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REPORT OF TWO SPONTANEOUS, RARE PHENOTYPIC TRAITS IN THE GENUS *PHILLYREA* L.

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ABSTRACT: In this note, I document two previously unreported, spontaneous and exceptionally rare phenotypic expressions affecting reproductive traits in adult plants of the Mediterranean genus *Phillyrea* (Oleaceae): (i) a morph with an abnormally elongated stigma and lobes transformed in two long branches (i.e. deeper-stigma phenotype), detected in a population of *Phillyrea latifolia* L. from NE Spain, and (ii) a morph with fruits lacking anthocyanins (i.e. colourless-fruit phenotype), in a population of *Phillyrea angustifolia* L. from SW Spain. Both phenotypes occurred at a very low frequency within their respective populations. Despite this, the novel traits acquired are discussed in an eco-evolutionary context, revealing their potential use as a study model, limited but suggestive, to test adaptive hypothesis in natural conditions. **Keywords:** phenotypic expression; adaptive value; anemophilous syndrome; selection mediated by frugivorous birds.

RESUMEN: Descripción de dos caracteres fenotípicos, raros y espontáneos en el género mediterráneo *Phillyrea* L. En esta nota documento la presencia de dos expresiones fenotípicas desconocidas, espontáneas y excepcionalmente raras, que afectan a caracteres reproductivos en plantas adultas del género mediterráneo *Phillyrea* (Oleaceae): (i) un morfo con un estigma anormalmente largo y lóbulos transformados en dos ramas (i.e. fenotipo de estigma profundo), detectado en una población de *Phillyrea latifolia* L. en el noreste de España, y (ii) un morfo con frutos carentes de antocianinas (i.e. fenotipo de frutos sin pigmentación), en una población de *Phillyrea angustifolia* L. del suroeste español. Ambos fenotipos se manifestaron con muy baja frecuencia dentro de sus respectivas poblaciones. A pesar de ello, se discute la adquisición de estos nuevos caracteres en un contexto eco-evolutivo, revelando su uso potencial como modelo de estudio con el que explorar hipótesis adaptativas en condiciones naturales. **Palabras clave:** expresión fenotípica; valor adaptativo; síndrome anemófilo; selección mediada por aves frugívoras.

INTRODUCTION

Novel phenotypic traits arising from any heritable source of biological variation are the substrate of all evolutionary change in nature (WONG & HOURY, 2006). Consequently, mechanistic and functional approaches to the existence and/or maintenance of innovative phenotypic forms within wild populations may have an important significance in evolutionary sciences, both at the micro- (FERNÁNDEZ & al., 2006; DORMONT & al., 2009; ZIERMANN & al., 2009) and macroevolutionary levels (BATEMAN & RUDDAL, 2006; RUDDAL & al., 2011). In this regard, field observation and recording of new phenotypic traits at local scale should not be underestimated, because of their value as possible starting-point to explore evolutionary processes in natural scenarios, beyond experimental manipulation of model-species in the laboratory.

In this note, I report the existence of two spontaneous, rare phenotypic traits within the genus *Phillyrea* L. (Oleaceae), related to floral structure and fruit display respectively: (i) a morph with an anomalous stigma bifurcated in two long branches, detected in *Ph. latifolia* (deeper-stigma phenotype, hereafter); and (ii) a morph with fruits lacking anthocyanins, in *Ph. angustifolia* (colourless-fruit phenotype, hereafter). To my knowledge, none of these phenotypic expressions have been described before (cf. DE JUANA, 2012).

STUDY SPECIES

Phillyrea L. is a circunmediterranean genus from an old lineage of miocenic origin, belonging to the olive-tree tribe (Oleaceae: Oleinae) (BESNARD & al., 2009; HONG-WA & BESNARD, 2013). At present, taxonomy considers only 2-3 species within this genus: *Ph. angustifolia* L., *Ph. latifolia* L. and, with controversy, *Ph. media* L. (FERRER-GALLEGO & al., 2014). However, recent molecular studies suggest its poly- or paraphyletic nature (HONG-WA & BESNARD, 2014), which will force to revise its taxonomy.

