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Quantitative genetic analysis of floral traits shows current limits but potential evolution in the wild

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The vast variation in floral traits across angiosperms is often interpreted as the result of adaptation to pollinators. However, studies in wild populations often find no evidence of pollinator-mediated selection on flowers. Evolutionary theory predicts this could be the outcome of periods of stasis under stable conditions, followed by shorter periods of pollinator change that provide selection for innovative phenotypes. We asked if periods of stasis are caused by stabilizing selection, absence of other forms of selection or by low trait ability to respond even if selection is present. We studied a plant predominantly pollinated by one bee species across its range. We measured heritability and evolvability of traits, using genome-wide relatedness in a large wild population, and combined this with estimates of selection on the same individuals. We found evidence for both stabilizing selection and low trait heritability as potential explanations for stasis in flowers. The area of the standard petal is under stabilizing selection, but the variability is not heritable. A separate trait, floral weight, presents high heritability, but is not currently under selection. We show how a simple pollination environment coincides with the absence of current prerequisites for adaptive evolutionary change, while heritable variation remains to respond to future selection pressures.

1. Introduction

Flowering plants exhibit a striking diversity in floral form and function, and because flowers are reproductive organs, the causes and dynamics of their evolution are crucial for understanding plant biodiversity. Much of the variation in floral traits at a macroevolutionary level is often interpreted as the result of adaptations to pollinators [1]. Experimental studies also confirm that many floral traits can be subject to selection by pollinators (reviewed by Parachnowitsch & Kessler [2] and Caruso *et al*. [3]). However, field studies measuring floral traits often find erratic evidence for strong pollinator-mediated selection in wild populations [4]. In their review, Harder & Johnson [4] found that only about one-third of the studies reported significant selection on floral traits. A possible reason for this paradox is the prevalence of periods of stasis, where pollinator-mediated selection on flowers is relaxed or limited due to stabilizing selection under stable conditions. These are then interrupted by more unstable periods where pollinator changes can provide selection for innovative phenotypes (e.g. [4,5]). The causes of evolutionary stasis, in this case for flowers but common in many types of traits and organisms, is one of the long-standing questions that have intrigued evolutionary biologists [6,7].

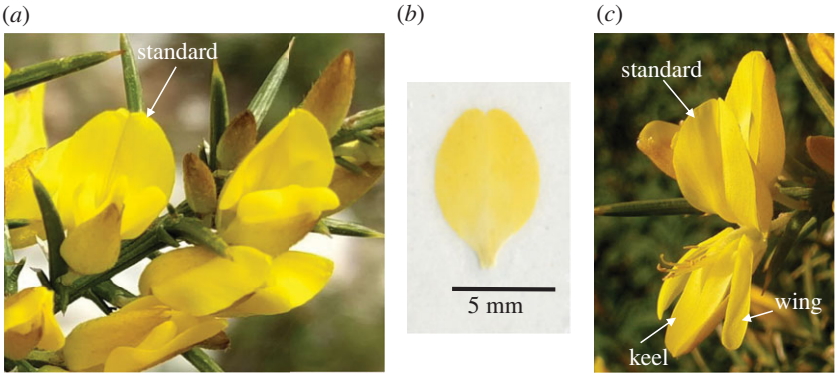

Figure 1. Flowers of *Ulex parviflorus*. (*a*) Flowers previous to a visit with standard petal extended and reproductive organs enclosed by the keel petals and calix. (*b*) Pressed standard petal. (*c*) Flower after being 'triggered' by a bee visit, showing all petals and exposed reproductive organs. Photo credits: (*a*) M. C. Castellanos; (*c*) J. Quiles.

Evolutionary theory's most basic prediction in quantitative trait evolution is captured by the breeder's equation ($R = S \times h^2$) where, for an evolutionary response to take place (R), phenotypic traits need to be under directional selection (S) and harbour enough heritable variation (h^2) for evolution to take place in the wild [8]. The multivariate extension of the breeder's equation reflects the fact that traits do not evolve in isolation and predicts a response determined by selection on multiple traits and a matrix of genetic variance [9]. In any of its forms, the breeder's equation has been found to be too simplistic to consistently explain periods of stasis (the missing response to selection) in many types of traits in wild animal and plant populations, suggesting that several other mechanisms are also involved (see [6,10]). However, our knowledge on even the fundamental aspects of the nature of selection and the presence of heritable variation in wild populations is still limited, particularly for plants. The breeder's equation is thus still useful by providing a good starting set of predictions, where periods of stasis can be the consequence of stabilizing or a lack of directional/disruptive selection on traits, or they can also be the result of low levels of heritable variation even if selection is present.

In the case of flowers, an appropriate model to study the role of these two non-exclusive scenarios is a plant with a single, reliable dominant pollinator. Under these stable conditions, floral traits can be expected to experience low levels of pollinator-driven selection. Heritable variation in floral traits has been shown for many species in greenhouse studies (reviewed in [11,12]), as well as in a few field studies [13–16]. If heritable variation is generally present, stabilizing selection, or a relaxation of selection of any kind could be the most likely explanation for stasis in floral traits in populations with stable environments. Stabilizing selection is one the main mechanisms invoked to explain periods of stasis; however, it is often hard to detect in microevolutionary studies [17].

Trait heritability in wild conditions could also be lower than the estimates under artificially reduced environmental variation. Traditional greenhouse and common garden studies of heritability allow for control of local environments and genetic background, but heritability values measured under controlled conditions can be systematically higher compared to wild conditions [18,19]. This can be caused by higher environmental variability in the field, as well as decreased expression of additive variance, or potential differences in survival in the field compared to the greenhouse, all leading to smaller heritability estimates. The alternative of measuring heritability directly in the field, although being more realistic, was until recently constrained by difficulties in either designing complex crossing and planting experiments (see [15]), or establishing relatedness among individual plants growing in the wild. This has now changed thanks to access to large and highly informative molecular markers that are distributed over the entire genome [20,21]. Using genome-wide markers to measure genetic similarity of plants growing in the wild (in the form of a genome-wide relatedness matrix, GRM), makes it possible to estimate the proportion of the phenotypic covariance that is explained by relatedness (i.e. heritability) in the focal trait [22]. This approach can incorporate environmental factors in the statistical estimation of heritability, to provide us with an ecologically realistic view of what plant populations are experiencing in natural conditions and help us understand the role of standing genetic variation in evolution [15,23].

