A stable pollination environment limits current but not potential evolution of floral traits

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Running title: Floral evolutionary potential

Abstract

Plants' vast variation in floral traits at a macroevolutionary level is often interpreted as the result of adaptation to pollinators. However, field studies often find no evidence of pollinator-mediated selection on flowers. This could be explained by periods of stasis, when selection is relaxed under stable conditions, followed by pollinator changes that provide innovative selection. We asked if periods of stasis are caused by stabilizing or absence of other forms of selection on floral traits, or by low trait heritability even if selection is present. We studied *Ulex parviflorus*, a plant predominantly pollinated by one bee species across its range. We measured heritability and evolvability of floral traits, using genome-wide molecular relatedness in a wild population, and combined this with estimates of selection. We found evidence for both stabilizing selection and low trait heritability as explanations for stasis in flowers. The area of the standard petal is under stabilizing selection, but the variability observed in the wild is not heritable. A separate trait, floral size, in turn presents high heritability, but is not currently under selection. We show how a stable pollination environment can lead to a lack of evolutionary change, yet maintain heritable variation to respond to future selection pressures.

Keywords: evolvability, floral evolution, heritability, keel flower, quantitative genetics in the wild, *Ulex parviflorus*

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 (Fenster et al. 2004). Experimental studies also confirm that many floral traits can be subject to selection by pollinators (reviewed by Parachnowitsch and Kessler 2010, Caruso et al. 2018). However, field studies measuring pollinator-mediated evolution of floral traits often find sporadic and erratic evidence for strong selection taking place in wild populations (Harder and Johnson 2009). In their review, Harder and Johnson found that only about 1/3 of the studies reported significant selection on floral traits. A possible reason for this 'paradox' is the likely prevalence of periods of stasis, where pollinator-mediated selection on flowers is relaxed under stable conditions, interrupted by more unstable periods where pollinator changes can provide innovative selection (e.g. Galen 1989, Harder and Johnson 2009).

For pollinator-mediated evolution to take place in the wild, floral phenotypic traits must not only be under selection but also harbour enough heritable variation. Periods of stasis can thus be the consequence of stabilizing or a lack of directional/disruptive selection on traits, or alternatively, they can also be the result of low levels of heritable variation even if selection is present. An appropriate model to study the role of these two non-exclusive scenarios would be a plant with a stable single dominant pollinator. Under these stable conditions, floral traits can be expected to experience low levels of pollinator-driven innovative selection, but still be heritable. Heritable variation in floral traits has been shown for numerous species in the greenhouse (reviewed in Ashman and Majetic 2006, Opedal 2018), and in a few field studies (Schwaegerle and Levin 1990, Mazer and Schick 1991, Campbell 1996, Galen 1996). Thus a relaxation of selection could be the most likely explanation for stasis in floral traits in populations with stable pollination environments.

However, it also possible that trait heritability is lower in wild conditions than indicated by 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 overestimated compared with wild conditions (Conner et al. 2003, Winn 2004). This can be caused by higher environmental variability in the field, as well as decreased expression of additive variance, or potential differences in survival, 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 designing complex crossing and planting experiments (see Campbell 1996), or in establishing relatedness among individual plants growing in the wild. This has now changed thanks to access to large and highly informative molecular markers (Castellanos et al. 2011, Stanton-Geddes et al. 2013). Using genome-wide markers to measure genetic similarity of plants growing in the wild (in the form of a relatedness matrix, *G*), it is possible to estimate the proportion of the phenotypic covariance that is explained by relatedness (i.e. heritability) in the focal floral trait (Ritland 1996). 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 genetic variation in evolution (Campbell 1996, Kruuk et al. 2014).

We study the consequences of a stable pollination environment on floral traits by focusing on a plant with a dominant pollinator, the Mediterranean gorse (*Ulex parviflorus*). Observations across its current distribution show that honey bees (*Apis mellifera*) are currently the prevailing pollinator, with consistently low visitation rates, including in areas with low human influence. Dominance of honey bee visitation was observed by Herrera (1988; no % of visits reported) and Reverté et al. (2016; 63% of visits were by honey bees) 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 pers com.). Pollinator-mediated selection on flowers is expected in this plant because *Ulex* and relatives (the large legume subfamily Faboidae) often have complex irregular butterfly-type flowers ("papilionoid" or "keel" flowers, Fig. 1) believed to be specialized on bee pollination, with traits that both enhance pollinator attraction and mechanical interactions that improve pollination (Westerkamp 1997). In such system, we predict 1) a relaxation of directional or disruptive (i.e. innovative) selection of floral traits, and/or 2) low trait heritability as a consequence of genetic erosion over time.

