternative functional explanations.

KEYWORDS

alternative strategies, color polymorphism, free-ranging population, mesocosm, *Podarcis muralis*, social behavior

1 | INTRODUCTION

Explaining the maintenance of phenotypic variability over time remains a central question in evolutionary biology. Population polymorphisms are a particularly widespread form of phenotypic variability (Galeotti, Rubolini, Dunn, & Fasola, 2003; Gray & McKinnon, 2007; Mckinnon & Pierotti, 2010; Roulin, 2004; Svensson, 2017). In polymorphic populations, individuals of the same sex and age may exhibit different phenotypes (e.g., color morphs) that are heritable, fixed in adults, and not condition-dependent (Galeotti et al., 2003; Mckinnon & Pierotti, 2010; Roulin, 2004). Selectively neutral polymorphisms are expected to be lost eventually due to stochastic processes (i.e., genetic drift; Roulin, 2004), and the long-term maintenance of polymorphisms within a population requires some form of balancing selection, for example, via nonrandom mating, source–sink dynamics, overdominance, or rare morph advantage (Galeotti et al., 2003; Roulin & Bize, 2007; Roulin, 2004; Svensson, 2017; Wellenreuther, Svensson, & Hansson, 2014).

Sexual selection often plays a major role in the maintenance of color polymorphisms (Roulin & Bize, 2007; Wellenreuther et al., 2014). Discrete variation among conspecifics in behavior or life histories associated with reproduction (termed alternative reproductive strategies, ARS) is frequently coupled with alternative color morphs (Ducrest, Keller, & Roulin, 2008; Roulin & Bize, 2007; Roulin, 2004; Shuster & Wade, 2003; Wellenreuther et al., 2014; Willink, Duryea, & Svensson, 2019; Zamudio & Sinervo, 2000). ARS are particularly frequent in males of polygynous (or polygynandrous) species, which experience a high variance in mating success and, thus, stronger sexual selection. In these species, the uneven distribution of fertilizations among males playing the conventional strategy allows the evolution of behavioral ARS (e.g., monogynist, satellite, sneaker) adapted to exploit distinct mating niches (Greenfield & Shelly, 2008; Shuster, 2008; Shuster, Briggs, & Dennis, 2013; Shuster & Wade, 2003; Taborsky, Oliveira, & Brockmann, 2008; Waltz, 1982). Genetically fixed strategies are favored whenever males tend to experience only one selective regime during their lifetime, so that specializing in alternative resources has higher fitness than being a generalist (Brockmann, 2001; Roulin, 2004; Zamudio & Sinervo, 2003). For instance, certain characteristic of the environment (e.g., heterogeneous distribution of resources, short breeding season) can interact with aspects of the species' ecology (e.g., short life span, adaptive site fidelity) producing resource-defense mating systems (i.e., territoriality) in which subordinate males are unlikely to disperse. Males of such species tend to experience a single social environment during their lifetime, promoting the evolution of fixed, rather than conditional, behavioral strategies (Shuster & Wade, 2003; Zamudio & Sinervo, 2003). Balancing selection can maintain these alternative strategies, even if genetically fixed, whenever they obtain equal average fitness across contexts. This can happen in a wide array of scenarios, such as marked seasonality or spatial environmental heterogeneity (Brockmann, 2001; Taborsky & Brockmann, 2010). In sympatry, ARS can obtain equal fitness through frequency-dependent selection (Gross, 1996; Shuster & Wade, 2003; Taborsky et al., 2008). Occasionally, two or more strategies can cycle in frequency over time if presenting a lower frequency confers a fitness advantage (negative frequency-dependent selection (NFDS; Brockmann, 2001; Roulin, 2004; Taborsky et al., 2008; Takahashi, Yoshimura, Morita, & Watanabe, 2010; Willink et al., 2019). Color polymorphism may participate of this evolutionary process and be maintained under two different conditions. On the one hand, alternative color morphs may be directly selected for because of an adaptive advantage they confer in the context of ARS (e.g., sexual mimicry in damselflies; Svensson, Willink, Duryea, & Lancaster, 2020; Willink et al., 2019). Alternatively, color morphs may be an indirect by-product of selection on other attributes related to the ARS (i.e., when genes involved in morphology, physiology, or behavior have pleiotropic effects on color production; Galeotti et al., 2003; Roulin & Bize, 2007; Roulin, 2004, 2016; Wellenreuther et al., 2014).

One of the best-studied cases of color polymorphic ARS is the side-blotched lizard, *Uta stansburiana*. Adult males of this species present one of three alternative throat colors (blue, orange, and yellow), each of which is associated with different sociospatial behaviors. Orange-throated males establish large territories overlapping with several females by outcompeting blue-throated males in territorial disputes. These vast territories make orange males vulnerable to losing fertilizations in favor of the nonterritorial yellow morph, which uses female mimicry to sneak copulations opportunistically. In turn, blue-throated males compensate their competitive disadvantage by guarding females directly and hence securing more fertilizations against the yellow sneaker males (Alonzo & Sinervo, 2001; Calsbeek & Sinervo, 2002a; Sinervo & Lively, 1996; Sinervo et al., 2006; 2007; Sinervo & Zamudio, 2001; Zamudio & Sinervo, 2000;). This dynamic gives rise to periodic oscillations in the relative frequencies of *U. stansburiana* male color morphs, in a cyclical “rock–paper–scissors” (RPS) game whereby each color morph, when predominant, is vulnerable to invasion by another color morph (Sinervo & Calsbeek, 2006; Sinervo & Lively, 1996). These results sparked a proliferation of studies aimed at detecting similar differences in reproductive behavior among the numerous species of lizards with color polymorphism (Bastiaans, Morinaga, Castañeda Gaytán, Marshall, & Sinervo, 2013; Fernández et al., 2018; Huyghe, Herrel, Adriaens, Tadić, & Van damme, 2009; Huyghe, Vanhooydonck, Herrel, Tadic, & Van Damme, 2007; Olsson, Healey, & Astheimer, 2007; Olsson, Stuart-Fox, & Ballen, 2013; San-Jose, Peñalver-Alcázar, Milá, Gonzalez-Jimena, & Fitze, 2014; Yewers, Pryke, & Stuart-Fox, 2016; Yewers, Stuart-Fox, & Mclean, 2018). For a number of reasons, morph-specific ARS, morph fluctuations, and rock–paper–scissors dynamics similar to those described in *Uta stansburiana* have been predicted to occur in Eurasian lacertids, particularly in wall lizards (genus *Podarcis*, family Lacertidae; Sinervo et al., 2007; Calsbeek, Hasselquist, & Clobert, 2010; Mangiacotti et al., 2019). First, ventral color polymorphisms involving three alternative colors (i.e., orange, white, and yellow) have been documented in adult individuals of at least 11 out of the 24 species currently recognized within the *Podarcis* genus, and is thus thought to have an ancestral origin (Andrade et al., 2019; Speybroeck, Beukema, Bok, Van der Voort, Velikov, 2016; Huyghe et al., 2007; Jamie & Meier, 2020; Pérez i de Lanuza, Bellati, Pellitteri-Rosa, Font, & Carretero, 2019; Runemark, Hansson, Pafilis, Valakos, & Svensson, 2010; Sacchi et al., 2007). Second, many of these species show high site fidelity, low interannual survival, and occupy habitats where resources relevant to reproduction (e.g., stone walls) are unevenly distributed (Barbault & Mou, 1988; Calsbeek et al., 2010; Carretero, 2007; Edsman, 1990, 2001; Font, Barbosa, Sampedro, & Carazo, 2012; Sinervo et al., 2007; Strijbosch, Bonnemayer, & Dietvorst, 1980). Third, males of many wall lizards experience strong intrasexual competition, mainly in the contexts of territorial disputes and sperm competition. Females seem to be attracted to high-quality and/or familiar patches of habitat rather than to males with certain phenotypic characteristics (Edsman, 1990, 2001; Font, Barbosa, et al., 2012). Moreover, behavioral observations and genetic analyses have confirmed that receptive females often mate with more than one male before oviposition, which results in a high incidence of multiple paternity (Heathcote et al., 2016; Oppliger, Degen, John-Alder, & Bouteiller-Reuter, 2007; Uller & Olsson, 2008). Consequently, adult males try to secure fertilizations by investing significant time and energy in the defense of territories offering resources valuable to females (such as basking spots, shelters, optimal egg-laying sites) against other males (Baird, 2013; Edsman, 1990; Font, Barbosa, et al., 2012). The outcome of this territorial disputes is crucial to male reproductive success, and patterns of shared paternity have often been found to reflect spatial and social dominance among males (MacGregor, Lewandowsky, et al., 2017; MacGregor, While, et al., 2017; Oppliger et al., 2007; Uller & Olsson, 2008; While et al., 2015). For these reasons, alternative color morphs in many wall lizards are often believed to represent the visible mark of heritable ARS involving differential sociospatial behaviors in males (Andrade et al., 2019; Calsbeek et al., 2010; Huyghe et al., 2007; Pérez i de Lanuza, Carretero, & Font, 2017; Sinervo et al., 2007).

The European common wall lizard (*Podarcis muralis*) shows the widest distribution within the genus *Podarcis*, and many populations exhibit a striking color polymorphism (Speybroeck, Beukema, Bok, Van der Voort, and Velikov, 2016). Adults of both sexes may show up to five alternative ventral color morphs: three uniform (pure) morphs, that is, orange (O), white (W), and yellow (Y), and two intermediate mosaics combining orange and white (OW) or yellow and orange (YO) (Pérez i de Lanuza, Font, & Carazo, 2013; 2019; Figure 1). These color morphs are fixed at maturity (Pérez i de Lanuza et al., 2013), and recent research suggests that orange and yellow color expression is caused by recessive homozygosity at two separate loci in the regulatory regions of two genes associated with pterin (SPR) and carotenoid (BCO2) metabolism, respectively (Andrade et al., 2019). Interestingly, each of these morphs is found in geographically distant sublineages of the species thought to have diverged up to 2.5 million years ago (Andrade et al., 2019; Salvi, Harris, Kaliontzopoulou, Carretero, & Pinho, 2013; Figure S1). Local morph composition shows considerable geographical variation, although white ventral coloration is typically the most common (>50%), while the orange and especially the yellow morph rarely predominate. The yellow and yellow-orange morphs are often the most infrequent, and in Pyrenean populations, they seem to be geographically restricted to a subset of localities (<50%) characterized by male-biased sex ratios and marked climatic seasonality (Pérez i de Lanuza et al., 2017; Pérez i de Lanuza, Sillero, & Carretero, 2018).