Phillyrea species inhabit thermo- to meso-mediterranean forests and macchia formations (RIVAS-MARTÍNEZ & al., 2002). Among woody flora from Mediterranean-type ecosystems, they present a set of morpho-functional features associated in the so-called pre-Mediterranean syndrome (HERRERA, 1992; VERDÚ & *al*., 2003). So, they are phanerophytes showing a deep root system, slow growth-rate from seed, long life-span and persistence after severe perturbation mediated by resprouting ability from subterranean buds (RUIZ-ROBLETO & VILLAR, 2005; VITALE & al., 2007); evergreen, sclerophyllous foliage, ecophysiologically well adapted to avoid drought effects (GRATANI & VARONE, 2004; SAURA-MAS & LLORET, 2007); small nectarless flowers, with a reduced perianth, relatively prominent stigma and exerted stamens with high pollen production, clearly adapted to anemogamy (HERRERA, 1987); and blue drupes with large seeds dispersed by birds (HERRERA, 1984). The most singular trait of *Phillyrea* species is their androdioecious nature, a rare, stable breeding system in which male and hermaphrodite individuals coexist within the same population (LEPART & DOMMÉE, 1992; ARONNE & WILCOCK, 1994).

RESULTS AND DISCUSSION

Phillyrea latifolia L. (deeper-stigma phenotype; fig. 1)

TARRAGONA: 31TCF3579. Vimbodí i Poblet, Prades Mountains, Valley of Torners, Solera dels Torner (Natural Reserve Barranc del Tillar), 850 m a.s.l., at 25% south-facing slope on district cambisol soil, 12-V-2005, *J.L. Medina-Gavilán*.

Ordinarily, hermaphrodite flowers of *Ph. latifolia* have a short style terminated by an elongate, bilobate stigma (<1 mm), whose length at maturity is almost half that of the anthers just before their dehiscence. Stigmatic lobes, though sometimes not conspicuous, are clearly acute (fig. 2). In fact, it has been taken as one of the differential trait for this species (AMARAL FRANCO & ROCHA, 1972; ANDRÉS, 2012).

In Solera dels Torner, one individual with all its flowers bearing an abnormal stigma was found within a dense forest community dominated by *Quercus ilex* L., *Phillyrea latifolia* L. and *Arbutus unedo* L. (cf. OGAYA & al., 2015 for a more detailed description). The stigma was extraordinarily long (=deep) -with a total length more than twice that of a typical one (2-5 mm)- and bifurcated, resembling a pitchfork (fig. 2). Likely, a mutation in a single (LIU & al., 2015) or a few (GOODWILLIE & al., 2006) quantitative trait loci accounts for the structural change on the trait 'stigma-depth' in this rare phenotype. In any case, female fertility at flower level (i.e. fruit-set) was similarly comparable to the ordinary phenotype.

A surface of 1200 m^2 containing 185 flowering trees of *Ph. latifolia* was surveyed on May 2005 in this zone, but no other individual with this trait was detected. It reveals the rarity of this morph. The population was apparently hermaphrodite, without known presence of male individuals (69% hermaphrodites, 31% unknown sex, n=268).

Phillyrea promotes xenogamy through a sophisticated homomorphic diallelic self-incompatibility system, largely conserved in Oleaceae (VERNET & al., 2016). Thus, any functional trait conducive to maximize the collection efficiency of compatible pollen is susceptible to be under natural selection for sexual success (FRIEDMAN & BARRETT, 2009). In principle, flowers of this rare phenotype expose a higher receptive surface to pollen grains dispersed by wind than its counterparts (NIKLAS, 1987; PAW U & HOTTON, 1989). Moreover, because of the elongated growth of the stigma, this abnormal increase in receptive area is not accompanied by a proportional increment in its diameter, which reduces boundary layer resistance for pollen deposition (WHITEHEAD, 1983).

Additionally, a deeper stigma could favour the selection of larger pollen grains, which are potentially more competitive due to increased pollen-tube growth rates in the autotrophic stage of their progression through the pistil (CRUDEN, 2009; WANG & al., 2016). This scenario is only possible as long as size of pollen grains from legitimate donors presents a biologically significant variation among themselves. In the case of *Phillyrea*, if the wide range of pollen sizes initially reported by RENAULT-MISKOWSKY & al. (1976) responds to variations generalized in natural populations, it could be hypothesized that the offspring of the deeper-stigma phenotype could have a certain competitive advantage from the basis of that the selection for fast pollen-tube growth may lead to an increase in progeny fitness (QUESADA & al., 1996).