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. We measured floral morphology and pollinator visits, along with natural selection, genetic correlation, evolvability, and heritability of the floral traits to 1) determine if floral traits in a stable pollination environment are currently under selection and show heritable

variation, thus evolving in response to selection, and 2) if not, to establish if the causes for a lack of evolutionary response are related to low selection, low heritability or both.

Materials and Methods

Study species and sampling locations

Ulex parviflorus Pourr. (Mediterranean gorse; Fabaceae) is a thorny perennial shrub that lacks true leaves in the adult stage and grows up to 2 m. Species in the genus *Ulex* have yellow hermaphroditic flowers visited and pollinated by large-bodied bees, in a similar way to other species in the tribe Genistae (Herrera 2001). Flowers do not produce nectar and the bees visit to collect pollen, but to be able to do so, they need to be heavy enough to actively trigger the explosive mechanism for pollen release. Reproductive organs in these flowers are enclosed by specialized petals, the keel and the wings (Fig. 1). The insect presses the keel petals with the hind legs and this pressure powerfully releases the concealed stamens and stigma upwards, placing a cloud of pollen grains on the ventral side of the bee. After a visit flowers do not recover their original shape, with stigmas and style now protruding from the keel, and are rarely visited by large bees again, but can receive visits by smaller insects like hoverflies and solitary bees. *Ulex parviflorus* is self-compatible but depends on pollinators to set fruit (Herrera 1987). Flowering starts in the winter and can last for a few months into the 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 (Pausas et al. 2012, Pausas and Moreira 2012, Pausas et al. 2017). The seeds form a persistent bank in the soil, where they remain dormant until the heat produced during a fire breaks dormancy and stimulates germination in post-fire conditions (Moreira et al. 2010). Current landscapes in eastern Spain are a mosaic of oldfields and postfire shrubland (Pausas & Millán 2019), where *Ulex parviflorus* is very abundant and distributed continuously from the lowlands up to 900m of altitude (Fig S1 in supplementary materials). As a consequence there is very low genetic differentiation in the study area (Moreira et al. 2014; see also Supplementary methods and Fig. S3), and different stands cannot be considered distinct populations. For our sampling, we selected six sites within this continuous population, aiming to capture the variability of mature *U. parviflorus* stands in the area (Table S1, Fig. S1). By sampling at different altitudes, for example, we include variability in floral traits along the elevational gradient (Fig. S2 in supplementary materials). At each site, we tagged 40 individual plants

(240 plants in total) for phenotypic and genotypic characterization as described below. Individuals were at least 5 m apart from each other and blooming at the time of sampling.

Pollinator censuses

To quantify the diversity of floral visitors and visitation rates, we ran multiple three-minute long pollinator censuses at different times of the day, for up to five hours of observations per locality, on two separate days during peak blooming in 2014. We also ran censuses in two localities in 2013, again during peak blooming (Montserrat and Cheste). Each census recorded the number and identity of visitors to patches of flowers on haphazardly chosen individuals. We counted the number of flowers included in each census to estimate the per-flower visitation rate.

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 more visible petal in these typical papilionoid flowers (Fig.1; standard petals are also often called flag or banner petals). We removed standards from all flowers when fresh, pressed them flat individually in a plant press until dry. We then used scanned images of the standards to measure their surface area with the Image-J analysis freeware (Schneider et al. 2012).

Flower size is important in the Genistae as it can determine the size of the insects that can visit the flowers (Herrera 2001, Córdoba and Cocucci 2011). Size was estimated as the dry weight of flowers (calyx and corolla) after removing the standard petal and the pedicel, and carefully brushing off all pollen grains. Flowers were pressed and oven-dried at 40ºC for 48 hours and weighed to the nearest 0.01 mg.

These traits were chosen because they can be expected to play an important role in the interaction with pollinators and thus be under natural selection driven by pollinators (see Study Species above). As is the case in many complex flowers, the two traits studied can be expected to co-vary (Herrera 2001), and analyses below are designed to take this into consideration. We have no reason to suspect that there is variability in these traits with flower age (see also Herrera 2001). We have never observed florivory in this species and thus doubt that herbivores will directly select for the two focal traits in this study.