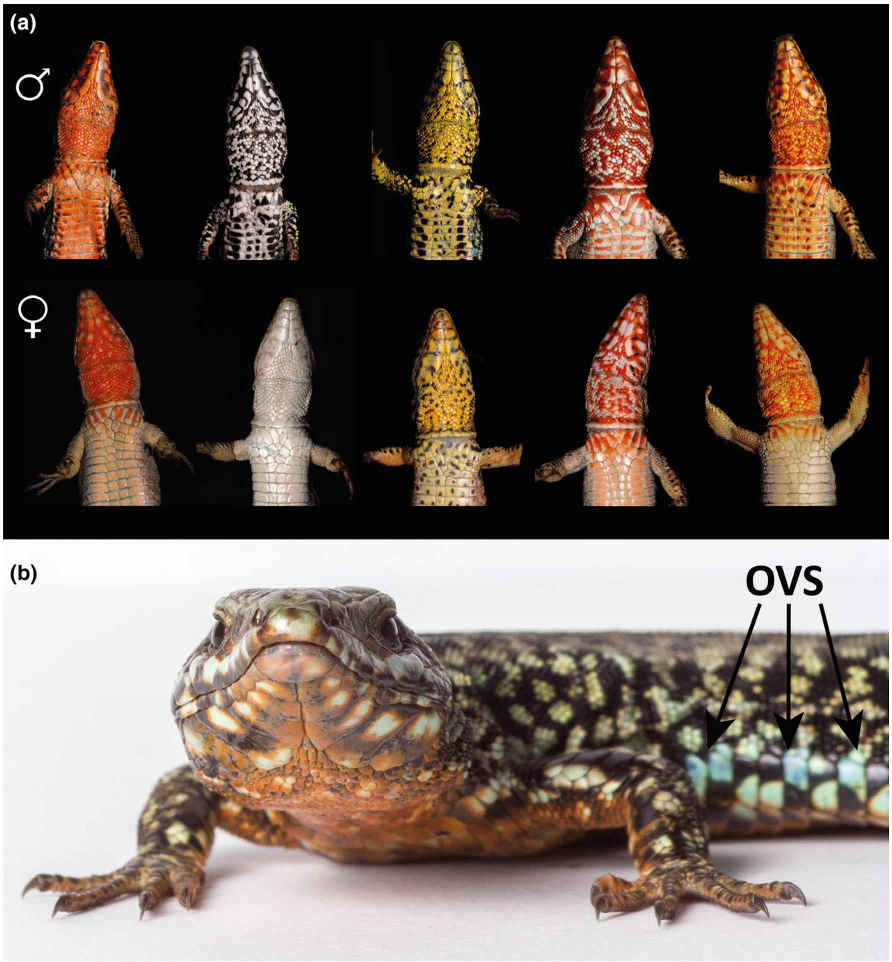

FIGURE 1 (a) Color variation in the ventral surface of adult *Podarcis muralis* lizards. (b) Close-up of an orange morph male showing UV-blue and black spots in its outer ventral scales (OVS)

At least the pure morphs in *P. muralis* are often assumed to reflect alternative behavioral or life-history strategies (e.g., Calsbeek et al., 2010; Galeotti et al., 2010; Scali et al., 2013; Zajitschek, Zajitschek, Miles, & Clobert, 2012). The colors are indeed well suited to function as color signals. They are highly conspicuous to the species visual system and heritable, and their ventral position allows the lizards to control their exposure through posture (Andrade et al., 2019; Pérez i de Lanuza, Carretero, & Font, 2016; Pérez i de Lanuza & Font, 2015, 2016). Moreover, the alternative colors show discrete variation and are chromatically discriminated as categorically distinct by conspecifics (Pérez i de Lanuza, Ábalos, Bartolomé, & Font, 2018; Pérez i de Lanuza et al., 2013), which makes them particularly suited to convey information about strategy (Tibbetts, Mullen, & Dale, 2017). Research on *P. muralis* has revealed several differences in morphological, physiological, and behavioral traits across color morphs (e.g., Calsbeek et al., 2010; Galeotti et al., 2013; Pérez i de Lanuza & Carretero, 2018; Sacchi, Mangiacotti, Scali, Ghitti, & Zuffi, 2017; Scali et al., 2013; Zajitschek et al., 2012). However, there is no clear evidence that these correlated traits reflect morph-specific strategies, whether in the context of sexual or natural selection. Furthermore, available studies have focused on morphology and physiology (Calsbeek et al., 2010; Galeotti et al., 2007, 2010; Galeotti, 2013; Pellitteri-Rosa, 2010; Sacchi, Mangiacotti, et al., 2017; Sacchi et al., 2007), while central aspects in the behavioral ecology of this species have received little attention (Abalos, Pérez i de Lanuza, Carazo, & Font, 2016; Pellitteri-Rosa et al., 2017; Sacchi et al., 2015; Sacchi et al., 2009). In particular, the interaction between sociospatial behavior, reproductive success, and shared paternity is key to ascertain whether *P. muralis* color morphs obtain their fitness using alternative behavioral strategies during the breeding season. If behavioral ARS underlie color polymorphism in *P. muralis*, the alternative color morphs may show equal reproductive success but differential investment in social dominance, territoriality, space use, and/or postcopulatory sexual behavior (e.g., mate-guarding), which often translate into morph-biased patterns of cosiring and clutch monopolization (Formica, Gonser, Ramsay, & Tuttle, 2004; Sinervo & Lively, 1996; Sinervo, Miles, Frankino, Klukowski, & DeNardo, 2000; Zamudio & Sinervo, 2000). However, no previous study has investigated the alignment of polymorphic coloration, social behavior, and reproductive performance in sufficient detail to draw firm conclusions about the existence of behavioral ARS in *P. muralis*. To fill this gap, we monitored morph differences in spatial behavior in a free-ranging polymorphic population from the eastern Pyrenees across a period of 5 years. We complemented this with a mesocosm experiment using ten experimental populations with balanced sex ratio and morph frequencies to study the spatial and sociosexual behavior of *P. muralis* pure color morphs in a controlled environment. Our experimental design was aimed to detect behavioral differences in space use or social behavior among the color morphs, as well as morph differences in shared paternity, rather than frequency-dependent effects on morph fitness. For this reason, we introduced the morphs in equal frequencies to optimize our sample size of individual lizards representing each morph within the enclosures. Incidentally, as the balanced morph ratios employed are highly unlikely to occur in natural populations, this design also allows us to test whether the higher prevalence of white morph lizards observed across the species distribution range results from some form of frequency-dependent fitness effect.

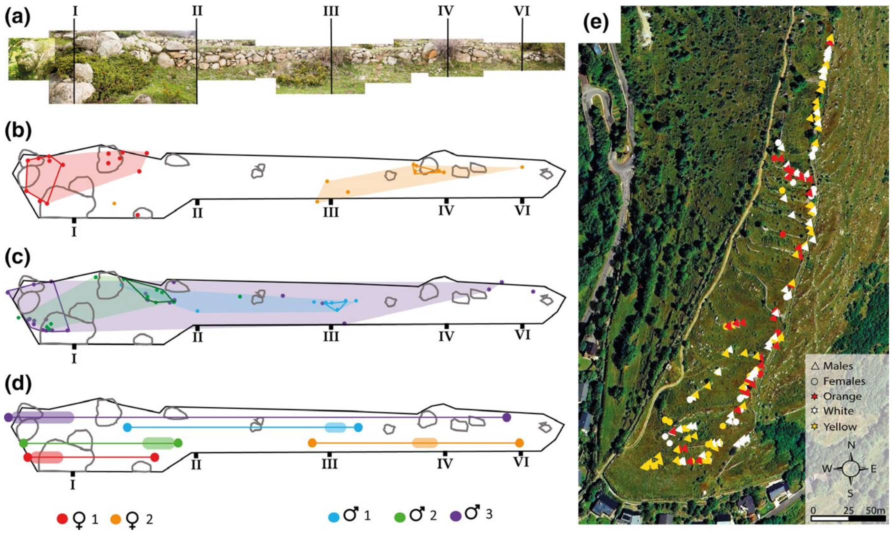

FIGURE 2 Space use in a free-ranging population of *P. muralis*. (a) Photographic composition of a stone wall in Angoustrine. Roman numbers mark reference points for precision. (b and c) Schematic representations of the wall vertical surface used as home- (color shades, 95% MCP) and core ranges (solid-line polygons, 50% MCP) by two females (b) and three males (c) during the breeding season of 2010. (d) Diagram of the linear home- and core range lengths estimated for each lizard as the width of the corresponding MCP (solid-lines = home range, color shades = core range). (e) Google Earth satellite image of the study site in Angoustrine (Map data: Institut Cartogràfic de Catalunya), with arithmetic center of each pure morph lizard core range during the period examined (367 lizards, 125 females, and 242 males)

2 | MATERIALS AND METHODS

2.1 | Spatial behavior in a free-ranging population of *Podarcis muralis*

During the spring seasons of 2006–2010, we collected data on the activity and spatial behavior of a population of wall lizards in Angoustrine (42°28′43″N, 1°57′12″E), eastern Pyrenees. The study site (ca 140 × 500 m = 7 ha; Figure 2) consists of a series of abandoned terraced fields characterized by granite outcrops and old dry-stone walls partially covered in vegetation (see Font, Barbosa, et al., 2012). Lizards were mostly sighted perching on the stone walls, usually remaining within the boundaries of a single wall for the whole breeding season. In any particular year, lizards showing at least six resightings on the same wall were considered resident, while lizards showing five or fewer resightings and/or sighted at walls located more than 100 m apart were considered nonresident transients (Edsman, 1990). We only considered lizards measuring at least 56 mm from snout to vent (SVL), which ensures they had developed full-blown adult ventral coloration (Figure S2; Pérez i de Lanuza et al., 2013).