We study the consequences of a simple pollination environment on natural selection and the heritability of floral traits by focusing on a plant with a single dominant pollinator, the Mediterranean gorse (*Ulex parviflorus*). *Ulex* and relatives (the large legume subfamily Faboidae) have complex irregular butterfly-type flowers ('papilionoid' or 'keel' flowers; figure 1) believed to be specialized on bee pollination, with traits that both enhance pollinator attraction and mechanical interactions that improve pollination success [24]. Over long periods of time, the evolution of flowers in this lineage was thus very likely shaped by adaptation to its pollinators. In contemporary timescales, observations across the current distribution of *U. parviflorus* show that honeybees (*Apis mellifera*) are currently the prevailing pollinator. Overall visitation rates to flowers are low in all areas studied, including in areas with low human influence. Dominance of honeybee visitation was observed by Herrera [25] and again decades later by Reverté *et al*. [26] in coastal populations in southern and eastern Spain, respectively, and has also been observed in inland populations in Cazorla, Spain (93% of visits; C. M. Herrera 2019, personal communication). In this currently simple pollination system, we predict (i) an absence of directional or disruptive selection on floral traits and predominance of stabilizing selection if any type of selection is present and/or (ii) low trait heritability as a consequence of pollinator-mediated selection in the past that might have reduced genetic variation.

To test these predictions, we measured trait heritability and natural selection on the same plant individuals in a

wild population; this allowed us to assess the potential for evolution in response to current and future selection. To our knowledge, this is the first time this approach is used successfully to study floral traits in the wild. We measured floral morphology and pollinator visits, along with estimates of natural selection, genetic correlations, evolvability and heritability of two floral traits. With this we determine if floral traits in a simple pollination environment currently have the potential to evolve in response to selection, and if not, if this is related to the nature of selection, low heritability or both.

2. Materials and methods

(a) Study species and sampling locations

Ulex parviflorus (Mediterranean gorse; Fabaceae) is a thorny perennial shrub that grows up to 2 m. Species in the genus *Ulex* have hermaphroditic flowers pollinated by large-bodied bees, like other species in the tribe Genistae [27]. Flowers are nectar-less, and bees visit to collect pollen, but to do so, they need to be heavy enough to trigger the explosive mechanism for pollen release. Reproductive organs are enclosed by specialized petals, the keel and the wings (figure 1). When an insect presses the keel with its hind legs, the concealed stamens and stigma are released with a powerful upward movement, placing a cloud of pollen grains on the ventral side of the bee. After a visit, flowers do not recover their original shape, with stamens and style now protruding from the keel, and are rarely visited by large bees again, but can receive visits from smaller insects. *Ulex parviflorus* is self-compatible but depends on pollinators to set fruit [28]. Flowering starts in the winter and can last for a few months into spring.

The species is widespread along the western Mediterranean coast from southern France to southern Portugal. It is a successful colonizer of oldfields resulting from abandoned human activities, as well as recently burnt areas, thanks to numerous adaptations to recruitment after fire [29–31]. The seeds form a persistent bank in the soil, until the heat produced during a fire breaks dormancy and stimulates germination [32]. Current landscapes in eastern Spain are a mosaic of oldfields and postfire shrubland [33], where *U. parviflorus* is very abundant and distributed continuously from the lowlands to 900 m (electronic supplementary material, figure S1). As a consequence of the high connectedness, there is very low genetic differentiation in the study area, so that the different sites cannot be considered distinct populations. This was already reported by Moreira *et al*. [34] for this same region, and is also confirmed by the new set of markers in this study (see detailed analysis of genetic structure in the electronic supplementary material). For our sampling, we selected six sites within this continuous population, aiming to capture the potential variability of *U. parviflorus* in the area (electronic supplementary material, table S1 and figure S1). By sampling at different altitudes, for example, we include variability in floral traits along the elevational gradient (electronic supplementary material, figure S2). We tagged 40 plants per site, chosen haphazardly (240 plants in total), for phenotypic and genotypic characterization. Individuals were at least 5 m apart and blooming at the time of sampling.

(b) Pollinator censuses

To quantify the diversity of floral visitors and visitation rates, we ran multiple 3 min pollinator censuses at different times of the day, for up to 5 h of observations per site, on two separate days during peak blooming in 2014 (plus extra censuses in two sites in 2013). Each census recorded the number and identity of visitors to patches of flowers on haphazardly chosen shrubs, including but not limited to the 40 tagged individuals. We counted the number of flowers surveyed in each census and the number of flowers visited to estimate the per-flower visitation rate.

(c) Floral phenotypes

We collected five haphazardly selected flowers from each individual plant for phenotypic characterization of two floral traits that function as proxies for flower showiness and flower size. The area of the upwards-facing petal, or standard, plays a key role in flower showiness, as it is the largest and most visible petal in these typical papilionoid flowers (figure 1; standard petals are often called flag or banner petals). We removed standards from all flowers when fresh, and pressed them flat individually in a plant press. We then used scanned images of the standards to measure their area with the ImageJ software [35].

Flower mass reflects the size of the flower and is important in the Genistae as it determines the size of the insects that can visit the flowers [27,36]. The complex floral shape makes it difficult to measure overall floral size directly, so instead, we estimated size as the dry weight of flowers (calyx and corolla) after removing the standard petal and the pedicel, and brushing off all pollen grains. Flowers were pressed and oven-dried at 40°C for 48 h and weighed to the nearest 0.01 mg.

The traits above were chosen because they are expected to play an important role in the interaction with pollinators and thus be under pollinator-driven natural selection (see 'Study species and sampling locations' above). As is often the case in complex flowers, the two traits are likely to covary, and analyses below take this into account. We have no reason to suspect that there is variability in these traits with flower age (see also [27]). We have not observed florivory in this species and thus assume that herbivores will not directly influence selection on the focal traits.

(d) Plant genotyping and single nucleotide polymorphism-based relatedness

Fresh twigs were collected from each tagged individual plant and dried in silica gel previous to DNA extraction. The extraction was performed using the Speedtools plant DNA extraction kit (Biotools), with small modifications for this highly lignified species. We used a genotyping-by-sequencing (GBS) protocol to identify single nucleotide polymorphisms (SNPs) across the genome [37]. Two Illumina libraries were built for each of our 240 individuals after separate digestions of genomic DNA with *PstI* and *EcoT22I*, to increase the number of high-quality SNPs. Library construction and HiSeq 2000 Illumina sequencing were performed by the Genomic Diversity facility at Cornell University (USA). We implemented SNP calling using the UNEAK pipeline [38] in the TASSEL v.3 software package [39], designed for datasets without a reference genome.

The final SNP dataset used for the analysis of relatedness below excluded loci that were genotyped in less than 90% of individual plants. The minimum allele frequency allowed to retain loci was set to MAF > 0.01. We also excluded individuals with low genotyping rates (under 85% of loci). After applying these filters, we further manually removed remaining loci with extreme values of observed heterozygosity (under 2% and higher than 98%), after estimating oHET with PLINK command '-Hardy' [40].