170 **Plant genotyping**

171 Fresh terminal twigs were collected from each tagged individual plant and dried in silica gel previous
172 to DNA extraction. The extraction was performed using the Speedtools plant DNA extraction kit
173 (Biotools, Madrid, Spain), with modifications to the manufacturer's protocol to optimize DNA
174 quantity and quality extracted for this highly lignified species. We used the Genotyping-by-
175 Sequencing (GBS) protocol to identify single nucleotide polymorphisms (SNPs) across the genome
176 (Elshire et al. 2011). Illumina libraries for our 240 individuals were constructed by digesting genomic
177 DNA with a restriction enzyme. The GBS protocol was followed twice for each plant after separate
178 digestions with *PstI* and *EcoT22I*, in order to increase the number of high quality SNPs. Library
179 construction and sequencing was performed by the Genomic Diversity facility at Cornell University
180 (USA). SNP calling was implemented using the UNEAK pipeline (Lu et al. 2013) in the TASSEL v.3
181 software package (Bradbury et al. 2007), designed for data sets without a reference genome.
182
183 The final SNP dataset used for the analysis of relatedness below excluded loci that were not
184 genotyped in at least 90% of individual plants. The minimum allele frequency allowed to retain loci
185 was set to MAF > 0.01. We also excluded individuals with low genotyping rates (under 85% of loci).
186 After applying these filters, we also manually removed remaining loci with extreme values of
187 observed heterozygosity (under 2% and higher than 98%), after estimating oHET with PLINK
188 command –Hardy (Purcell et al. 2007).
189
190 **Fitness estimates and phenotypic selection**

191 We estimated fruit set in the same 40 individual plants in each locality as a proxy for female
192 reproductive success. For this, we labelled a representative flowering twig in each plant during
193 flowering peak. When fruits were already developing (browning capsules) a few weeks later, we
194 collected the labelled twig in a paper envelope. Back in the laboratory we measured 10 cm of twig to
195 calculate a) the number of fruits developing normally, and b) scars left by all flowers produced by
196 the twig, clearly visible under a dissecting microscope. From this we calculated fruit set as the
197 proportion of flowers that develop into a fruit. The majority of fruits had one (71% of 3200 fruits
198 examined) or two seeds (25%), with a mean number of 1.22 seeds/fruit across all individuals.
199
200 We estimated selection parameters to test for both linear and non-linear selection on the two floral
201 traits, using fruit set as the response fitness variable in the models. Because floral weight and
202 standard area show a significant phenotypic correlation (even though floral weight did not include
203 the standard, Pearson $r = 0.43$, $P < 0.001$), we estimated selection gradients in addition to selection

204 differentials. Selection differentials provide univariate estimates of selection without considering
205 other traits, while gradients provide estimates on correlated traits. By estimating the four selection
206 parameters - standardized linear (S), and quadratic (c) selection differentials, and standardized linear
207 (β) and quadratic (γ) selection gradients - we can explore direct and indirect selection on the floral
208 traits. Linear parameters test for directional selection, while quadratic parameters measure
209 potential stabilizing or disruptive selection.
210
211 We used generalised additive models (GAM) to measure selection parameters on absolute fitness
212 values, following the approach developed by Morrisey and Sakreda (2013). This approach provides
213 quantitative estimates of selection differentials and gradients for non-normal fitness components,
214 testing for both linear and quadratic selection. We fitted GAMs for binomial fruit set data (fruits
215 developed in relation total flowers), using a logit link function and assuming a binomial error
216 distribution with the *mgcv* package in R. We used univariate GAMs to estimate selection
217 differentials, and included both floral traits into a bivariate model to estimate selection gradients. To
218 control for local effects, we included locality as a random factor in all models. Models included
219 additive spline effects on all factors. Differential and gradient parameters were estimated based on
220 numerical approximations of first and second partial derivatives of relative fitness, averaged over
221 the distribution of observed phenotype. To calculate the significance of selection differentials and
222 gradients, we used the bootstrap approach (n= 1000 samples) implemented in the *gsg* package in R
223 (Morrissey and Sakrejda 2013).
224
225 **SNP-based relatedness and quantitative genetic parameters**
226 Pairwise relatedness between all pairs of individuals was estimated from the similarity of their SNP
227 genotypes. To estimate G, the genome-wide relatedness matrix among all pairs of individuals, we
228 used the realized relatedness method of VanRaden (2008) and Astle and Balding (2009) as
229 implemented in the *kin* function of package *synbreed* in *R* (Wimmer et al. 2012; see details in
230 Supplementary methods). Relatedness values under this approach are a measure of excess allele
231 sharing compared to unrelated individuals. As a consequence, negative values can be common and
232 correspond to individuals sharing fewer alleles than expected given the sample.
233 To estimate additive genetic variance (and then heritability and evolvability) we used a linear
234 mixed 'animal model' approach to model the phenotypic variance in floral traits while including the
235 variance explained by relatedness (Wilson et al. 2010). We included the elevation above sea level as
236 a fixed effect to account for environmental variability among plants, because elevation is the main
237 factor that varies among localities (Fig. S2) and this could affect floral traits as in other species