To examine potential intermorph differences in activity levels, for each lizard we counted the total number of sightings, the mean days elapsed between consecutive sightings, and the mean distance between consecutive sightings. As frequently done when a species' habitat is physically constrained (e.g., river fauna; Ahlers, Heske, Schooley, & Mitchell, 2010; Kornilev, Dodd, & Johnston, 2010; Kramer, 1995), we calculated a one-dimensional measure of home range size for each lizard inhabiting a particular stone wall. We operationally defined the width of the 95% minimum convex polygon (MCP) encompassing the lizard's cluster of resightings on a stone wall as the lizard's linear home range size, and the width of the 50% MCP as the linear core range in which the animals were most frequently observed (Figure 2; Grassman, Tewes, Silvy, & Kreetiyutanont, 2005; Heupel, Simpfendorfer, & Hueter, 2004; Powell, 2000). To determine what fraction of male core ranges constitutes a territory (Maher & Lott, 1995), we defined the exclusive range of each male as the part of its core range that did not show overlap with the core range of any other male (i.e., territory; Kerr & Bull, 2006). Then, for each male with a reliable linear home range estimate (≥17 sightings; see Appendix S1) we measured spatial overlap by counting the number of resident females whose core ranges overlapped at least partially with either the home range, or the exclusive range of the focal male. To account for the vertical dimension of the lizards' home ranges, we also calculated the mean perching height of each resident lizard sighted.

2.2 | Mesocosm experiment

2.2.1 | Lizard capture and housing

We captured 190 lizards (100 females and 90 males) by noosing from 12 polymorphic localities spread across the Cerdanya Valley (Eastern Pyrenees). In each of these localities, we captured 2–8 lizards (SVL ≥ 56 mm) showing each of the pure color morphs (O, W, Y) so as to avoid a geographical bias in our sample. No lizards were captured from populations lacking any of the pure color morphs. To ensure captured females were not gravid, we captured females at the end of the previous breeding season (September 2017), and transferred them to the Statión d'Ecologie Théorique et Expérimentale (SETE, Moulis, France). There, we housed females in groups of 3–5 coming from the same locality in outdoor circular plastic tanks (170 cm diameter, 60 cm high), where they were kept under natural conditions for 130 days (Bestion, Teyssier, Aubret, Clobert, & Cote, 2014; Le Galliard, Ferriere, & Clobert, 2005). In May 2018, after an artificial hibernation period (see Appendix S1), we reinstalled the females in the outdoor tanks for 2 weeks while we captured the males.

2.2.2 | Morphometry

Two days before the onset of the experiment, we measured SVL (0.1 mm) and mass (±0.01 g) of each lizard with a ruler and a spring balance (Pesola, Schindellegi, Switzerland). Using a digital caliper (±0.01 mm; Mitutoyo, Telford, UK), we quantified interlimb length (ILL) in females, and two head measurements in males: length (HL) and width (HW) (Olsson, Shine, Wapstra, Ujvari, & Madsen, 2002). We also removed ~5 mm from the tail tip of each individual and preserved the tissue in 90% ethanol for genetic analyses.

2.2.3 | Experimental enclosures and egg incubation

To study social behavior and mating patterns in ten experimental populations of *P. muralis*, we released 180 lizards of either sex into ten experimental enclosures at the Metatron research facility (Caumont, France; Legrand et al., 2012). Within each of these enclosures, we created two types of sites that varied in structural complexity. Each site consisted of a wooden pallet (~1.2 m^2) with differing number of bricks, cinderblocks, rocks, and logs piled above, which acted both as shelter and as basking sites (Figure S3.). We arranged high- and low-quality sites (respectively HQ and LQ) in two rows of three pallets along the N-S axis, separated by a line of six rocks (which we also considered as LQ habitat) (MacGregor & While, et al., 2017). We then surrounded the area with a plastic barrier (70 cm high) to prevent any escapes or intrusions. In total, each experimental cell had 47 m^2.

On 23 May 2018, we released nine males (3O:3W:3Y) within each of the enclosures (simultaneously and always from the southeast corner). We monitored male behavior (see below) for 7 days before releasing nine females (3O:3W:3Y) within each enclosure. Due to posthibernation mortality, the white female morph was underrepresented in two of the ten experimental enclosures (5o:1w:3y). Prior to release, we marked each lizard permanently on the ventral scales using a disposable medical cautery unit (Ekner, Sajkowska, Dudek, & Tryjanowski, 2011) and drew a dorsal number with a toluene xylene-free permanent marker to facilitate individual recognition during behavioral observations (see Video S1 in the Appendix S1; Ferner & Plummer, 2016). To

TABLE 1 Partial ethogram used during behavioral observations to collect data on social interactions within the experimental enclosures

Behavior	Description
Approach[a]	Movement toward a nonfleeing conspecific
Display	Gular extension, back-arching, shoulders raised, head down, sagittal compression (any combination)
Bite	One or more bites to another individual (excluding tail grab)
Retreat[a]	Movement away from a nonchasing conspecific
Chase	Rapidly following another FLEEING lizard
Flight	Fast-paced movement to withdraw from a CHASING lizard
Foot shakes II[b]	Sequence of front-leg waves in the air or onto the substrate
Tail grab	A male bites the tail or inguinal region of a female. Often followed by copulation
Tail shake	Shaking entire tail (or its posterior portion) swiftly from side to side
Mating	Two lizards engage in copulation
Coperching	Two or more lizards lying together in close vicinity (<15 cm; >30 s)
Cloacal drag	Pulling body forward while keeping cloaca in contact with substrate

[a] We classified the mode of locomotion used as either running (fast-paced) or any other mode of locomotion (slow-paced).

[b] *Podarcis muralis* lizards perform four types of foot shake displays (named I, IIa, IIb, and III; see Font et al., 2012 and references therein), of which two (IIa and IIb) are given in a social context. We only recorded these two types of foot shakes. Type IIa: rapid large amplitude vertical movements of front legs frequently performed by females in male–female interactions (belly-down, head-up posture). Losers of male–male agonistic interactions often perform this type of foot shakes, which are hence considered in this context as submissive/appeasement displays (see Font & Desfilis, 2002; Aragón, López, & Martín, 2006 for details in other *Podarcis* lizards). Type IIb: Performed by males when approaching females (limbs extended, often displaying; Pérez i de Lanuza, Font, et al., 2016).

minimize the noise introduced by size asymmetries and prior social interactions, we allowed a maximum SVL difference of 2 mm (within-sexes) and only put lizards together in the same experimental enclosure if they had been captured at least 300 m apart.

On 22 June, we released the males at their capture location (previously determined using a GPS device) and housed females individually in the laboratory until oviposition (see Appendix S1). We lost 22 clutches due to females laying eggs before we retrieved them from the enclosures (12 females) or because they failed to produce a clutch (10 females). These lost clutches were evenly distributed across enclosures (χ^2 = 14.667, p = .10) and female morphs (orange = 8, white = 8, yellow = 6). For the remaining 68 females, we counted the number of fertile and infertile eggs within each clutch by noting the presence of a calcified shell and vascularization 48 hr after oviposition (Köhler, 2006). We incubated the resulting 230 fertile eggs in plastic cups filled with moist coco husk (1:2 coco:water by weight) and covered with a perforated lid at a constant temperature of 28°C (Memmert GmbH + Co.KG incubator, Schwabach, Germany). Upon hatching, each of the 209 born juveniles was measured (SVL), weighted, sampled for DNA, permanently marked, and released at the outdoor tanks in the SETE Moulis. For 21 embryos that died before hatching, we obtained DNA samples via dissection of the eggs. Average clutch size was 5.57 ± 0.20 eggs, average fertilization success (fertile eggs/clutch size) was 67%, and average hatching success (hatched/fertilized eggs) was 90%.

2.2.4 | Behavioral observations

From 23 May to 22 June, we conducted observations of spatial and social behavior at the natural peak activity hours for the lizards (9.30–14.30; 16.30–19.30), spacing consecutive visits to the same enclosure at least 1 hr and ensuring an even distribution of observations across the different time periods. Two researchers (JA and AB) recorded the identity, position, and behaviors of the lizards participating in social interactions using a behavior sampling rule in recording sessions lasting 40 min. A social interaction was considered to occur whenever a marked lizard in our visual range directed any of the behaviors listed in Table 1 toward a conspecific. During interactions, we recorded the first occurrence of the behaviors performed by each lizard. Consecutive interactions involving the same lizards were recorded as different events whenever the participants remained further than 30 cm apart for longer than 2 min. To ensure interobserver reliability, JA and AB collected behavioral data together for the first 6 days of the experiment (Cohen's *kappa* ± $\mathrm{CI}_{95\%}$ = 0.87 ± 0.05; Kaufman & Rosenthal, 2009). A third observer (OL) performed sequential rounds visiting all the enclosures every 2.5 hr to collect data on the lizards' spatial behavior. Using scan sampling, we determined the identity and location of every lizard in sight on a scale map of the enclosure that included the six wooden pallets. Each enclosure was observed from a starting position located 1 m from the plastic barrier surrounding it for 5 min, and then walking around it (randomizing direction between consecutive visits) to record lizards that were not visible from the starting position. To balance sampling effort across enclosures, scanning of a single enclosure was restricted to a maximum period of 15 min after the first lizard was spotted.