In short, this wild mutant could serve to explore some of these interesting ecological hypothesis in the frame of micro-evolutionary processes for anemogamous long-lived species, despite the severe limitation of having a single individual.

Phillyrea angustifolia L. (colourless-fruit phenotype; fig. 3)

SEVILLE: 29SQB4723. Aznalcázar, Monte "Grupo ordenado de Aznalcázar" (SE-50001-AY), close to Cañada Real de los Isleños (cuartel A, tramo III, subtramo e), 18 m a.s.l., on typic fragixeralf soil, 1-IX-2003, *J.L. Medina-Gavilán*.

Ripen fruits of *Phillyrea* are purple to blackish-blue drupes, with anthocyanins as pigments responsible for these colours (AYRANCI & ERKAN, 2013). Exceptionally, two individuals bearing yellowish-white fruits were found in a large population of *Phillyrea angustifolia* (n=217) located at a cleared zone (≈4 ha) within a forest of *Pinus pinea* L. (cf. AVILÉS & al., 2015 for a comprehensive description of the territory).

The study population presents a marked biannual pattern of fruiting (cf. HERRERA & *al*., 1998: 588), where years with dominance of vegetative growth alternate with years of massive crop of flowers and fruits. In the latter, apparent differences in fruit size and abortion rate between both morphs were not detected, including observations on seed loss produced by the galling-insect *Probruggmanniella phillyreae* (=*Schizomyia phillyreae*) (cf. TRAVESET, 1994). In contrast, fruit removal rate by frugivorous passerines, and therefore seed dispersal, shows important differences between morphs. Indeed, yellowish-white fruits remained on the crown for several months until their natural abscission, whereas most blue fruits were removed in only two months (September-October).

Anthocyanins content in fruits is well discriminated by European blackcaps (*Sylvia atricapilla*) and garden warblers

(*S. borin*) (SCHAEFER & al., 2008, 2014), which are one of the most common dispersers for seeds of *Ph. angustifolia* (HERRERA, 1984). Moreover, a recent study performed in this territory shows that those passerines species select darker and less chromatic fruits as a reliable indicator of lipid content (SCHAEFER & al., 2014). These results may explain why colourless-fruits of *Ph. angustifolia* mutants are not eaten by birds. Analogously, a rare colourless-fruit individual of *Olea europaea* (f. *leucocarpa*) present in the zone is also neglected by passerine dispersers (pers. obs.). Nevertheless, TRAVESET & al. (2001) did not find differences in fruit removal by birds when they studied a natural population of *Myrtus communis* with a similar colour-fruit dimorphism, suggesting a variable behaviour of frugivorous bird community dependent on environmental factors (e.g. frequency of morphs).

According to the rarity of the colourless-fruit phenotype in *Ph. angustifolia*, with a frequency of 0.003 at study area (n=800), the inhibition of anthocyanin synthesis could be produced by a mutation encoding an enzyme common to biosynthetic pathway of other phenolic compounds, whose deleterious pleiotropic effects prevent its fixation at population level (SOBEL & STREISFELD, 2013). However, ecological constraints, through the avoidance of seed dispersal and germination facilitation by birds, must be also considered as causes of its exceptionality. In sum, these colourless-fruits mutants may be an interesting model on testing ecoevolutionary hypothesis under natural conditions.

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Fig. 1. Deeper-stigma phenotype (*Phillyrea latifolia*)

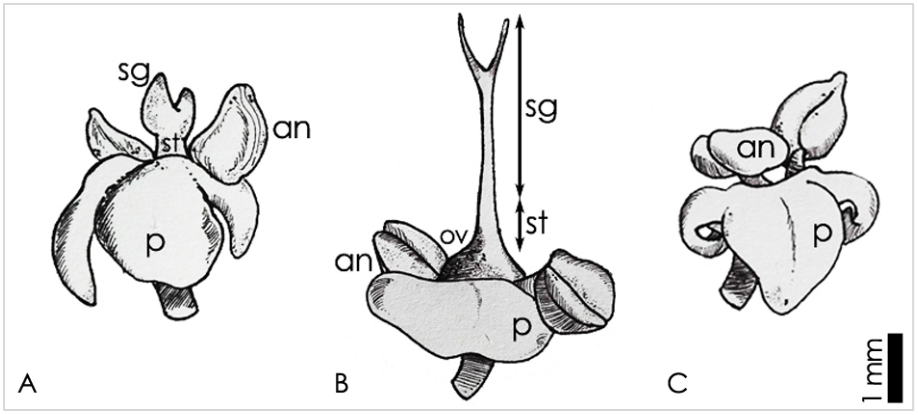

Fig. 2. Morphological differences among hermaphrodite (A), deeper-stigma (B) and male (C) morphs in *Phillyrea latifolia.* sg: stigma; st: style; an: anthers; p: petals.