238 (Herrera 2005). In addition to the additive genetic effects (see model below), models included two
239 more random effects: the site of origin of each plant, to account for unmeasured local
240 environmental effects that could co-vary with genetic variation, and the individual identity to
241 account for intra-individual effects (a "permanent environment" effect in Wilson et al. 2010),
242 because we had five flower replicates per plant. We ran a univariate model for each of the two
243 floral traits studied, specified as:
244

245 $$y = X\beta + Z_1 a + Z_2 s + Z_3 i + e$$

246
247 where y is the vector of floral trait values, β is the vector of fixed effects (with X as the incidence
248 matrix), Z_1, Z_2 and Z_3 are incidence matrices for the random effects *a* (individual identity to partition
249 additive genetic effects), s (the locality), i (individual identity to model intra-individual effects caused
250 by differences among replicate flowers from the same individual), and e is the residual error. The
251 variance-covariance structure of random factor *a* in the model is defined by $G \cdot V_a$, where G is the
252 genome-wide relatedness matrix between plant pairs, and V_a is the additive variance to be
253 estimated. To test for the effect of not including the spatial and environmental predictors in the
254 models, we also ran a 'naïve' version of each model that included only the relatedness and individual
255 effects (Castellanos et al. 2015). We ran Bayesian animal models using package *MCMCglmm* for *R*
256 (Hadfield 2010) with both floral weight and standard petal area modeled as continuous traits. For
257 modelling the standard area, we used parameter expanded priors for the distribution of variance
258 components following the χ^2 distribution with one degree of freedom. Each analysis was iterated
259 long enough to obtain 5000 independent chains (see supplementary methods and Table S2 for
260 model details, scripts and prior selection).
261
262 Narrow sense heritability (h^2) was then estimated as the proportion of the total phenotypic
263 variance assigned to the individual (i.e. to the additive genetic variance, V_a):

$$h^2 = \frac{V_a}{V_a + V_s + V_i + V_e}$$

264 where V_S is the variance explained by the site of origin, V_i is the intra-individual variance in the trait,
265 and V_e is the residual variance. We also estimated the narrow sense evolvability (*e*), i.e. the mean-
266 standardized additive genetic variance, $e = V_a / x^2$, where *x* is the trait mean. *e* reflects the expected
267 percentage of change of a trait under a unit strength of selection per generation (Houle 1992,
268 Hansen et al. 2003) and provides an estimate of evolvability that is independent of trait variation
269 and comparable across traits.
270

271 In addition, we estimated the genetic correlation (r_G) between floral weight and standard area by
272 running a bivariate animal model in *MCMCglmm*. In this case we used the same fixed and random
273 factors as in the univariate models above (see supplementary methods for prior information).
274

275 Results

276 Pollinators

277 We recorded 364 visits to 22522 censused flowers in 28 hours of observations across the six *U.*
278 *parviflorus* localities. Of those, 331 (92%) were visits by the honeybee *Apis mellifera*. Further 25 visits
279 were by *Bombus sp.* individuals (7%). The remaining 3 visits were to already open flowers by small
280 coleoptera and a hoverfly, both unlikely to contact stigmas and carry out pollination. Across sites, we
281 found an average visitation rate of 0.015 (±0.057) visits per 3-minute census to an individual flower,
282 which translates into a visit every 3.3 hours, on average. Visitation rates were similar when
283 comparing localities, except for one where visits were significantly more frequent (Simat average
284 visitation rate= 0.03 visits per census).
285

286 Floral phenotypes and selection

287 Flowers show considerable variation in the two traits measured, flower weight and standard petal
288 area, both within and across localities. A variance partition analysis showed that the variance in both
289 traits across the five flowers sampled per plant was negligible, so the selection analysis below was
290 run using mean floral values for each individual plant (see also Herrera 2001).
291

292 We found no evidence of linear directional selection on floral traits, either in univariate models (s
293 coefficients) or models of correlated selection incorporating both floral variables (β coefficients,
294 Table 1). However, we found evidence for univariate quadratic effects in both traits (c coefficients)
295 but only standard area shows significant quadratic gradients (γ coefficients). This suggests that floral
296 weight is not under direct selection, while there is strong evidence for stabilising selection on
297 standard petal area (Fig. 2).
298