Pairwise relatedness between all pairs of the remaining 225 individuals was estimated from the similarity of their SNP genotypes. To estimate GRM, the GRM among all pairs of individuals, we used the realized relatedness method of VanRaden [41] and Astle & Balding [42] as implemented in the *kin*

function of package *synbreed* in *R* [43] (see details in electronic supplementary material, methods). Relatedness values under this approach are a measure of excess allele sharing compared to unrelated individuals. As a consequence, negative values can be common and correspond to individuals sharing fewer alleles than expected given the sample.

(e) Fitness estimates and phenotypic selection

We estimated fruit set in the 40 individual plants in each of the six sites as a proxy for female reproductive success. We labelled a representative flowering twig per plant during peak flowering and collected it a few weeks later when fruit capsules were beginning to brown. In the laboratory, we measured 10 cm of twig to count (i) the number of fruits developing normally and (ii) scars left by all flowers produced by the twig, clearly visible under a dissecting microscope. From this we calculated fruit set as the proportion of flowers that develop into a fruit. The majority of fruits had one (71% of 3200 fruits examined) or two seeds (25%), with a mean number of 1.22 seeds per fruit across all individuals.

We estimated selection parameters to test for both linear (directional selection) and nonlinear (stabilizing or disruptive) selection on the two floral traits, using fruit set as the response fitness variable in the models. Because floral weight and standard area are significantly correlated (even though floral weight did not include the standard, Pearson $r = 0.43$, $p < 0.001$), we estimated selection gradients in addition to selection differentials. Selection differentials provide univariate estimates of selection without considering other traits, while gradients provide estimates on correlated traits [9]. By estimating the four selection parameters—standardized linear (S), and quadratic (c) selection differentials, and standardized linear (β) and quadratic (γ) selection gradients—we can explore direct and indirect selection on the floral traits.

We used generalized additive models (GAMs) to measure selection parameters on absolute fitness values, following the approach by Morrissey & Sakrejda [44]. This approach provides quantitative estimates of selection differentials and gradients for non-normal fitness components, testing for both linear and quadratic selection. We fitted GAMs for binomial fruit set data (fruits developed in relation to total flowers), using a logit link function and assuming a binomial error distribution with the *mgcv* package in R. We used univariate GAMs to estimate selection differentials, and included both floral traits into a bivariate model to estimate selection gradients. To control for potential unmeasured local effects, we included site (as random factor) and elevation (as a fixed factor, see below) in all models. Models included additive spline effects on all factors. Models included additive spline effects on all factors. Differential and gradient parameters were estimated based on numerical approximations of first and second partial derivatives of relative fitness, averaged over the distribution of observed phenotype. We used mean floral values for each individual plant (see 'Results' for more details). To calculate the significance of selection differentials and gradients, we used the bootstrap approach ($n = 2000$ samples) implemented in the *gsg* package in R [44].

(f) Quantitative genetic parameters

To estimate additive genetic variance (and then heritability and evolvability) we used a linear mixed 'animal model' approach to model the phenotypic variance in floral traits while including the variance explained by relatedness [45]. We included the elevation above sea level as a fixed effect to account for environmental variability among plants, because elevation is the main factor that varies among sites (electronic supplementary material, figure S2) and this can specially affect floral traits as seen in other species [46]. In addition to the additive genetic effects (see model below), models included two more random effects: the site of origin of each plant, to account for unmeasured local environmental effects that could covary with genetic variation, and the individual identity to account for inter-individual effects (a 'permanent environment' effect in [45]). We had five flower replicates per plant, so the residual error represents within-individual variation. We ran a univariate model for each of the two floral traits studied, specified as

$$y = X\beta + Z_1 a + Z_2 s + Z_3 i + \text{error},$$

where y is the vector of floral trait values, β is the vector of fixed effects (with X as the incidence matrix), Z_1, Z_2 and Z_3 are incidence matrices for the random effects a (individual identity to partition additive genetic effects), s (the site), i (individual identity to model intra-individual effects caused by differences among replicate flowers from the same individual) and error is the residual error. The variance–covariance structure of random factor a in the model is defined by GRM·V_a, where GRM is the genome-wide relatedness matrix between plant pairs, and V_a is the additive variance to be estimated. To test for the effect of not including the spatial (elevation) and environmental (site) predictors in the models, we also ran a 'naive' version of each model that included only the relatedness and individual effects [47]. We ran Bayesian animal models using package *MCMCglmm* for *R* [48] with both floral weight and standard petal area modelled as continuous traits. For modelling the standard area, we used parameter-expanded priors for the distribution of variance components following the χ^2 distribution with one degree of freedom. Each analysis was iterated long enough to obtain 5000 independent chains (see electronic supplementary material, methods and table S2 for model details, scripts and prior selection).

Narrow sense heritability (h^2) was then estimated as the proportion of the total phenotypic variance assigned to the individual (i.e. to the additive genetic variance, V_a):

$$h^2 = \frac{V_a}{V_a + V_s + V_i + V_{\text{error}}},$$

where V_S is the variance explained by the site of origin, V_i is the inter-individual variance in the trait associated with the permanent environment and V_{error} is the residual intra-individual variance. We also estimated the narrow sense evolvability e, i.e. the mean-standardized additive genetic variance, $e = V_a/x^2$, where x is the trait mean; e reflects the expected percentage of change of a trait under a unit strength of selection per generation [49,50] and provides an estimate of evolvability that is independent of trait variation and comparable across traits. We estimated MCMC 95% credible intervals and use to determine the precision of our estimates.

In addition, we estimated the genetic correlation (r_G) between floral weight and standard area by running a bivariate animal model in *MCMCglmm*. In this case, we used the same fixed and random factors as in the univariate models above (see electronic supplementary material, methods, for prior information).

Finally, we calculated the statistical power of our heritability and genetic correlation estimates using the GCTA-GREML Power Calculator at shiny.cnsgenomics.com/gctaPower [51]. This analysis uses the GCTA approach by Yang *et al.* [52], also a population-based estimation method of heritability that differs from our models in using unrelated individuals only, and no repeated measures per individual. Even so, it can provide an indirect power estimation for this study.

3. Results

(a) Pollinators

In 569 surveys, we recorded 364 insect visits to 22 522 censused flowers in 28 h of observations across the six

Table 1. Directional and quadratic selection coefficients (±s.e.) for the two floral traits studied. n.s., not significantly different from zero, asterisks indicate significant coefficients.

	differential		gradient	
trait	directional, s	quadratic, c	directional, β	quadratic, γ
standard petal area	−0.004 ± 0.003 n.s.	−0.001 ± 0.000 ***	−0.066 ± 0.039 n.s.	−0.086 ± 0.029 **
flower weight	−0.008 ± 0.032 n.s.	−0.069 ± 0.023 **	−0.055 ± 0.048 n.s.	−0.043 ± 0.021 n.s.
interaction				0.001 ± 0.004 n.s.