2.2.5 | Behavioral analyses

We classified the interactions according to their sociosexual context into four types: intrasexual competitive and noncompetitive, and male–female reproductive and nonreproductive. Intrasexual interactions were deemed competitive whenever one lizard (i.e., the loser) used fast-paced locomotion to flee from another lizard (i.e., the winner) showing display behavior and/or physical aggression (i.e., display, bite, or chase). In males, where competitive encounters were numerous, we used the R package BradleyTerry2 to fit a Bradley–Terry model to the observed matrix of contest outcomes within each enclosure to obtain an individual index of social dominance for every male (further details in Abalos et al., 2016; Firth & Turner, 2012; Stuart-Fox, Firth, Moussalli, & Whiting, 2006). To examine potential nontransitive relations of dominance among male color morphs, we also fitted three logistic mixed models (one for each morph) on the contest outcome of heteromorphic encounters and tested whether the probability of winning against other morphs differed from even odds. Male–female interactions were classified as reproductive when the lizards engaged in sex-specific display behaviors (i.e., ♂: display; ♀: foot shakes, tail shake), copulatory behavior (i.e., tail grab, mating), or prolonged physical vicinity (i.e., coperching). To examine the effect of morph combination on the frequency of male–female reproductive interactions, we used social network analysis on the compiled version of SOCPROG (Whitehead, 2009) (Appendix S1).

Positional data were used to examine the putative effect of color morph on activity, space use and overlap with conspecifics. To account for habitat use within the enclosures, we estimated range areas by adjusting the smoothing factor in a fixed-kernel contour analysis until it matched the area of the 95% MCP (smoothing multiplier = 0.75, matrix cell number = 40; Kie, 2013; Row & Blouin-Demers, 2006; MacGregor, Lewandowsky, et al., 2017; MacGregor, While, et al., 2017). Lizards with fewer than nine sightings (N = 3) were excluded from the analysis (see Appendix S1). For each lizard, we calculated range size and overlap with conspecifics both at the 95% (home range) and at the 50% (core range) isopleth levels. Each lizard was assigned to a high- or low-quality site based on the position where the 50% kernel estimate indicated peak density. Because of the high lizard density within the enclosures, male-exclusive areas were peripheral and uninformative, so we did not conduct further analyses on them. When calculating home range estimates, we excluded the positional data collected during the first 6 days of the experiment to allow for an acclimation period. All spatial analyses were conducted in Ranges 9 (Anatrack Ltd., UK; Kenward, Casey, Walls, & South, 2014).

2.2.6 | Parentage analyses

We isolated DNA from tail-tip samples using the DNeasy 96 Blood & Tissue Kit (Qiagen, Valencia, CA, USA), obtaining a final elution volume of 150 μl in AE buffer. We then combined the primers of six microsatellite loci described in *P. muralis* (Heathcote, Dawson, & Uller, 2014; Richard et al., 2012) into two different multiplexes (MPA: Pm16, Pm09, PmurC168; MPB: Pm19, Pm14, PmurC038) and ran standard PCR with 26 cycles and a final extension step of 30 min at 60°C. Forward primers were labeled with different fluorescent dyes (FAM, NED, HEX). Diluted PCR products (1:5) were genotyped together with an internal ladder (Red ROX-500) on an ABI 3130 genetic analyzer (Applied Biosystems Inc.). One researcher (HL) scored the alleles for every adult and juvenile lizard in Geneious 7.0.4 (Biomatters, available at http://www.geneious.com), which we used to conduct parentage analysis in Cervus 3.0 (Kalinowski, Taper, & Marshall, 2007; Marshall, Slate, Kruuk, & Pemberton, 1998). We assigned paternity based on the log-likelihood statistic of each mother–father–offspring trio (LOD scores), using two confidence levels (strict: 95%, relaxed: 80%) and the nine males within each enclosure as candidate fathers. Critical LOD scores were determined by running a simulation paternity analysis based on 100,000 offspring with known mothers and nine candidate fathers. We could reliably assign paternity to every offspring examined (strict: 209 juveniles, relaxed: 229 juveniles).

To quantify individual fitness, we operationally defined two variables based on the results of the paternity analysis: mating success (i.e., the overall number of different mates with whom a lizard conceived offspring) and reproductive success (i.e., the total number of embryos/hatchlings sired). Since selection will depend on relative rather than absolute fitness, we then divided the fitness measures of each lizard by the mean for all same-sex conspecific within its enclosure. In addition, to evaluate intermorph differences in sperm competition intensity, for each male we determined the average number of competitors with which he shared paternity of a clutch.

2.2.7 | Statistical analyses

We ran linear mixed models using the *lme4* package (Bates, 2014) in R (R Core Team, 2019), and model selection was conducted using backward single-term deletions ($p < .05$) of the saturated model followed by model comparisons via likelihood-ratio tests (at $\alpha = 0.05$). All numerical variables were centered and scaled before running the models (Schielzeth, 2010). We checked that all response variables conformed to homoskedasticity and normality assumptions before assuming a Gaussian distribution in model fitting. For some variables that did not conform to these assumptions even after transformation, we fitted models using different distributions (Appendix S1).

2.2.8 | Power analysis

Using G*Power (Erdfelder, Faul, & Buchner, 1996) and the methodology provided by Thalheimer and Cook (2002), we determined the effect size for an array of published morph differences detected in *U. stansburiana* and other polymorphic lizards thought to present some form of ARS (Table S1). We then used G*Power to calculate the smallest effect size that our sample size from the free-ranging population allowed us to detect (sensitivity analysis), and the sample size required to detect biologically meaningful differences among morphs in the mesocosm experiment (a priori required sample size). We chose the more conservative approach of conducting these a priori analyses in G*Power instead of by simulation since this latter approach requires the researcher to directly determine estimates for both fixed and random effects, for which we had no previous reliable information (Green & Macleod, 2016). However, to better accommodate for the mixed-model statistical design of our experiments, we additionally used the estimates obtained here to run a simulation-based analysis of power on the probability of detecting medium-sized (Cohen's *d* > 0.5) and large (Cohen's *d* > 0.8) effects with growing sample sizes (Haenlein & Kaplan, 2004; Hoenig & Heisey, 2001; O'Keefe, 2007). We created two artificial LMMs using the *simr* package in R (Green & Macleod, 2016), one corresponding to the free-ranging population and another corresponding to the mesocosm experiment. In the former, we replicated the terms and parameters of the standardized model exploring morph differences in home range size. In the latter, we replicated the terms and parameters of the standardized model exploring morph differences in social dominance (see Appendix S1). Following Green and Macleod (2016), we then modified the standardized estimate for the morph factor (i.e., effect size) to either 0.5 or 0.8, and conducted a power analysis by running 1,000 simulations at 10 different levels of sample size (range = 5–50 lizards within each morph).

3 | RESULTS

3.1 | Spatial behavior in a free-ranging population of *Podarcis muralis*

In total, we accumulated 5,046 sightings of 472 different lizards. Eighty-seven lizards were observed more than 1 year (maximum = 3 years, 21 lizards). Out of those, 76 (87.4%) were found on the same wall as the previous year, seven (8%) moved between neighboring walls, and only four (4.6%) changed to a nonadjoining wall between years. Only 181 males and 101 females were large enough (SVL ≥ 56 mm) to be included in the analyses about morph differences (Table S2). For each variable considered, we provide separate measures of centrality and dispersion for males and females in Table S3. Residents represented 59.6% of both adult male and female lizards, and no color morph was overrepresented among resident or transient lizards (GLMM (binomial): $\chi^2 = 1.60$, $p = .81$). Movements between walls were similarly frequent among color morphs (GLMM (gamma), $\chi^2 = 2.80$, $p = .59$). Color morphs did not differ in the total number of resightings accumulated, the mean days elapsed between consecutive resightings, or the mean distance between consecutive relocations ($p > .28$; see Table S4 for more details and effect size).

We could calculate reliable estimates of linear home- and core ranges for 83 lizards, but decided to exclude mixed-morph lizards from the analyses due to their scarcity. The final dataset consisted of 70 lizards: 18 females and 52 males with at least 17 resightings (Table S2). Neither sex showed significant differences in SVL among color morphs (LMM: $\chi^2 = 6.61$, $p = .16$). Males had both larger linear home ranges and core ranges than females, and also perched higher on the stone walls ($p < .01$; Table S4). Morphs did not differ in the size of their home- and core ranges, neither in males (LMM: home ranges: $\chi^2 = 4.31$, $p = .19$; core ranges: $\chi^2 = 2.41$, $p = .30$), nor in females (LMM: home ranges: $\chi^2 = 0.44$, $p = .80$; core ranges: $\chi^2 = 3.09$, $p = .21$). Similarly, mean perching height did not differ among color morphs ($\chi^2 = 1.01$, $p = .60$; Table S4). In males, we did not find significant intermorph differences in the number of females within their linear home- or core range (GLMM (gamma): $\chi^2 < 1$, $p > .3$). Likewise, males of different color morphs did not differ in the size of their exclusive ranges (i.e., the fraction of core range that is not shared with any other male) or in the number of female core ranges partially included within those ranges ($p > .35$; Table S4).

3.2 | Mesocosm experiment

3.2.1 | Morphology and color traits

None of the morphometric traits examined (reported to be under intrasexual selection in male wall lizards; Baird, 2013; Pérez i de Lanuza, Carazo, & Font, 2014; While et al., 2015) were found to differ among color morphs in our sample of experimental males (Table S5). In females, neither SVL nor ILL (both positively correlated with fecundity; Kratochvíl, Fokt, Rehák, & Frynta, 2003; Olsson et al., 2002) varied with color morph, but white morph females (before reproduction) were found to be significantly heavier than orange females (Table S5).