299 Genomic markers and population genetic structure

300 The GBS sequencing approach yielded a large number of polymorphic SNPs across individuals
301 (261,775 SNPs before quality filtering). After MAF and heterozygosity filtering, we retained 10,421
302 high-quality SNPs that were present in at least 90% of individuals across all localities. The analyses
303 below use this dataset to estimate genomic relatedness; however, we also tested for the effect of
304 retaining a larger number of SNPs (with presence in at least 50% of the individuals, which leads to a

305 higher number of genotypes imputed by *synbreed*, see Supplementary methods). Analysis with this
306 larger dataset produced the same qualitative results, suggesting that retaining more (but highly
307 imputed) markers did not add valuable information on the relatedness among our study plants.
308 Therefore, all analyses below use the smaller dataset with 10,421 SNPs.
309
310 **Heritability, evolvability and genetic correlation**
311 Pairwise relatedness among sampled individuals varied markedly and was overall relatively low
312 (average values ranging from -0.09 to 0.79, but with most values <0.2), even within locality (Fig. S4),
313 supporting the prevalence of outcrossing in this species. The low population genetic structure and
314 the presence of variance in relatedness provide the conditions for a reliable estimation of heritability
315 in the field in this species (Ritland 1996).
316
317 We found significant estimates of heritability and evolvability in flower weight (h^2 = 0.14, e = 0.42%;
318 Table 2). For standard area, our models instead detected very low additive variance, yielding very
319 low h^2 and e in this case (h^2 = 0.001, e < 0.001%; Table 2). For both traits, Deviance Information
320 Criterion (DIC) values for the heritability naïve models were larger than for the complete model
321 (Table S2), indicating a better fit for the latter. The naïve models included only the relatedness
322 among individuals and neither environmental nor spatial predictors, and showed estimated h^2 values
323 substantially higher than our final estimates (Table 2).
324
325 Our bivariate analysis found a low genetic correlation between the two floral traits that is
326 indistinguishable from zero (r_G = 0.06); in addition, credible intervals were large (-0.139 to 0.381), so
327 we cannot confidently support the presence of a genetic correlation.
328
329 ## Discussion
330 We provide an example of a stable pollination environment that has led to a lack of innovative
331 selection, yet maintaining enough heritable variation for responding to possible novel selection
332 pressures, at least in some traits. In *Ulex parviflorus*, we found evidence for both stabilizing selection
333 and low trait heritability as alternative explanations for lack of evolution in flowers. Specifically, the
334 area of the standard petal is currently under stabilizing selection, but the variability we observe in
335 the field is not heritable. Floral weight, in turn, presents high heritability, but is not currently under
336 selection.
337

338 Stable pollinator communities are potentially a common feature for many plant species under even
339 environmental conditions. For the particular case of *Ulex parviflorus*, current evidence shows that
340 honey bees are the most frequent pollinators in all surveyed populations, including the one studied
341 here and other localities (Herrera 1988, Reverté et al. 2016, C.M. Herrera pers com). Other species in
342 the genus, including *U. europaeus, U. minor* and *U. galli*, present a higher diversity of large bees
343 among their visitors (several species of *Bombus* and *Andrena*; Kirchner and Bullock 1999, Bowman et
344 al. 2008, Falk 2011). The dominance of honey bees in *Ulex parviflorus* populations could be seen as a
345 consequence of the large anthropogenic influence across its range; however, *U. parviflorus*
346 populations in an area with low human influence and high pollinator diversity (Sierra de Cazorla, see
347 Herrera 2018) corroborates the predominance of honey bees as pollinators of this species.
348 Regardless of the reasons for the low pollinator diversity, our study provides evidence on how stable
349 conditions can lead to lack of current evolution in floral traits.
350
351 On the opposite side of the spectrum, field studies that do detect pollinator-mediated directional
352 selection on unmanipulated floral traits often focus on plants that are exposed to changing
353 pollinators, either in different parts of the species range (Herrera et al. 2006, Anderson et al. 2010)
354 or in hybrid contact zones where there is selection against hybridization (Campbell et al. 2018).
355 Taken together, current evidence supports the idea that pollination-driven floral evolution takes
356 place mostly during evolutionarily innovative periods driven by to changing pollinators.
357
358 Stabilizing selection is expected in floral traits that influence the accuracy of the flower-pollinator
359 interaction (Cresswell 2000, Armbruster et al. 2009). It is difficult to establish how common
360 stabilizing selection is on floral traits in wild plants, because studies do not measure non-linear
361 selection as often as directional selection (Harder and Johnson 2009, Caruso et al. 2018). For the
362 standard petal in *Ulex*, we detected stabilizing selection for intermediate surface area. The size of
363 this "flag" petal is expected to play an important role on pollinator attraction by increasing the floral
364 colourful display (Fig. 1), so that selection against smaller sizes is expected. Too large standard petals
365 could be selected against if they incur a higher cost for the plant. This cost could be even higher if
366 large standard petals are developmentally restricted to overall larger flowers; however, our genetic
367 correlation estimates suggest that the association of standard petal area with floral size is weak. This
368 is consistent with a previous study that carefully dissected the role of the different petals in another
369 keel flower; in *Collaea argentina,* Córdoba et al. (2015) found that the standard petal is not
370 functionally integrated with another set of floral traits that collectively regulate the enclosing
371 mechanism of stamens and pistil. That is, the mechanics of protecting the enclosed rewards in these