**$p < 0.01$ and ***$p < 0.001$.

U. parviflorus sites. Of those, 331 (92%) were visits by the honeybee *A. mellifera*. Further 25 visits were by *Bombus sp.* individuals (7%). The remaining three visits were to already open flowers by small Coleoptera and a hoverfly, both unlikely to contact stigmas and carry out pollination. Across sites, we found an average visitation rate of 0.015 visits per 3-min census to an individual flower (with the majority of the censuses, 459 out of 569, showing zero visits), which translates into one visit every 3.3 h on average. Visitation rates were similar across sites, except for one site where visits were significantly more frequent (Simat average visitation rate = 0.03 visits per census). This higher visitation did not translate into higher reproductive success compared to other sites.

(b) Floral phenotypes and natural selection

Flowers showed variation in the two traits measured, flower weight and standard petal area, both within and across sites. A variance partition analysis showed that most of the variation is among individuals and across sites (33% and 53%, respectively, for floral weight, and 42% and 40% for standard petal area). This means that there is high within-plant repeatability in both traits ($R = 86\%$ and 80%, respectively) showing low variability across the five flowers measured per individual. High repeatability can lead to attenuation of the selection estimates [53], but their simulations suggest this is unlikely to be a problem in this case of high R. To avoid overcomplicating the models, we ran the selection analysis below using mean floral values for each individual plant (see also [27]). The coefficient of variation (CV, the standard deviation divided by the mean) of these mean values was similar for the two traits (flower weight CV = 21.1%, standard petal area CV = 21.2%).

We found no evidence of linear directional selection on floral traits, either in univariate models (s coefficients) or models of correlated selection incorporating both floral variables (β coefficients, table 1). However, we found evidence for univariate quadratic effects in both traits (c coefficients) and quadratic gradients (γ coefficients) for standard area. This suggests that floral weight is not under direct selection, while there is strong evidence for stabilizing selection on standard petal area (figure 2).

(c) Genomic markers

The GBS sequencing approach yielded a large number of polymorphic SNPs across individuals (261 775 SNPs before quality filtering). After MAF and heterozygosity filtering, we retained 10 421 high-quality SNPs that were present in at least 90% of individuals across all sites. The analyses below use this dataset to estimate genomic relatedness; however, we also tested for the effect of retaining a larger number of SNPs (with presence in at least 50% of the individuals, which leads to a higher number of genotypes imputed by *synbreed*, see electronic supplementary material, methods). Analysis with this larger dataset produced the same qualitative results, so that retaining more (but highly imputed) markers did not add valuable information on the relatedness among our study plants. Therefore, all analyses below use the smaller dataset with 10 421 SNPs.

(d) Heritability, evolvability and genetic correlation

Pairwise relatedness among sampled individuals varied markedly and was overall relatively low (average values ranging from −0.09 to 0.79, but with most values less than 0.2), even within sites (electronic supplementary material, figure S4); this supports the prevalence of outcrossing in this species. The low population genetic structure and the presence of variance in relatedness provide the conditions for a reliable estimation of heritability in the field [22].

We found significant estimates of heritability and evolvability in flower weight ($h^2 = 0.14$ with 0.03–0.34 95% credible intervals [CI], $e = 0.42\%$ with 0.1–1.2% CI; table 2). For standard area, our models instead detected very low additive variance, yielding very low h^2 and e in this case ($h^2 = 0.001$ with 0–0.27 CI, $e = 0.009\%$ with 0–1.9% CI; table 2). For both traits, deviance information criterion (DIC) values for the heritability naive models were slightly larger than for the complete model (electronic supplementary material, table S2), indicating that environmental factors explain part of the variance. The naive models included only the relatedness among individuals and neither environmental nor spatial predictors, and showed estimated h^2 values substantially higher than our final estimates (table 2). Variance components and their CIs are shown in electronic supplementary material, table S3.

Our sample size for estimating the variance components is limited (less than 240 plants) and thus the statistical power was low to detect very low values of heritability (power = 0. 09 for h^2 values of 0.1; see full power analysis in electronic supplementary material, table S5). Even with this limited power, we were able to estimate a significant h^2 value for flower weight in this population. Our bivariate analysis found a low genetic correlation between the two floral traits that is indistinguishable from zero ($r_G = 0.06$). Credible intervals were large (−0.139 to 0.381), so we cannot support the presence of a genetic correlation. For

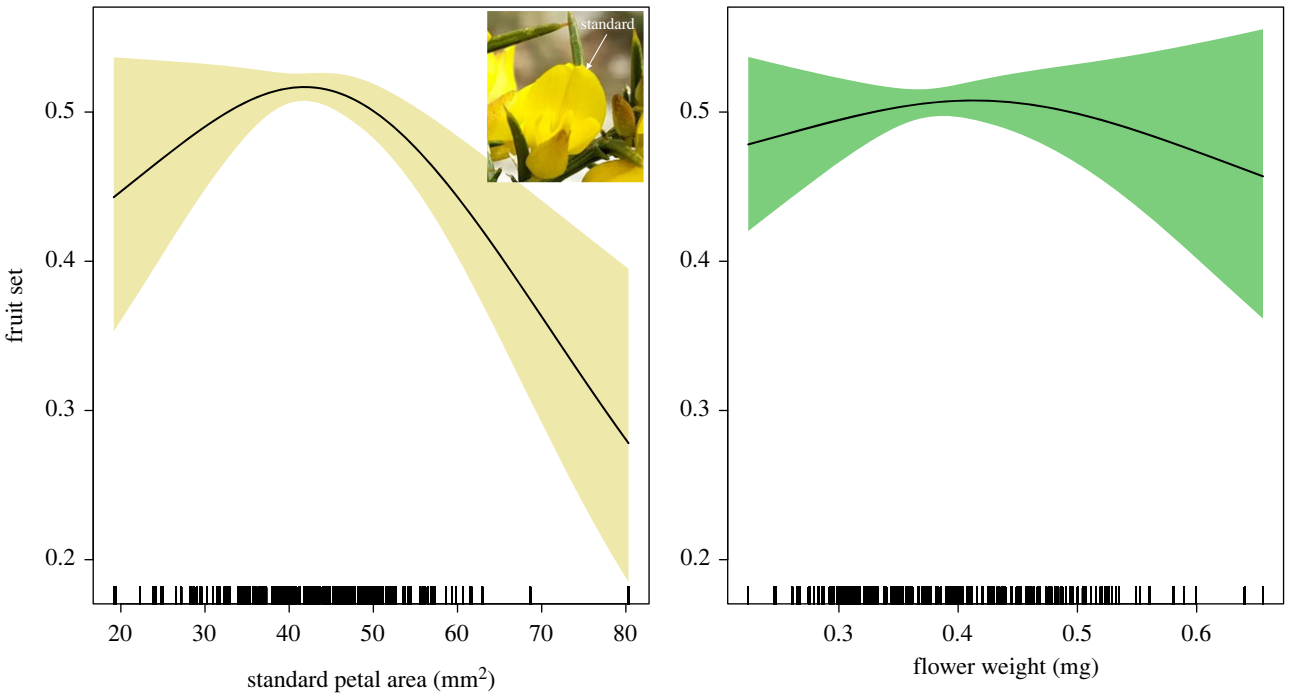

Figure 2. Fruit set as a function of the two floral traits measured: (*a*) standard petal area and (*b*) flower weight (a proxy for floral size). Lines are univariate generalized additive model fits with shaded areas showing 95% confidence intervals.