3.2.2 | Spatial behavior

Overall, we accumulated 7,190 resightings of the marked lizards in 655 scan samplings. The total number of resightings per lizard differed significantly between sexes (males were resighted more often), but not among color morphs (GLMM (negative binomial): sex: $\chi^2 = 57.11$, $p < .001$; morph: $\chi^2 = 0.81$, $p = .67$). Likewise, we found a strong intersexual difference in the ability to settle in high- or low-quality sites, but no intermorph difference (GLMM (binomial): sex: $\chi^2 = 56.38$, $p < .001$; morph: $\chi^2 = 1.37$, $p = .50$; Figure 3). In fact, even though lizards were evenly distributed among sites (HQ: $N = 91$, LQ: $N = 89$), females had three times higher odds of settling in HQ sites (OR = 3.26), whereas only highly dominant males managed to occupy HQ sites (Figure S4). Specifically, an increase of one *SD* in social dominance among males meant 4.5 times higher odds of settling in HQ sites ($p < .001$; Table S6). Males settled in HQ pallets did not differ in body size, weight, or head variables from males settled in LQ pallets (LMM: $\chi^2 < 1$, $p > .2$).

As expected, males had larger home- and core ranges than females, and lizards settling in HQ sites occupied smaller areas than lizards in LQ sites (LMM on k50: sex $\chi^2 = 34.95$, $p < .001$; pallet quality: $\chi^2 = 7.64$, $p = .006$). In males, variation in home- and core range size was significantly explained by social dominance ($p < .001$; Table S6), but not by color morph ($p > .20$; Table S6). In females, we found significant differences in home- and core range areas among female color morphs, with white morph females showing the largest areas ($p < .001$; Table S7). Male–female spatial overlap was not affected by color morph, but was significantly associated with site quality in both sexes ($p < .01$; Tables S6 and S7). Males established in HQ sites overlapped with 3.0 ± 1.2 more females, and females established in LQ sites overlapped with 1.7 ± 1.0 more males.

3.2.3 | Intrasexual competition

We recorded 927 intrasexual interactions (614 in males and 384 in females). Competitive interactions were more common among males (*N* = 543; 88% of total male–male interactions) than among females (*N* = 25; 7%), which were often observed in groups engaged in prolonged coperching in the vicinity of a male (*N* = 338, 88%). In males, display posturing and/or foot shakes (IIa, appeasement, Table 1) were observed in 60% of these competitive encounters, a third of them (36%) ended with a rapid chase/flight, and 16% involved physical aggression (i.e., bites). Display behavior and bites were usually exhibited only by the winning lizard (display: *N* = 307, 91% only by winner; bite: *N* = 89, 70% only by winner), while foot shakes were almost exclusively performed by losing males (*N* = 70, 93% only by loser) with no differences among morphs (χ^2 = 3.07, *p* = .22). No morph combination was overrepresented among these contests (χ^2 = 5.63, *p* = .40). We found no evidence of an intermorph difference in the index of social dominance estimated from the Bradley–Terry model (*p* = .68; Table S6 and Figure 4). After dealing with pseudoreplication (200 different pairs of rivals; Table S8), we found no effect of morph combination on the outcome of heteromorphic contests (GLMM (binomial): orange: χ^2 = 0.33, *p* = .56; white: χ^2 = 1.83, *p* = .18; yellow: χ^2 = 0.88, *p* = .35). In fact, for either of the morphs involved in these combinations, the probability of winning did not differ significantly from even odds (Figure 4).

3.2.4 | Male–female interactions and parentage

In total, we recorded 1,230 male–female interactions, of which 1,098 were deemed as reproductive because they involved the exchange of sex-specific behaviors (441), prolonged coperching (551), and/or copulatory behavior (153).

Male color morphs did not differ in the number of females with which they interacted, engaged in coperching, or engaged in copulatory behavior (*p* > .57; Table S9). Unsurprisingly, males settled in HQ sites engaged in reproductive interactions more frequently (LMM: χ^2 = 36.91, *p* < .001) and with a higher number of females than males settled in LQ sites (*p* < .001; Table S9; Figure 5). We found no difference in relative reproductive success or relative mating success among male color morphs (*p* > .19; Table S9). Males settled in HQ sites showed significantly higher relative reproductive success (*p* < .001), but not relative mating success (*p* = .107; Table S9). Sperm competition intensity faced by each individual male was also independent of color morph (*p* = .56), but significantly higher in low-quality sites (*p* = .001; Table S9). No morph combination in male cosirings was more prevalent than expected by chance (χ^2 = 2.13, *p* = .83; Table S10). Results from the analysis of male fitness are summarized in Figure 6.

Female color morphs did not vary in the number of males encountered in reproductive interactions, eggs produced, or fertilization success (*p* > .11; Table S11). Body mass and ILL (but not color morph, *p* = .71) were significantly related to laying date, with heavier and longer females laying their clutches sooner than the rest (*p* = .014; Table S11). Although we found high levels of multiple paternity within the experimental enclosures (81% of clutches), female color morphs did not differ in the number of sires fathering offspring

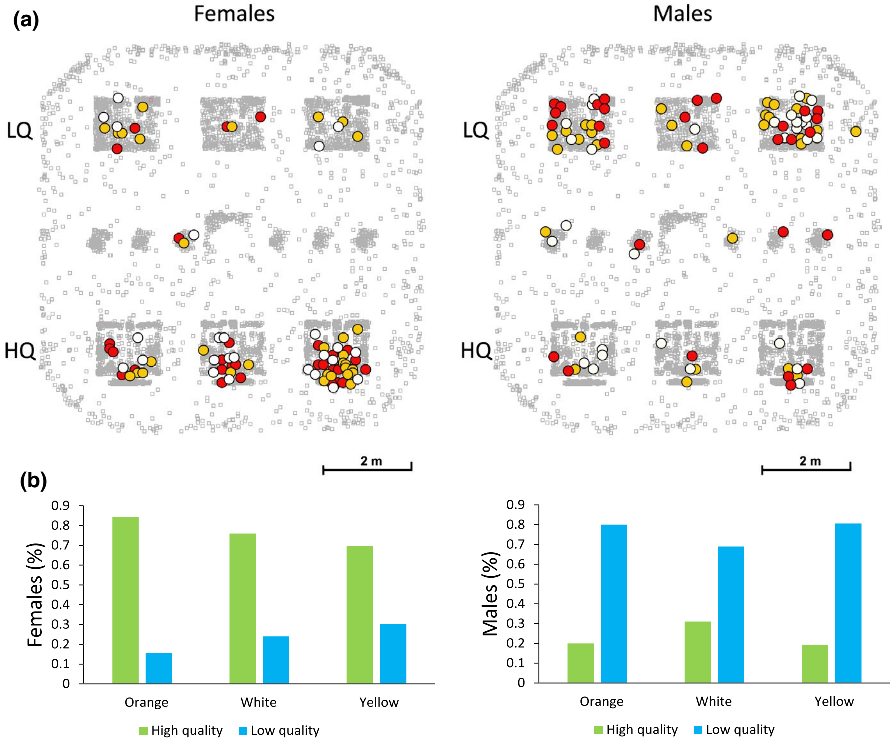

FIGURE 3 Distribution of the lizards among high- and low-quality sites in the experimental enclosures. (a) Position of the peak density of resightings for each male and female (filled circles), plotted on a background schematic diagram of an experimental enclosure obtained by pooling together every resighting of a lizard collected during the experiment (gray squares). The orange, white, or yellow fill of the circles represents color morph. (b) Barplots showing the relative frequency of males and females of each color morph that settled in high- or low-quality sites

in their clutches (LMM: $\chi^2 = 2.84$, $p = .24$), nor in the number of viable juveniles conceived (LMM: $\chi^2 = 4.31$, $p = .12$). Relative measures of fitness yielded similar results ($p > .16$; Table S11). We found a significant effect of habitat quality on some aspects of female social behavior and reproductive parameters: Females established in LQ sites interacted with a higher number of males showed higher levels of multiple paternity, and their clutches contained a smaller fraction of unfertilized eggs ($p < .05$; Table S11).

Parentage was significantly predicted across enclosures by both of the association networks based on social behavior during male–female interactions (coperchings: $\chi^2 = 51.91$, $p < .001$; copulation attempts: $\chi^2 = 45.40$, $p < .001$). However, neither of the behavioral association networks nor the resulting parentage network were found to be affected by morph combination (coperchings: $\chi^2 = 0.69$, $p = .69$; copulation attempts: $\chi^2 = 0.83$, $p = .83$; parentage: $\chi^2 = 0.32$, $p = .32$; Figure 7). We found a significant interaction of the parental morph combination over juvenile body mass (LMM: $\chi^2 = 12.91$, $p = .012$). Splitting the dataset by female morph, we found that this result was exclusively driven by a nonsignificant tendency of yellow males to sire heavier offspring than orange males when coupled with white females (LMM: $\chi^2 = 6.28$, $p = .09$). We found no effect of male or female morph alone on juvenile mass (LMM: $\chi^2 < 1$, $p > .5$).

3.2.5 | Power analysis

The sensitivity analysis in G*Power estimated a minimum detectable effect size of Cohen's $d = 0.46$ ($N = 181$) and Cohen's $d = 0.88$ ($N = 52$) for activity and space use differences (respectively) between male color morphs in the free-ranging population. For the mesocosm experiment, we estimated that a sample size of 90 males and females would allow us to detect medium-sized (Cohen's $d = 0.66$) intrasexual differences in behavior and fitness among color morphs with a standard statistical power of 0.80. These effect sizes are at the lower end of the range of effect sizes (Cohen's $d = 0.49–2.32$), which we calculated from the literature (Table S1), suggesting that we had enough statistical power to detect even subtle but biologically meaningful differences among morphs. Accordingly, results from the two simulation-based analyses of power showed that our sample sizes were high enough to detect biologically relevant differences among color morphs (power > 0.80 to detect medium-sized and large effect sizes). In fact, introducing the observed coefficients for the fixed and random factors in the simulations and plotting the expected increment in power at different sample sizes revealed a higher statistical power for the data presented here than the more conservative estimates obtained in G*Power (Figure S5).