372 flowers can be independent of pollinator attraction as we expected, and selection can vary across
373 floral parts.
374
375 Floral morphological traits are often found to present heritable variation (reviewed by Ashman and
376 Majetic 2006, Opedal 2018); however, most of the studies in these reviews were performed in
377 controlled environments. Our field estimates of heritability fall within the lower range of those
378 summarized in Fig. 1 of Altman and Majetic (2006), as expected from field values compared to
379 greenhouse estimates. We found that flower weight shows significant heritability, but no detectable
380 heritability in the standard petal area. Comparing petals in papilionoid flowers, Herrera (2001) found
381 that the standard had higher phenotypic variance than other petals across Genisteae, and argued
382 that its role in pollination was smaller than for the keel petals, in a similar way as Córdoba et al.
383 (2015). This and our results suggest that this petal might be prone to high environmentally-induced
384 variation, which increases the exposure to stabilising selection, but does not lead to evolutionary
385 change.
386
387 Heritability estimates have been criticised as poor standardized measures of evolutionary potential
388 in realistic ecological settings, in part because of the covariance between environmental and genetic
389 effects (Houle 1992, Hansen et al. 2011). In this study, we estimate heritability directly in the field,
390 statistically controlling for environmental variation, and in the same individuals used to estimate
391 natural selection. In this context, field heritability estimates provide a very useful approach to
392 understand the current evolutionary potential at the population level, precisely because we are
393 interested in the role of environmental effects on the phenotypic variance, as exposed to natural
394 selection. An alternative measure of evolutionary potential, evolvability, uses the mean of trait
395 values to standardize the additive genetic variance and provides a comparable estimate of
396 proportional change in a trait value after selection (Hansen et al. 2003). Our estimates of evolvability
397 here confirm our findings in heritability, also showing near-zero evolutionary potential for the
398 standard petal area, but higher values for flower weight. In the latter case, evolvability is estimated
399 to be significant but small (under 1% of the trait mean value), suggesting that change in this trait
400 would not be fast unless submitted to strong selection. This value of evolvability is within the range
401 of evolvability values estimated for floral size specifically across plant species, as summarised in a
402 recent review (Opedal 2018).
403
404 Our estimate of genetic correlation between the two focal traits suffers from a low sample size to
405 run a bivariate animal model and needs to be interpreted with caution. However, the lack of a

genetic correlation is not surprising given that we cannot detect significant additive genetic variation in one of the trait (the area of the standard petal). This does contrast with the fact that there is a significant phenotypic correlation between the two traits, but as suggested by previous studies, phenotypic correlations are not always good predictors of genetic correlations, even in highly integrated organs as flowers (Gómez et al. 2009). Again, this is consistent with the decoupling of petals found in a related species with keel flowers (Córdoba et al. 2015). It is thus possible that the phenotypic correlation is caused by shared environmental factors that affect both traits in *Ulex* flowers, further confirming the importance of studying evolutionary potential in field realistic conditions.

Even though we could not detect a genetic correlation between the two floral traits studied here, a caveat in our analysis is that we do not include selection on other (unmeasured) potentially correlated traits. Another potential source of problems is that *Ulex* flowers are hermaphroditic and thus likely subject to selection via both male and female reproductive success. Our estimates of selection here are based on fruit set alone, and we cannot rule out that the two focal traits might be under selection through male function (van Kleunen and Burczyk 2008). 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 works using large sets of molecular markers to study quantitative genetics in wild populations, mostly focused on animals (Perrier et al. 2018), but also on plants (Castellanos et al. 2015). Studies comparing the accuracy of SNP-based relatedness matrices compared to pedigrees are consistently showing that they can be very good approximations, as long as a large number of markers and a good sample of individuals is available (Bérénos et al. 2014, Perrier et al. 2018). 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 specific case of flowers, our findings suggest that low-diversity pollination environments as those caused by anthropogenic pollination declines can lead to reduced selection pressures, reduced opportunity for selection, and stasis (Caruso et al. 2018), while exposure to new pollinators can lead to novel evolutionary change.