Table 2. Estimates of heritability h^2 and evolvability *e* (with 95% credibility intervals, CI) for floral traits in wild *Ulex parviflorus*. 'Naive' heritability models did not include spatial or environmental predictors.

	naive h^2 model		final h^2 model		evolvability	
	h^2	CI	h^2	CI	*e*	CI
standard petal area	0.76	0.60–0.81	0.001	0.00–0.27	<0.001%	0.00–1.91
flower weight	0.71	0.60–0.80	0.14	0.03–0.34	0.42%	0.11–1.21

this genetic correlation analysis, the power to detect non-zero values was also low (power = 0.05, given our sample size and estimated values of heritability).

4. Discussion

We provide an example of a stable pollination environment that is associated with phenotypic traits that lack the capacity for contemporary adaptive change, yet maintain enough heritable variation for responding to possible novel selection pressures, at least in one important trait. This follows general theoretical expectations and exemplifies two of the main expected biological explanations for stasis [6], with evidence for both stabilizing selection and low trait heritability as explanations for the potential lack of evolution. Our study focuses on flower traits, but the findings can be relevant to any trait under stable conditions, in the context of the missing response to selection. In *U. parviflorus*, the area of the standard petal is currently under stabilizing selection, but the variability we observe in the field is not heritable. Floral weight, in turn, presents significant heritability, but is not currently under selection.

Simple pollinator communities are a common feature of many plant species under stable environmental conditions. A single pollinator that is also abundant can lead to low pollen limitation and thus reduce the chances for selection on floral traits. However, a low diversity in pollinators does not necessarily imply stability in terms of selection, because few pollinator species that are functionally different (e.g. belong to different taxonomic groups) and vary in space or time, could provide increased opportunities for selection. In the case of *U. parviflorus*, we did observe spatial variation in visitation rates, but this did not lead to changes in reproductive success in those localities. All current evidence shows that honeybees are the dominant pollinators in all surveyed populations, including the one studied here and other distant localities, often sampled decades apart [25,26] (C. M. Herrera 2019, personal communication). Other species in the genus, including *U. europaeus*, *U. minor* and *U. galli*, are visited by a higher diversity of large bees (several species of *Bombus* and *Andrena* [54–56]). The dominance of honeybees in *U. parviflorus* populations could be seen as a consequence of the large anthropogenic influence across its range; however, populations in an area with low human influence and high pollinator diversity (Sierra de Cazorla; see [57]) corroborate the dominance of honeybees as pollinators of this species across its current distribution. Regardless of the reasons for low pollinator diversity, all this suggests that the pollination environment is overall stable for *U. parviflorus* and our

study provides evidence on how this stability can lead to lack of current evolution in floral traits. On the opposite side of the spectrum, field studies that do detect directional selection on unmanipulated floral traits often focus on plants that are exposed to changing pollinator environments, either in different parts of the species range [58–60], or in hybrid contact zones where there is selection against hybrid phenotypes [61]. Taken together, current evidence supports the idea that pollination-driven floral evolution takes place mostly during evolutionarily innovative periods driven by changing pollinators.

Stabilizing selection is one of the main potential causes of stasis in wild populations, and is also expected to be important in the particular case of floral traits that influence the accuracy of pollen deposition during the flower–pollinator interaction [62,63]. It is difficult to assess how common stabilizing selection is on floral (or many other) traits in wild plant populations, because studies rarely measure nonlinear selection [3,4], in addition to the overall difficulty of detecting it [17]. For the standard petal in *Ulex*, we detected stabilizing selection for intermediate surface area. The size of this 'flag' petal is expected to play an important role in pollinator attraction by increasing the floral colour display (figure 1), so that selection against smaller sizes can be expected. Too large standard petals could be selected against if they incur a higher cost for the plant. This cost could be even higher if large standard petals were developmentally only possible as part of overall larger flowers; however, our preliminary genetic correlation estimates suggest only a weak association between standard petal area and floral weight. This is consistent with a previous study that carefully dissected the role of the different petals in another keel flower; in *Collaea argentina*, Córdoba *et al*. [64] found that the standard petal is not functionally integrated with another set of floral traits that collectively regulate the enclosing mechanism of stamens and pistil. That is, the mechanics of protecting the enclosed rewards in these flowers can be independent of pollinator attraction as we expected, and selection can vary across floral parts.

Floral morphological traits often present high levels of additive genetic variation (reviewed by Ashman & Majetic [11] and Opedal [12]); however, most of the studies in these reviews were performed in controlled environments where h^2 estimates are not directly relevant to evolution in the wild. Our field estimates of heritability fall within the lower range of those summarized in fig. 1 of Ashman & Majetic [11], as expected for field estimates, where environmental variation is higher. We found that flower weight shows significant heritability, but there was no detectable heritability in the standard petal area, that is in turn under stabilizing selection. In the case of the latter, we cannot completely rule out that heritability is present but very small, because our sample size provides low statistical power to detect very small values of h^2. Comparing petals in papilionoid flowers, Herrera [27] found that the standard had higher phenotypic variance than other petals across Genisteae, and argued that its role in pollination was smaller than for the keel petals, in a similar way as Córdoba *et al*. [64]. This and our results suggest that this petal might be prone to high environmentally induced variation. It is also likely that long-term stabilizing selection has reduced the additive genetic variance in this trait, leading to the low h^2 values we detected, and consistent with the theoretical expectations for variance reductions under stabilizing selection (although this is not always the case [65]).

Heritability estimates have been criticized as poor standardized measures of evolutionary potential in realistic ecological settings, in part because of the covariance between environmental and genetic effects [49,66]. In this study, we estimate heritability directly in the field, statistically controlling for environmental variation, and in the same individuals as those used to estimate natural selection. In this context, field heritability estimates provide a useful approach to understand the current evolutionary potential at the population level, because we are interested in the role of environmental effects on the phenotypic variance, as exposed to natural selection. An alternative measure of evolutionary potential, evolvability, uses the mean of trait values to standardize the additive genetic variance (as opposed to standardizing by the total phenotypic variance) and provides a comparable estimate of proportional change in a trait value after selection [50]. Our estimates of evolvability are within the range of values estimated for floral size across plant species [12]. These values confirm our findings for heritability, that is, near-zero evolutionary potential for the standard petal area, but higher values for flower weight. In the latter case, evolvability is estimated to be significant but small (under 1% of the trait mean value), suggesting that change in this trait would be slow unless submitted to strong selection.