4 | DISCUSSION

Overall, our results from both a longitudinal field study and an enclosure experiment argue against the hypothesis that *P. muralis* color morphs reflect alternative reproductive strategies (ARS) involving differential sociosexual behavior and space use. In territorial species such as *Podarcis* lizards, resource-holding potential, spatial behavior, and activity are expected to vary across males employing alternative strategies (Calsbeek & Sinervo, 2002a, 2002b; Molnár, Bajer, Szövényi, Török, & Herczeg, 2016; Noble, Wechmann, Keogh, & Whiting, 2013; Sinervo et al., 2000; Sinervo & Svensson, 2002; Sinervo & Zamudio, 2001; Zamudio & Sinervo, 2000). In this study, we did not find any evidence that color morphs differ in resource-holding potential (i.e., social dominance, agonistic behavior, territoriality), space use (i.e., site fidelity, home range size, overlap with conspecifics), or activity (i.e., frequency of resightings, distance between consecutive resightings).

No color morph was overrepresented among resident or transient lizards in the field, and we did not observe differences in either intermorph resighting propensity, distance between consecutive resightings, or interannual site fidelity. Furthermore, color morphs showed

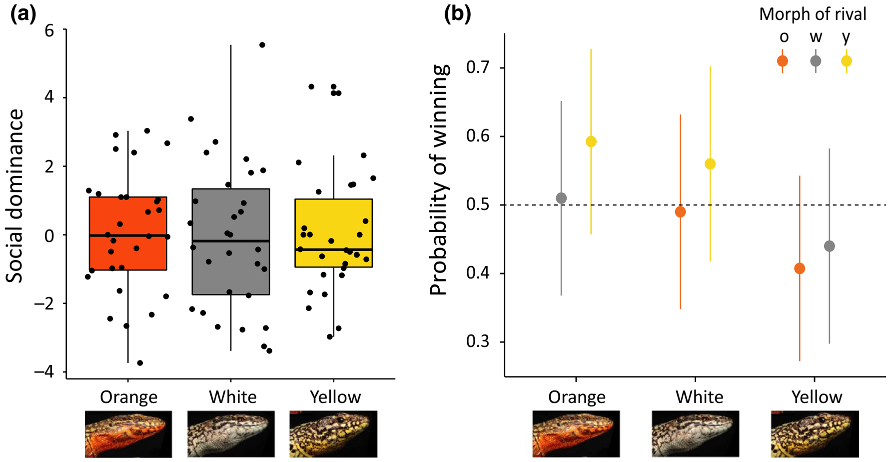

FIGURE 4 Male–male competitive interactions. (a) Boxplot of social dominance by color morph. Boxes indicate the interquartile range (IQR, 50% of data). Horizontal lines represent the median, and bars extend to 1.5 times the IQR. A jittered dot cloud shows the value of the variable of interest for each lizard in our dataset. (b) Mean plot showing the probability of winning for each morph combination according to the predicted values of the logistic mixed models. Bars extend to the $CI_{95\%}$. The horizontal dotted line marks 50% probability

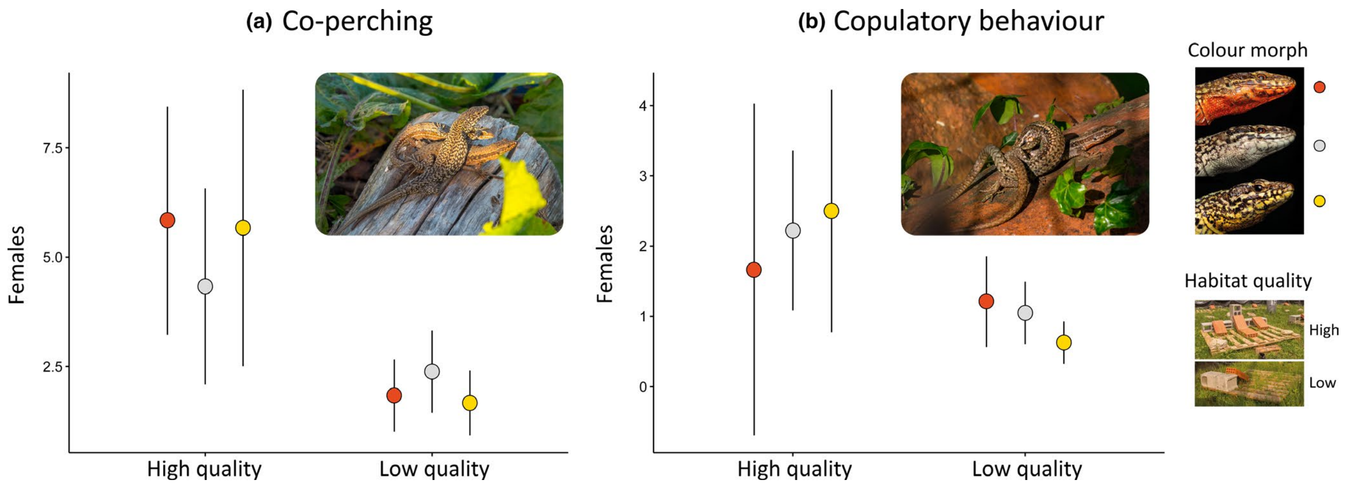

FIGURE 5 Variation in the number of different females with which males engaged in either coperching (a) or copulatory behavior (b, copulation and tail grabs). Males settled in high-quality pallets interacted with a significantly higher number of individual females, while male color morphs did not differ in sociosexual behavior. Bars extend to the $CI_{95\%}$

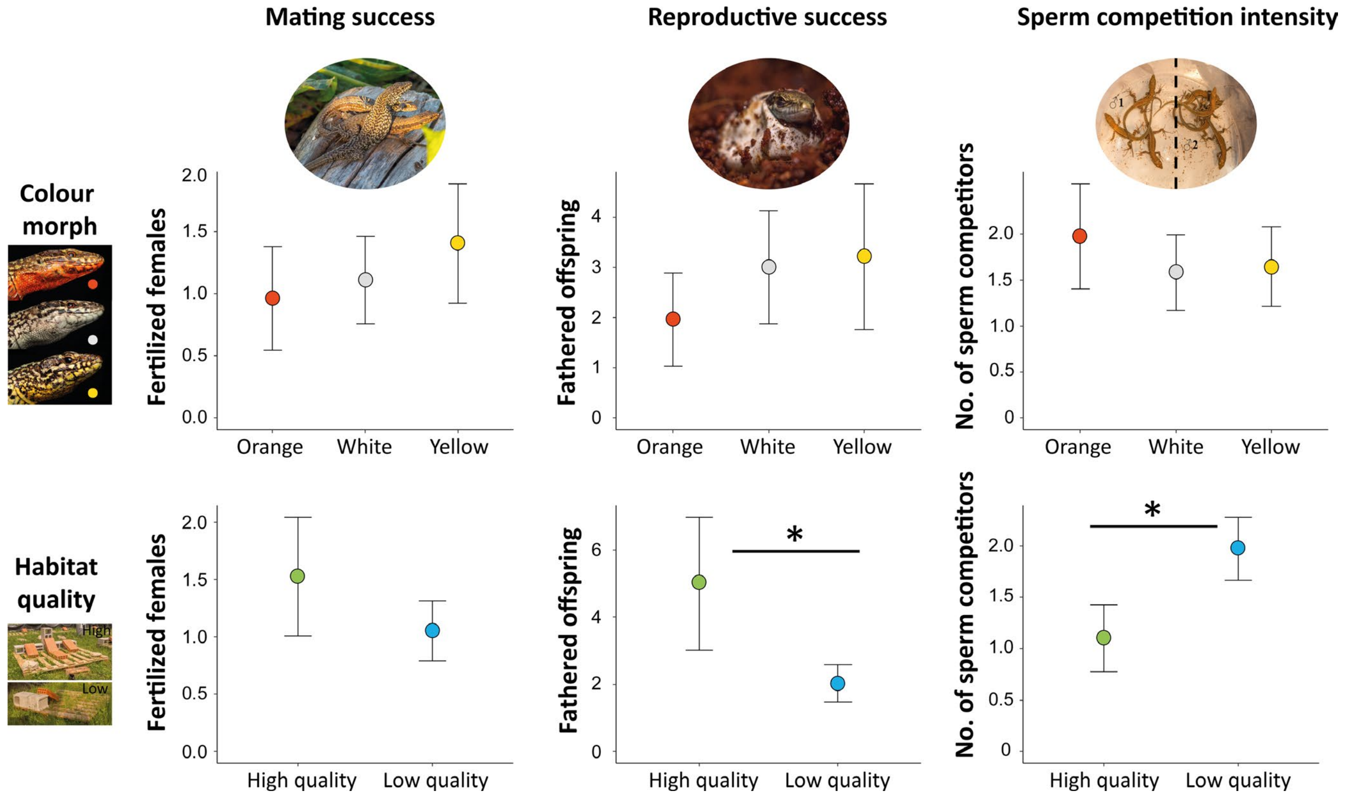

FIGURE 6 Variation in male individual fitness among alternative color morphs (up) and between sites of different quality (below). Bars extend to the $CI_{95\%}$. Significant differences are marked with an asterisk ($p < .001$)

similar home range size and male–female overlap both in natural conditions and in experimental enclosures. In both sexes, alternative color morphs obtained similar relative fitness within the enclosures (which would be necessary for their maintenance over time), but crucially, this was not associated with different behavioral strategies. In line with previous evidence on the behavioral ecology of territorial lizards (Baird, 2013; Baird, Timanus, & Sloan, 2003), males competed fiercely to settle in high-quality sites irrespective of their color morph, and the subset of successful dominant males (23%) engaged in coperching with a higher number of females, experienced significantly lower levels of sperm competition, and ultimately achieved higher reproductive success. In sum, while lizards were strongly attracted to high-quality sites (both in the field and in the mesocosm experiment), we did not find any evidence that color morph played a role in securing access to them or in the ability to exclude other conspecifics from its use. In fact, we did not find an effect of color morph on the outcome of male–male competitive interactions. These results contrast previous evidence suggesting lower fighting ability