Conclusion

Relative stasis can be prevalent in contemporary populations, yet heritable phenotypic variance can be present; in combination with potential genetic correlations, this provides the potential to respond to novel selection. Selection on floral traits is not restricted to pollinators, as herbivores and abiotic factors can also be agents of selection (reviewed by Caruso et al. 2018). Regardless of the source of selection, our findings contribute to explain the macroevolutionary patterns of floral evolution where novel phenotypes are ubiquitous (exceptions are often related to very generalised pollination that is stable over evolutionary time, see Vasconcelos et al. 2019). Populations can experience stable conditions with undetectable innovative selection, but at the same time harbour genetically based variability to evolve under new conditions.

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592 **Table 1** Directional and quadratic selection differentials and coefficients (± standard errors) for the
593 two floral traits studied.

Trait	Differential		Gradient	
	Directional, S	Quadratic, c	Directional, β	Quadratic, γ
Standard petal area	-0.003 ± 0.003 ns	**-0.002 ± 0.000** ***	-0.041 ± 0.039 ns	**-0.102 ± 0.029** ***
Flower weight	-0.018 ± 0.033 ns	**-0.070 ± 0.023** **	-0.022 ± 0.047 ns	-0.038 ± 0.021 ns
Interaction				0.000 ± 0.002 ns

594

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

	Naïve 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

Figure legends

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) MC Castellanos, (c) J. Quiles).

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 generalised additive model fits with shaded areas showing 95% confidence intervals.