Our estimate of genetic correlation between the two focal traits needs to be interpreted with caution as our sample size was relatively low. However, the lack of a genetic correlation is not surprising given that we cannot detect significant additive genetic variation in one of the traits (the area of the standard petal). This contrasts with the fact that there is a significant phenotypic correlation between the two traits (although relatively low $r = 0.43$), but as suggested by previous studies, phenotypic correlations are not always good predictors of genetic correlations, even in highly integrated organs as flowers [67]. Again, this is consistent with the decoupling of petals found in a related species with keel flowers [64]. It is thus possible that the phenotypic correlation is caused by shared environmental factors that affect both traits in *Ulex* flowers, further emphasizing the importance of studying evolutionary potential in field conditions.

Even though we could not detect a genetic correlation between the two floral traits studied here, our analysis does not include selection on other (unmeasured) potentially correlated traits. Another potential caveat is that our estimates of selection are based on fruit set alone (as a proxy for reproductive success), and we cannot rule out that the two focal traits might be under selection through the male function [68]. However, the two traits studied here can be expected to affect pollen dispersal in similar ways as pollen deposition (and thus seeds sired), because the trigger mechanism forces both male and female reproductive organs to make contact with the bees at the same time. This means that factors affecting seed set and seed sire are probably highly related in keel flowers.

This study adds to a series of recent work using large sets of molecular markers to study quantitative genetics in wild populations, mostly focused on animals [69], but also on plants [47,70]. Studies comparing the accuracy of SNP-based relatedness matrices compared to pedigrees are consistently showing that they can be very good approximations of continuous genetic relatedness, depending on the specie's life history, and that they can provide higher analytical flexibility

[69,71,72]. This is, therefore, an exciting time for studying the evolution of traits directly in the wild because field-based estimates of evolutionary potential provide new avenues to understand basic evolutionary questions (such as stasis and the role of plasticity in trait variation), but also the potential for wild organisms to respond to new selection pressures including those imposed by anthropogenic environmental change. In the case of flowers, the broad implications of our findings are that low-diversity pollination environments as those caused by anthropogenic pollinator declines might lead to reduced selection pressures, reduced potential to respond to selection, and stasis, while exposure to new pressures could lead to novel evolutionary change.

(a) Final remarks

Our approach in this study attempts to capture microevolutionary response in the wild assuming that measures of selection and heritability are sufficient and constant enough to predict microevolutionary change. This might not always be case, as other factors, such as a low a correlation between traits and fitness, genetic constraints, non-genetic inheritance and plasticity, are not included in the breeder's equation (reviewed in [10]). Nevertheless, in this study we provide results for a study system that are consistent with both theoretical predictions and observations in the field. Selection on floral traits is not restricted to pollinators alone (see [3] and electronic supplementary material, figure S2 for environmentally related variation), but regardless of the source of selection, our findings contribute to the open question of explaining macroevolutionary patterns of floral evolution where novel phenotypes are ubiquitous (exceptions are often related to very generalized pollination that is stable over evolutionary time, see [73]). Populations can experience periods of stasis, but heritable phenotypic variance can remain present in some traits. In combination with potential genetic correlations, this provides the potential to respond to novel selection. However, to fully understand evolutionary responses to rapid environmental change, more studies in the wild are urgent.

Data accessibility. Datasets and R code for all analyses are available in the Dryad Digital Repository [74]. Supplementary methods, figures and tables are provided in the electronic supplementary material [75]. Raw sequence data can be accessed from the NCBI Sequence Read Archive (SRA) with BioProject accession PRJNA951080.

Authors' contributions. M.C.C.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, resources, validation, visualization, writing—original draft, writing—review and editing; J.M.-P: data curation, formal analysis; P.Z.: data curation, formal analysis; J.M.B.: data curation, methodology, resources; J.C.: data curation, methodology, resources; J.G.P.: conceptualization, formal analysis, funding acquisition, methodology, project administration, resources, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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References

1. Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD. 2004 Pollination syndromes and floral specialization. *Annu. Rev. Ecol. Evol. Syst.* **35**, 375–403. (doi:10.1146/annurev.ecolsys.34.011802.132347)
2. Parachnowitsch AL, Kessler A. 2010 Pollinators exert natural selection on flower size and floral display in *Penstemon digitalis*. *New Phytol.* **188**, 393–402. (doi:10.1111/j.1469-8137.2010.03410.x)
3. Caruso CM, Eisen KE, Martin RA, Sletvold N. 2018 A meta-analysis of the agents of selection on floral traits. *Evolution* **73**, 4–14.
4. Harder LD, Johnson SD. 2009 Darwin's beautiful contrivances: evolutionary and functional evidence for floral adaptation. *New Phytol.* **183**, 530–545. (doi:10.1111/j.1469-8137.2009.02914.x)
5. Galen C. 1989 Measuring pollinator-mediated selection on morphometric floral traits: bumblebees and the alpine sky pilot, *Polemonium viscosum*. *Evolution* **43**, 882–890.
6. Merilä J, Sheldon B, Kruuk L. 2001 Explaining stasis: microevolutionary studies in natural populations. *Genetica* **112**, 199–222. (doi:10.1023/A:1013391806317)
7. Estes S, Arnold SJ. 2007 Resolving the paradox of stasis: models with stabilizing selection explain evolutionary divergence on all timescales. *Am. Nat.* **169**, 227–244. (doi:10.1086/510633)
8. Falconer DS, Mackay T. 1996 *Introduction to quantitative genetics*. New York, NY: Longman.
9. Lande R, Arnold SJ. 1983 The measurement of selection on correlated characters. *Evolution* **37**, 1210–1226. (doi:10.2307/2408842)
10. Pujol B *et al.* 2018 The missing response to selection in the wild. *Trends Ecol. Evol.* **33**, 337–346. (doi:10.1016/j.tree.2018.02.007)
11. Ashman T, Majetic C. 2006 Genetic constraints on floral evolution: a review and evaluation of patterns. *Heredity* **96**, 343–352. (doi:10.1038/sj.hdy.6800815)
12. Opedal ØH. 2018 The evolvability of animal-pollinated flowers: towards predicting adaptation to novel pollinator communities. *New Phytol.* **221**, 1128–1135. (doi:10.1111/nph.15403)
13. Schwaegerle KE, Levin DA. 1990 Quantitative genetics of seed size variation in *Phlox*. *Evol. Ecol.* **4**, 143–148. (doi:10.1007/BF02270911)
14. Mazer SJ, Schick CT. 1991 Constancy of population parameters for life-history and floral traits in *Raphanus sativus* L. II. Effects of planting density on phenotype and heritability estimates. *Evolution* **45**, 1888–1907. (doi:10.2307/2409838)
15. Campbell DR. 1996 Evolution of floral traits in a hermaphroditic plant: field measurements of heritabilities and genetic correlations. *Evolution* **50**, 1442–1453. (doi:10.2307/2410882)
16. Galen C. 1996 Rates of floral evolution: adaptation to bumblebee pollination in an alpine wildflower, *Polemonium viscosum*. *Evolution* **50**, 120–125. (doi:10.2307/2410786)
17. Haller BC, Hendry AP. 2014 Solving the paradox of stasis: squashed stabilizing selection and the limits of detection. *Evolution* **68**, 483–500. (doi:10.1111/evo.12275)
18. Conner JK, Franks R, Stewart C. 2003 Expression of additive genetic variances and covariances for wild radish floral traits: comparison between field and greenhouse environments. *Evolution* **57**, 487–495.
19. Winn AA. 2004 Natural selection, evolvability and bias due to environmental covariance in the field in an annual plant. *J. Evol. Biol.* **17**, 1073–1083. (doi:10.1111/j.1420-9101.2004.00740.x)
20. Castellanos MC, Alcántara JM, Rey PJ, Bastida JM. 2011 Intra-population comparison of vegetative and floral trait heritabilities estimated from molecular markers in wild *Aquilegia* populations. *Mol. Ecol.* **20**, 3513–3524.