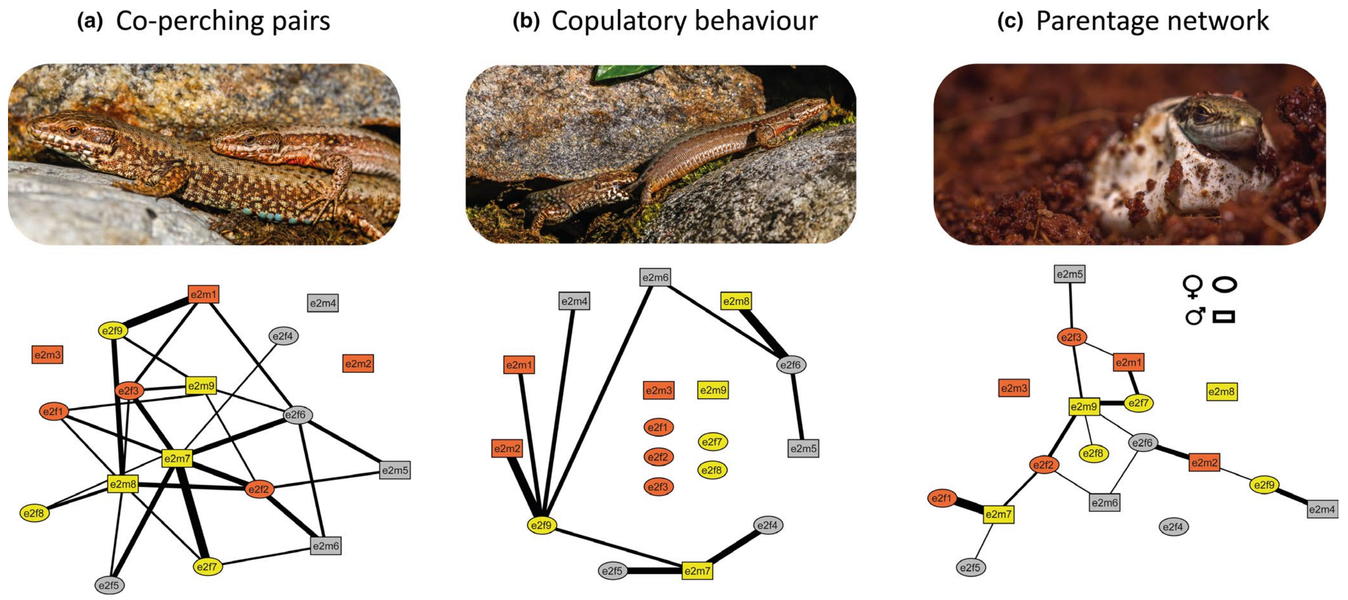

FIGURE 7 Example network diagrams from one of our experimental enclosures based on (a) coperching pairs, (b) copulatory behavior (i.e., interactions involving tail grabs and/or matings), and (c) the resulting parentage network. Each node represents an individual lizard, with shape and color denoting sex and color morph, respectively. Alphanumeric codes within the nodes correspond to the unique ID of each lizard within the enclosure. The thickness of the lines connecting nodes characterizes the number of social interactions (a, b) or offspring (c) between each dyad of lizards. Unconnected nodes represent lizards that we did not observe to engage in coperching or copulatory behaviors (a, b), or did not reproduce (c)

in orange morph males during laboratory-staged encounters (Abalos et al., 2016), likely because any differences between size-matched morphs meeting at a neutral arena are overridden by the effect of size asymmetries and residency status when confrontations occur under more natural conditions (Stuart-Fox & Johnston, 2005). Similarly, Sacchi et al. (2009) reported no effect of color morph on aggressive behavior during laboratory-staged contests when the experimental design allowed for size and residency asymmetries. Previous studies have reported larger body sizes in orange morph lizards with respect to white (Calsbeek et al., 2010; Sacchi et al., 2007), with some authors suggesting an advantage of orange morph lizards in male–male competition for preferred territories and hence reproductive success (Calsbeek et al., 2010). The size difference, however, may result from miscategorizing subadult lizards as pertaining to the white morph (i.e., the lizards' ventral surface appears white to the human eye before achieving sexual maturity), leading to the conflation of any possible morph difference with the expected size asymmetry between younger and older lizards. For instance, orange morph lizards from our study population in Angoustrine are only 1.7 ± 0.3 mm larger than white morph lizards in the free-ranging population of Angoustrine (1942 adult SVL > 56 mm lizards), which represents a 2.6% of the average SVL in adult lizards. There is, in fact, no evidence for biologically relevant differences among male *P. muralis* morphs neither in morphology or sex-specific coloration (i.e., UV-blue ventrolateral spots; Pérez i de Lanuza et al., 2014), and in this study, we did not observe differential use of agonistic behaviors during intrasexual competitive interactions.

The existence of ARS in a polymorphic territorial species does not necessarily imply that color morphs must differ in territoriality or aggressive behavior (Shuster & Wade, 2003). ARS in males of polygynandrous species are often expressed as differential sexual behaviors (e.g., mate-guarding) or physiological adaptations (e.g., increased testis size) representing alternative solutions to the trade-off between securing fertilizations and acquiring new mates (Formica et al., 2004; Shuster, 2008; Taborsky, 2001; Taborsky & Brockmann, 2010). For example, in the Australian painted dragon (*Ctenophorus pictus*), yellow morph males have larger testis and strongly outperform orange males in laboratory-staged sperm competition trials, despite the absence of differential territory-acquisition abilities between both morphs (Healey & Olsson, 2008; Olsson, Schwartz, Uller, & Healey, 2009). In contrast, *P. muralis* male morphs within experimental enclosures showed similar time allocation between guarding females and acquiring new mates, and no difference in the number of mates sired, and experienced similar levels of sperm competition. In *U. stansburiana*, the interplay between the usurper, guarding, and sneaker strategies leads to morph-biased patterns of shared paternity, with yellow sneaker males obtaining almost all of their reproductive success from cosiring clutches with orange males, while blue guarding males show low overall levels of cosiring (especially with yellow males; Sinervo & Zamudio, 2001; Zamudio & Sinervo, 2000). Here, we found no evidence of a similar bias, with no morph combination in cosired clutches being more prevalent than expected by random association. In fact, given the absence of differences in precopulatory behavior, the similar reproductive success achieved by males of the three color morphs indirectly argues against the existence of physiological adaptations in the context of postcopulatory sexual selection (e.g., larger testis and ejaculates, which would have biased paternity in the absence of differential social behavior). Further research could directly address this question by studying reproductive physiology in *P. muralis* color morphs and staging realistic sperm competition trials across morphs.

While most research on color polymorphism and ARS concerns males, females are also often polymorphic. Differential female breeding strategies, such as the different solutions to the trade-off between egg size and number described in the female color morphs of *U. stansburiana* (Alonzo & Sinervo, 2001), have also been suggested to occur in *P. muralis*. One study of an Italian population found that, in captivity, yellow females laid relatively larger clutches of smaller eggs than white morph females (Galeotti et al., 2013). Our results also contradict this hypothesis, as we found no difference among female morphs in clutch size or juvenile mass. Unexpectedly, white morph females roamed across larger areas than females from the other morphs. Rather than alternative strategies in space use, we think this difference may result from white morph females being heavier (and likely more advanced in their ovarian cycle) when released into the enclosures. This could have prompted exploratory behavior in the search for suitable egg-laying sites earlier in this morph. Whether this unexpected result is artefactual or derives from differences in the timing of reproduction among female morphs should be examined in future studies. Overall, our results constitute strong evidence against the existence of ARS concerning male–male aggression, spatial dominance, sexual behavior, or breeding strategy in *P. muralis* color morphs.

Even if color morphs do not reflect ARS, nonrandom mating with respect to color can contribute to the stability of polymorphic systems over time (Galeotti et al., 2003; Roulin, 2004; Wellenreuther et al., 2014). Mate preferences may vary among individuals if the expected benefits derived from mating with differently colored individuals are a function of the chooser's morph (e.g., genetic compatibility) or vary relative to other factors (e.g., time, space, population density; Mckinnon & Pierotti, 2010; Roulin, 2004; Wellenreuther et al., 2014). In polymorphic Pyrenean populations of *P. muralis*, homomorphic pairs of males and females occur more frequently than heteromorphic pairs, irrespective of local morph diversity (Pérez i de Lanuza et al., 2013; Pérez i de Lanuza, Font, & Carretero, 2016). This assortative pairing suggests a role of color morph in mate choice, but is not sufficient to demonstrate its existence (Roulin & Bize, 2007; Roulin, 2004; Wellenreuther et al., 2014). In fact, color-assortative pairing can also occur in the absence of mate choice, for example, if phenotypically similar lizards tend to cluster together within populations as a consequence of similar environmental constraints or population viscosity (Roulin, 2004; Wellenreuther et al., 2014). Here, we did not find evidence of morph assortativity in the male–female social interactions observed within the enclosures. Previous research using laboratory-staged mate choice trials has already reported the absence of color-assortative preferences toward differently colored males in *P. muralis* females (Sacchi et al., 2015). However, we think that our results constitute a more realistic perspective of male–female dynamics in nature, since mounting evidence suggests that the initiation and outcome of precopulatory male–female interactions in lizards are almost completely under male control (Andrews, 1985; Heathcote et al., 2016; Noble & Bradley, 1933; Olsson, 2001; Olsson & Madsen, 1995; Olsson et al., 2013; Tokarz, 1995). Following our results, we deem unlikely that the color-assortative pattern observed in the wild (>60% of pairings at our study site; see Pérez i de Lanuza et al., 2013) results from the lizards actively choosing to pair with similarly colored partners. Rather, assortative pairing could result indirectly from some form of clustering in the spatial distribution of color morphs in natural populations, due to population viscosity or ecophysiological constraints (Lindsay et al., 2019; Pérez i de Lanuza, Sillero, et al., 2018; Svensson, 2017; Svensson, Abbott, Gosden, & Coreau, 2009; Wellenreuther et al., 2014).