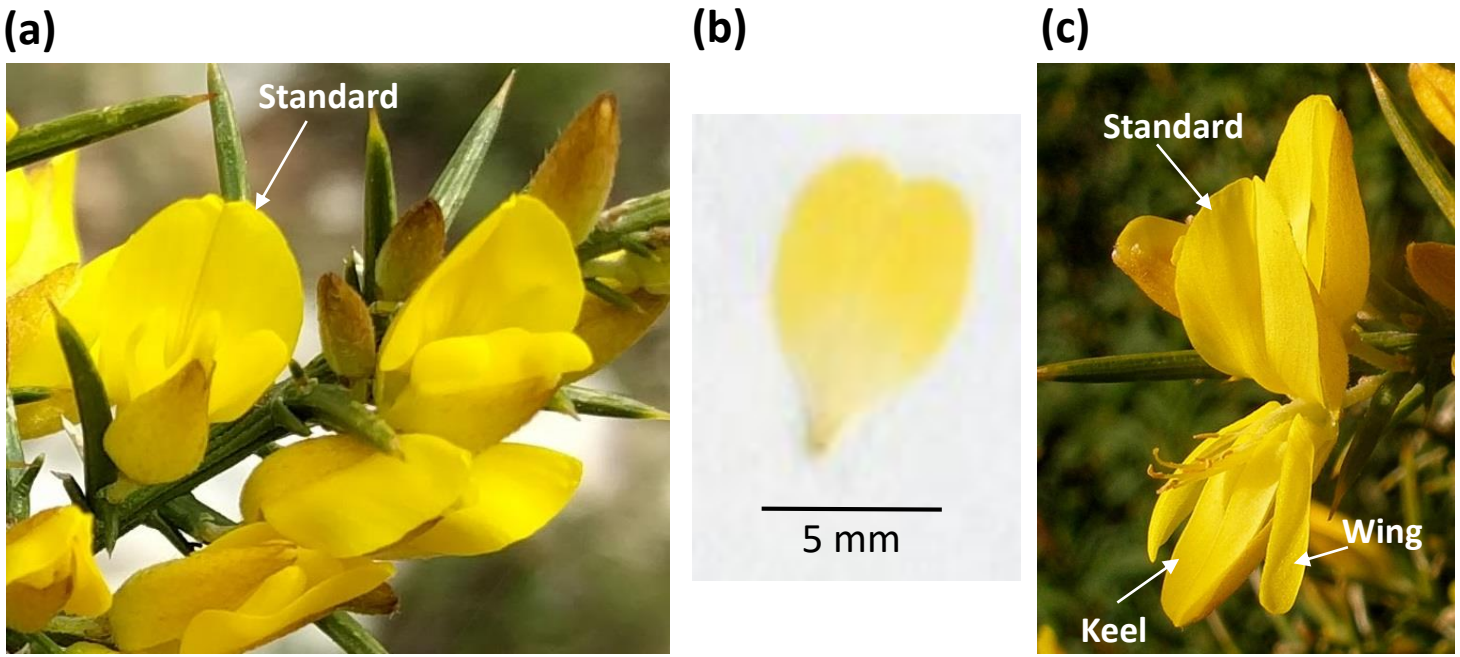

Figure 1

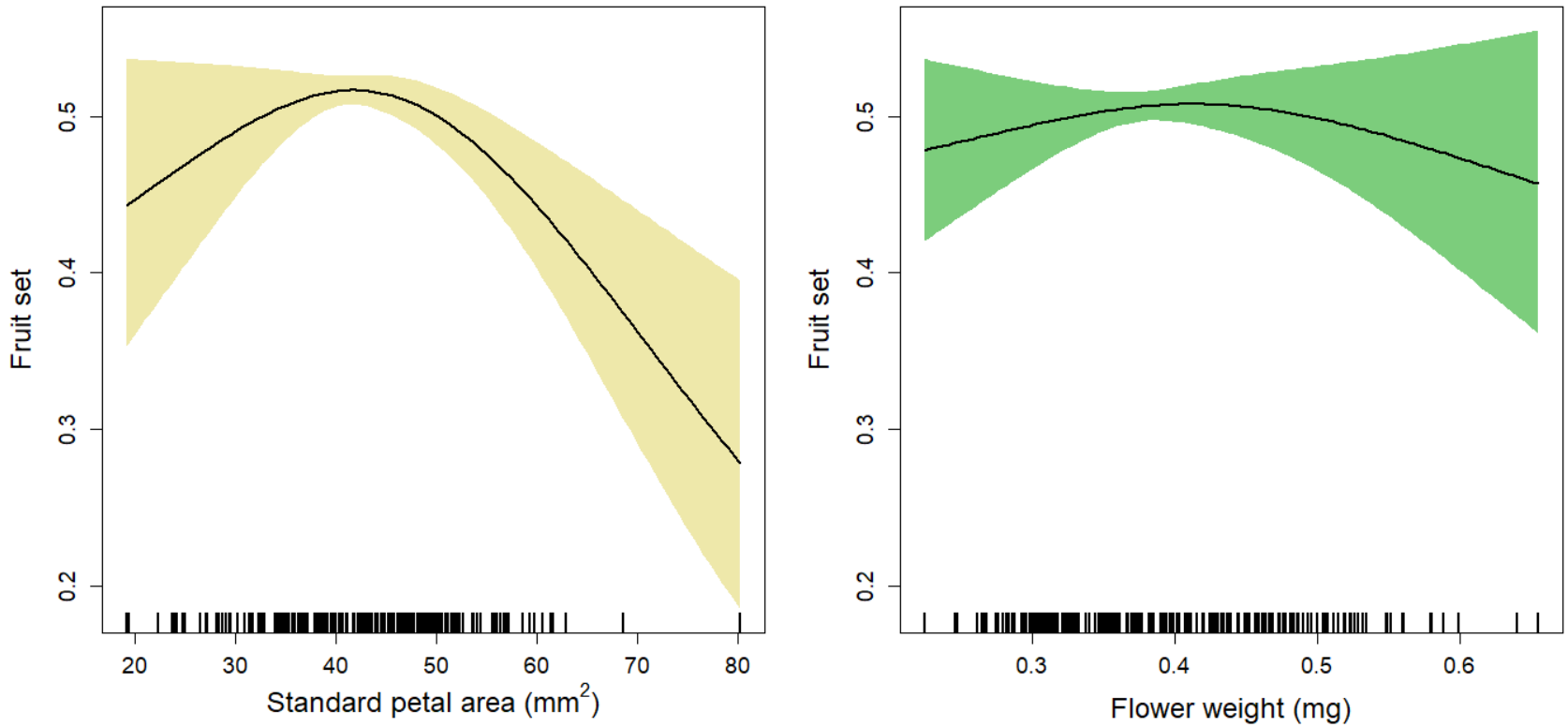
Fruit set
0.2
0.3
0.4
0.5
20
30
40
50
60
70
80
Standard petal area (mm^2)
Fruit set
0.2
0.3
0.4
0.5
0.3
0.4
0.5
0.6
Flower weight (mg)

Fig. 2