21. Stanton-Geddes J, Yoder JB, Briskine R, Young ND, Tiffin P. 2013 Estimating heritability using genomic data. *Methods Ecol. Evol.* **4**, 1151–1158. (doi:10.1111/2041-210X.12129)
22. Ritland K. 1996 Marker-based method for inferences about quantitative inheritance in natural populations. *Evolution* **50**, 1062–1073. (doi:10.2307/2410647)
23. Kruuk LE, Charmantier A, Garant D. 2014 The study of quantitative genetics in wild populations. In Charmantier, A, Garant D, Kruuk LEB (eds), *Quantitative genetics in the wild* (eds A Charmantier, D Garant, LEB Kruuk), pp. 1–15. Oxford, UK: Oxford University Press.
24. Westerkamp C. 1997 Keel blossoms: bee flowers with adaptations against bees. *Flora* **192**, 125–132. (doi:10.1016/S0367-2530(17)30767-3)
25. Herrera J. 1988 Pollination relationships in southern Spanish Mediterranean shrublands. *J. Ecol.* **76**, 274–287. (doi:10.2307/2260469)
26. Reverté S, Retana J, Gómez JM, Bosch J. 2016 Pollinators show flower colour preferences but flowers with similar colours do not attract similar pollinators. *Ann. Bot.* **118**, 249–257. (doi:10.1093/aob/mcw103)
27. Herrera J. 2001 The variability of organs differentially involved in pollination, and correlations of traits in Genisteae (Leguminosae: Papilionoideae). *Ann. Bot.* **88**, 1027–1037. (doi:10.1006/anbo.2001.1541)
28. Herrera J. 1987 Flower and fruit biology in southern Spanish Mediterranean shrublands. *Ann. Mo. Bot. Gard.* **74**, 69–78. (doi:10.2307/2399263)
29. Pausas JG, Alessio GA, Moreira B, Corcobado G. 2012 Fires enhance flammability in *Ulex parviflorus*. *New Phytol.* **193**, 18–23. (doi:10.1111/j.1469-8137.2011.03945.x)
30. Pausas JG, Moreira B. 2012 Flammability as a biological concept. *New Phytol.* **194**, 610–613. (doi:10.1111/j.1469-8137.2012.04132.x)
31. Pausas JG, Keeley JE, Schwilk DW. 2017 Flammability as an ecological and evolutionary driver. *J. Ecol.* **105**, 289–297. (doi:10.1111/1365-2745.12691)
32. Moreira B, Tormo J, Estrelles E, Pausas JG. 2010 Disentangling the role of heat and smoke as germination cues in Mediterranean Basin flora. *Ann. Bot.* **105**, 627–635. (doi:10.1093/aob/mcq017)
33. Pausas JG, Millán MM. 2019 Greening and browning in a climate change hotspot: the Mediterranean Basin. *BioScience* **69**, 143–151. (doi:10.1093/biosci/biy157)
34. Moreira B, Castellanos MC, Pausas JG. 2014 Genetic component of flammability variation in a Mediterranean shrub. *Mol. Ecol.* **23**, 1213–1223. (doi:10.1111/mec.12665)
35. Schneider CA, Rasband WS, Eliceiri KW. 2012 NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **9**, 671. (doi:10.1038/nmeth.2089)
36. Córdoba SA, Cocucci AA. 2011 Flower power: its association with bee power and floral functional morphology in papilionate legumes. *Ann. Bot.* **108**, 919–931. (doi:10.1093/aob/mcr196)
37. Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE. 2011 A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS ONE* **6**, e19379. (doi:10.1371/journal.pone.0019379)
38. Lu F, Glaubitz J, Harriman J, Casstevens T, Elshire R. 2012 TASSEL 3.0 universal network enabled analysis kit (UNEAK) pipeline documentation. *White Pap.* **2012**, 1–12.
39. Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES. 2007 TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics* **23**, 2633–2635. (doi:10.1093/bioinformatics/btm308)
40. Purcell S *et al.* 2007 PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559–575. (doi:10.1086/519795)
41. VanRaden PM. 2008 Efficient methods to compute genomic predictions. *J. Dairy Sci.* **91**, 4414–4423. (doi:10.3168/jds.2007-0980)
42. Astle W, Balding DJ. 2009 Population structure and cryptic relatedness in genetic association studies. *Stat. Sci.* **24**, 451–471. (doi:10.1214/09-STS307)
43. Wimmer V, Albrecht T, Auinger H, Schoen C. 2012 *synbreed*: a framework for the analysis of genomic prediction data using R. *Bioinformatics* **28**, 2086–2087. (doi:10.1093/bioinformatics/bts335)
44. Morrissey MB, Sakrejda K. 2013 Unification of regression-based methods for the analysis of natural selection. *Evolution* **67**, 2094–2100. (doi:10.1111/evo.12077)
45. Wilson AJ, Réale D, Clements MN, Morrissey MM, Postma E, Walling CA, Kruuk LEB, Nussey DH. 2010 An ecologist's guide to the animal model. *J. Anim. Ecol.* **79**, 13–26. (doi:10.1111/j.1365-2656.2009.01639.x)
46. Herrera J. 2005 Flower size variation in *Rosmarinus officinalis*: individuals, populations and habitats. *Ann. Bot.* **95**, 431–437. (doi:10.1093/aob/mci041)
47. Castellanos MC, González-Martínez SC, Pausas J. 2015 Field heritability of a plant adaptation to fire in heterogeneous landscapes. *Mol. Ecol.* **24**, 5633–5642. (doi:10.1111/mec.13421)
48. Hadfield JD. 2010 MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *J. Stat. Softw.* **33**, 1–22. (doi:10.18637/jss.v033.i02)
49. Houle D. 1992 Comparing evolvability and variability of quantitative traits. *Genetics* **130**, 195–204. (doi:10.1093/genetics/130.1.195)
50. Hansen TF, Armbruster WS, Carlson ML, Pélabon C. 2003 Evolvability and genetic constraint in *Dalechampia* blossoms: genetic correlations and conditional evolvability. *J. Exp. Zool.* **296B**, 23–39. (doi:10.1002/jez.b.14)
51. Visscher PM, Hemani G, Vinkhuyzen AA, Chen G-B, Lee SH, Wray NR, Goddard ME, Yang J. 2014 Statistical power to detect genetic (co)variance of complex traits using SNP data in unrelated samples. *PLoS Genet.* **10**, e1004269. (doi:10.1371/journal.pgen.1004269)
52. Yang J, Lee SH, Goddard ME, Visscher PM. 2011 GCTA: a tool for genome-wide complex trait analysis. *Am. J. Hum. Genet.* **88**, 76–82. (doi:10.1016/j.ajhg.2010.11.011)
53. Dingemanse NJ, Araya-Ajoy YG, Westneat DF. 2021 Most published selection gradients are underestimated: why this is and how to fix it. *Evolution* **75**, 806–818. (doi:10.1111/evo.14198)
54. Kirchner F, Bullock J. 1999 Taxonomic separation of *Ulex minor* Roth. and *U. gallii* Planch.: morphometrics and chromosome counts. *Watsonia* **22**, 365–376.
55. Bowman G, Tarayre M, Atlan A. 2008 How is the invasive gorse *Ulex europaeus* pollinated during winter? A lesson from its native range. *Plant Ecol.* **197**, 197–206. (doi:10.1007/s11258-007-9370-1)
56. Falk S J. 2011 A survey of the bees and wasps of fifteen chalk grassland and chalk heath sites within the East Sussex South Downs. See https://www.bwars.com/sites/www.bwars.com/files/diary_downloads/East_Sussex_Downs_Bees%26Wasps.pdf.
57. Herrera CM. 2018 Complex long-term dynamics of pollinator abundance in undisturbed Mediterranean montane habitats over two decades. *Ecol. Monogr.* **89**, e01338.
58. Herrera CM, Castellanos MC, Medrano M. 2006 Geographical context of floral evolution: towards an improved research programme in floral diversification. In *Ecology and evolution of flowers* (eds LDB Harder, CH Spencer), pp. 278–294. Oxford, UK: Oxford University Press.
59. Anderson B, Alexandersson R, Johnson SD. 2010 Evolution and coexistence of pollination ecotypes in an African Gladiolus (Iridaceae). *Evolution* **64**, 960–972. (doi:10.1111/j.1558-5646.2009.00880.x)
60. Mackin CR, Peña JF, Blanco MA, Balfour NJ, Castellanos MC. 2021 Rapid evolution of a floral trait following acquisition of novel pollinators. *J. Ecol.* **109**, 2234–2246. (doi:10.1111/1365-2745.13636)
61. Campbell DR, Faidiga A, Trujillo G. 2018 Clines in traits compared over two decades in a plant hybrid zone. *Ann. Bot.* **122**, 315–324. (doi:10.1093/aob/mcy072)
62. Cresswell JE. 2000 Manipulation of female architecture in flowers reveals a narrow optimum for pollen depostion. *Ecology* **81**, 3244–3249. (doi:10.1890/0012-9658(2000)081[3244:MOFAIF]2.0.CO;2)
63. Armbruster WS, Hansen TF, Pélabon C, Pérez-Barrales R, Maad J. 2009 The adaptive accuracy of flowers: measurement and microevolutionary patterns. *Ann. Bot.* **103**, 1529–1545. (doi:10.1093/aob/mcp095)
64. Córdoba SA, Benitez-Vieyra S, Cocucci AA. 2015 Functional modularity in a forcible flower mechanism: relationships among morphology, biomechanical features and fitness. *Evol. Ecol.* **29**, 719–732. (doi:10.1007/s10682-015-9783-6)
65. Johnson T, Barton N. 2005 Theoretical models of selection and mutation on quantitative traits. *Phil. Trans. R. Soc. B* **360**, 1411–1425. (doi:10.1098/rstb.2005.1667)
66. Hansen TF, Pélabon C, Houle D. 2011 Heritability is not evolvability. *Evol. Biol.* **38**, 258. (doi:10.1007/s11692-011-9127-6)