Our results also offer evidence against the existence of strong frequency-dependent effects on morph fitness. As stated before, by introducing the color morphs in equal frequencies within the enclosures we simulated a situation that is rarely observed in any of the different *P. muralis* lineages showing color polymorphism. Such balanced morph frequencies were never observed in natural populations from eastern Pyrenees (examined in Pérez i de Lanuza et al., 2017, Pérez i de Lanuza, Sillero, et al., 2018, $N = 116$ localities), where white morph lizards usually predomínate (e.g., morph frequency ranges: orange = 0%–60%; white = 27%–92%; yellow = 0%–25%; orange-white = 0%–27%; yellow-orange = 0%–13%), and only 3.45% of the localities show a morph other than white as the most common. Additionally, morph frequencies do not seem to experience substantial interannual variation, with the same rank order being maintained in the study population of Angoustrine for the last 6 years (Figure S1). If color morphs are, in fact, under some form of frequency-dependent selection, the frequencies observed in natural populations may reflect a selective equilibrium where each morph obtains equal average fitness. By using a 1:1:1 morph ratio in our experimental setup, we simulated a displacement from such equilibrium frequencies, which should have resulted in a selective pullback, and hence higher fitness in white morph lizards (Roulin, 2004; San-Jose et al., 2014; Sinervo et al., 2007; Svensson, 2017). In contrast, we did not find significant differences in fitness among color morphs, suggesting that strong frequency-dependent effects on morph fitness are unlikely to be the prime determinant of morph relative frequencies in *P. muralis* natural populations. This study is primarily aimed at detecting differences in sociosexual behavior among male morphs, and we acknowledge that our experimental design is not tailored to test for frequency-dependent effects on fitness. In fact, testing for a rare (NFDS) or a common morph advantage with a mesocosm design would require to introduce each morph consistently in lower or higher frequency across the enclosures (Roulin, 2004; Svensson, 2017; Wellenreuther et al., 2014). Additionally, selection on color morphs is often dependent on both biotic (demography, sex ratio) and abiotic factors (environmental conditions), as well as on the population morph composition and relative morph frequencies (Forsman, Ahnesjö, Caesar, & Karlsson, 2008; Gosden & Svensson, 2008, 2009; McLean & Stuart-Fox, 2014; McLean, Stuart-fox, & Moussalli, 2015; Svensson, 2017; Svensson et al., 2020; Willink et al., 2019). Future studies should examine the environmental dependence of morph fitness in populations characterized by extreme morph compositions and socioecological contexts (i.e., varying sex ratio, density, and environmental conditions), for example, by combining field observations with the experimental alteration of these same parameters in enclosure experiments.

The maintenance of color polymorphism may be possible through genetic mechanisms entirely independent of sociosexual behavior. For instance, if heterozygosity at genes coding for color polymorphism provides fitness benefits (i.e., overdominance), and the advantages of heterozygosity only concern viability selection (e.g., survival to adulthood), color morphs would be maintained in the population even if morphs mated at random (Krüger, Lindström, & Amos, 2001; Roulin & Bize, 2007; Roulin, 2004; Wellenreuther et al., 2014). In a breeding experiment conducted on captive *P. muralis* lizards from Italian polymorphic populations, morph pair combination was found to affect fertilization success, hatching success and newborn quality (i.e., juvenile mass; Galeotti et al., 2013). Here, we found a weak effect of color morph combination on juvenile mass, but the low sample size ($N = 44$) is insufficient to draw firm conclusions. To examine the role of genetic compatibility and overdominance on stabilizing color polymorphism in future research, we would need to estimate juvenile fitness and interannual survival at the genotypic (rather than the phenotypic) level, as the fitter heterozygotes could be phenotypically indistinguishable from other genotypes (Gratten et al., 2008; Johnston et al., 2013; Tregenza & Wedell, 2000).

Despite drawing substantial interest from evolutionary biologists, the evolutionary causes and consequences of lacertid color polymorphisms are still poorly understood. Alternative reproductive strategies have been suggested to occur in the Dalmatian wall lizard (*Podarcis melisellensis*), where orange males have been found to present larger body size, disproportionately large heads, and higher fighting ability in size-matched contests staged in the laboratory (Huyghe et al., 2007, 2009; Huyghe, Vanhooydonck, Herrel, Tadić, & Van Damme, 2012). In contrast, in the European common lizard (*Zootoca vivipara*), interpopulation differences in morph composition and rapid morph cycles have been explained by the cumulative effect of two frequency-dependent mechanisms starkly different from ARS (morph-biased female mate choice and offspring survival; San-Jose et al., 2014; Sinervo et al., 2007). Meanwhile, differences in morph composition among island populations of the Skyros wall lizard (*Podarcis gaigeae*) have been found to be fall within that expected under neutral genetic divergence, and genetic drift could thus not be rejected as an explanation of the pattern (Runemark et al., 2010). Lastly, most of the evidence suggesting the existence of physiological or behavioral morph differences in *P. muralis* comes from studies conducted on the southern Alps sublineage (Galeotti, 2013; Sacchi, Mangiacotti, et al., 2017; Sacchi, Scali, et al., 2017; Galeotti et al., 2007; Scali et al., 2016), which is only distantly related to the western European lineage found in Pyrenees (Gassert et al., 2013; Giovannotti, Nisi-Cerioni, & Caputo, 2010; Schulte, Gassert, Geniez, Veith, & Hochkirch, 2012). These observations, together with the high prevalence and ancient origin of color polymorphisms in wall lizards (Andrade et al., 2019; Arnold, Arribas, & Carranza, 2007; Jamie & Meier, 2020), suggest the intriguing possibility that genes coding for the expression of the alternative color morphs might become linked to genes that influence other functionally relevant traits (i.e., physiology, behavior, life history, development) only at times, and hence be under selection only in some environments or in some lineages (i.e., *Podarcis* species). Linkage disequilibria are expected to decay rapidly if not counteracted by strong and chronic correlational selection, and genetic drift is very effective in leading to the loss of polymorphism (especially in small populations; Gray & McKinnon, 2007; Mckinnon & Pierotti, 2010; Sinervo & Svensson, 2002; Svensson, 2017). Hence, this evolutionary scenario would cause correlations between color and other phenotypic traits to vary either in space or in time, and even lead to morph loss in some populations or lineages. Polymorphism loss has likely occurred in wall lizards. Despite their putative ancestral origin (Andrade et al., 2019), color morphs are apparently absent in some *Podarcis* species (Speybroeck et al., 2016), and the polymorphic species that have been examined often show marked geographical variation in morph diversity (Jamie & Meier, 2020; MacGregor, Lewandowsky, et al., 2017; Pérez i de Lanuza, Sillero, et al., 2018; Runemark et al., 2010). However, due to its high genetic diversity, effective population sizes in *P. muralis* (and likely in other wall lizards) have been estimated to be sufficiently large ($Ne > 4 \times 10^6$; Yang et al., 2020) to allow for the long-term persistence of a largely neutral trait under intermittent selection contingent on the environment. Local morph extinctions could thus be counteracted by immigration from larger populations where selectively neutral color expression could resist the eroding effect of genetic drift for longer periods, and interpopulation differences in morph composition would be mainly driven by the environmental and genetic constraints of color expression (Gray & McKinnon, 2007; Mckinnon & Pierotti, 2010; Roulin et al., 2004). Recent results showing the recessive genetic basis of orange and yellow ventral coloration in *P. muralis* with respect to white (Andrade et al., 2019) could provide a simple explanation for the marked bias toward the white morph observed in natural populations (Pérez i de Lanuza et al., 2017, Pérez i de Lanuza, Ábalos, et al., 2018, Pérez i de Lanuza et al., 2019; Figure S1). Future research should investigate the possibility of spatially or temporally varying correlations between polymorphic color expression and other phenotypic differences in *Podarcis* lizards, as well as evaluate the relative importance of selection and genetic drift in shaping interpopulation differences in morph composition and relative frequencies (Runemark et al., 2010).

In conclusion, our results do not warrant the frequent assumption that behavioral ARS underlie the maintenance of ventral color morphs in the European common wall lizard. In the wake of the *U. stansburiana* model, much effort has been devoted to detect intermorph differences suggestive of behavioral ARS in polymorphic lizards (Calsbeek et al., 2010; Fernández et al., 2018; Healey, Uller, & Olsson, 2007; Yewers et al., 2016). However, these studies have often painted a much more complex picture involving several evolutionary processes, of which ARS may represent but one in many mechanisms explaining the vast diversity of lizard color polymorphisms (Carpenter, 1995; Huyghe et al., 2012; McLean et al., 2015;

San-Jose et al., 2014). We should therefore reassess the allegedly central role of ARS in explaining the maintenance of phenotypic variability in nature, and broaden the perspective to incorporate other hitherto overlooked processes.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

Javier Abalos: Conceptualization (equal); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); visualization (lead); writing–original draft (lead); writing–review and editing (equal). **Guillem Pérez i de Lanuza:** Conceptualization (equal); supervision (equal); validation (equal); writing–review and editing (equal). **Alicia Bartolomé:** Data curation (equal); investigation (equal); methodology (equal); validation (equal); writing–review and editing (equal). **Océane Liehrmann:** Investigation (equal); methodology (equal); writing–review and editing (supporting). **Hanna Laakkonen:** Investigation (equal); methodology (equal); writing–review and editing (supporting). **Fabien Aubret:** Conceptualization (equal); validation (equal); writing–review and editing (equal). **Tobias Uller:** Conceptualization (equal); validation (equal); writing–review and editing (equal). **Pau Carazo:** Conceptualization (equal); validation (equal); writing–review and editing (equal). **Enrique Font:** Conceptualization (equal); supervision (equal); validation (equal); writing–review and editing (equal).

DATA AVAILABILITY STATEMENT

The datasets used and analyzed in this study are available from Dryad: https://doi.org/10.5061/dryad.j0zpc86bx

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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