Fig. 3. Comparative between normal and colourless-fruit phenotypes (*Phillyrea angustifolia*)

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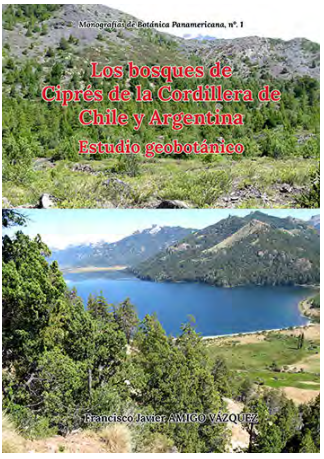

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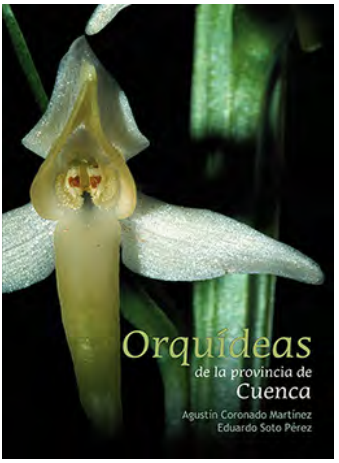

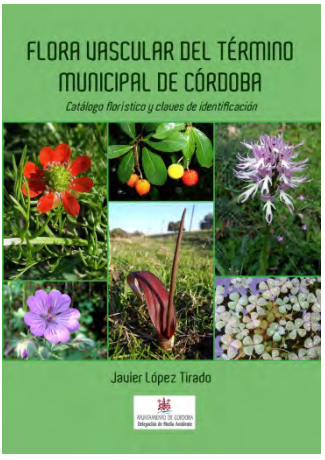

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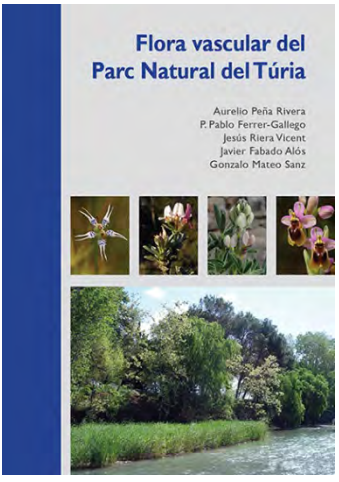

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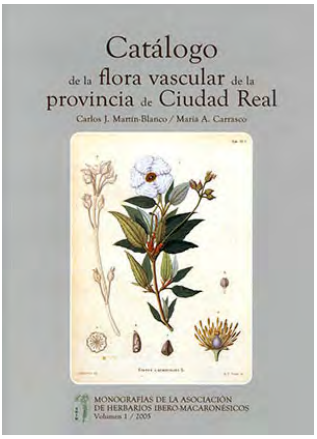

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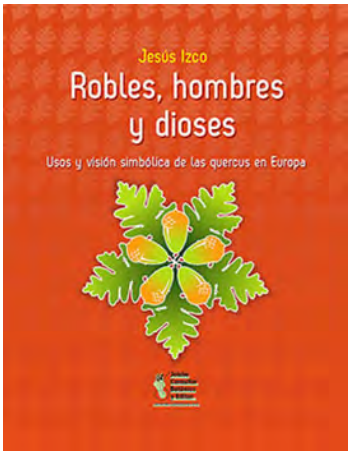

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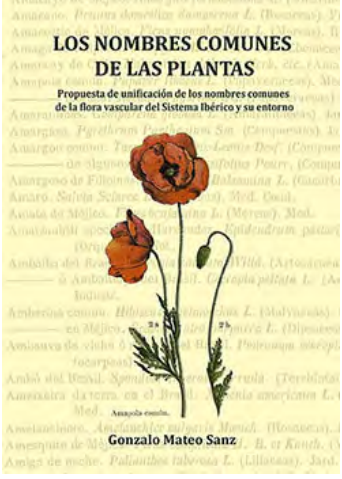

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