67. Gómez J, Abdelaziz M, Muñoz-Pajares J, Perfectti F. 2009 Heritability and genetic correlation of corolla shape and size in *Erysimum mediohispanicum*. *Evolution* **63**, 1820–1831. (doi:10.1111/j.1558-5646.2009.00667.x)
68. van Kleunen M, Burczyk J. 2008 Selection on floral traits through male fertility in a natural plant population. *Evol. Ecol.* **22**, 39–54. (doi:10.1007/s10682-007-9157-9)
69. Perrier C, Delahaie B, Charmantier A. 2018 Heritability estimates from genomewide relatedness matrices in wild populations: application to a passerine, using a small sample size. *Mol. Ecol. Resour.* **18**, 838–853. (doi:10.1111/1755-0998.12886)
70. Johnston SE, Chen N, Josephs EB. 2022 Taking quantitative genomics into the wild. *R. Soc.* **289**, 20221930.
71. Bérénos C, Ellis PA, Pilkington JG, Pemberton JM. 2014 Estimating quantitative genetic parameters in wild populations: a comparison of pedigree and genomic approaches. *Mol. Ecol.* **23**, 3434–3451. (doi:10.1111/mec.12827)
72. Gienapp P, Fior S, Guillaume F, Lasky JR, Sork VL, Csilléry K. 2017 Genomic quantitative genetics to study evolution in the wild. *Trends Ecol. Evol.* **32**, 897–908. (doi:10.1016/j.tree.2017.09.004)
73. Vasconcelos TNC, Chartier M, Prenner G, Martins AC, Schönenberger J, Wingler A, Lucas E. 2019 Floral uniformity through evolutionary time in a species-rich tree lineage. *New Phytol.* **221**, 1597–1608. (doi:10.1111/nph.15453)
74. Castellanos MC. 2023 Data from: Quantitative genetic analysis of floral traits shows current limits but potential evolution in the wild. *Dryad Digital Repository*. (doi:10.5061/dryad.9zw3r22jp)
75. Castellanos MC, Montero-Pau J, Ziarsolo P, Blanca JM, Cañizares J, Pausas JG. 2023 Quantitative genetic analysis of floral traits shows current limits but potential evolution in the wild. Figshare. (doi:10.6084/m9.figshare.c.6533977)