Running title: Reproduction of *Primula bergidensis*

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**EFFECT OF PLANT TRAITS AND POPULATION STRUCTURE ON THE FEMALE
REPRODUCTIVE SUCCESS OF THE ENDEMIC *PRIMULA ELATIOR* SUBSP.
BERGIDENSIS (PRIMULACEAE)**

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Abstract

This study explores the possible causes of variation in female reproductive success of the subspecific taxon *Primula elatior* subsp. *bergidensis*, a distylic endemic to the north-western Iberian Peninsula, by analysing both vegetative and reproductive traits. In three populations we marked vegetative and reproductive individuals either by mapping the spatial position of every individual (in one population), or by establishing permanent quadrats (in the remainder two populations). We recorded floral morph (pin or thrum), width and length of the largest leaf, scape length and number of flowers produced; all individuals were monitored to estimate number of fruits and seeds produced. Results show that morph ratio did not differ significantly from 1:1 in any of the populations. The number of flowers per plant varied between populations, and longer scape length was associated with higher fruit set in all populations. Plant size, scape length and population spatial structure all had major effects on reproductive success, but the strength—and in some cases the direction—of the effects varied among populations.

Key words

endemic, heterostyly, morph, *Primula elatior* subsp. *bergidensis*, reproductive success, spatial population structure.

Abbreviations

DOP: **distance** to the nearest scape of **opposite** morph

FNOP: number of **flowers** in the **nearest** scape of **opposite** morph

SOP: number of **scapes** of **opposite** morph in a radius of 1 m

FOP: number of **flowers** of **opposite** morph in a radius of 1 m

STOT: **total** number of **scapes** of both morphs in a radius of 1 m

FTOT: **total** number of **flowers** of both morphs in a radius of 1 m

Introduction

The analyses of the factors that determine the spatial variation in reproductive success in plant populations constitute a central aspect in the evolutionary ecology of plants. In plants with a fragmented or isolated distribution, often with few individuals per population, knowledge of the factors governing reproductive success is critical for evaluating population viability. Hence, there is increasing interest in studies of the reproductive biology of especially sensitive species (rare or endemic), with the aim of designing strategies that guarantee adequate conservation of their populations (Synge 1981, Schemske et al. 1994). The viability of plant populations basically depends on the number of individuals in the population and their reproductive success, these two factors being closely related (Iriondo et al. 2003).

Both female and male reproductive success in plant populations is often simultaneously influenced by the intrinsic characters of the individuals and by the characteristics of the population (see De Jong and Klinkhamer 1994, Mitchel 1994, Brys et al. 2004 and references therein). In heterostylous plants with well-established dimorphic incompatibility systems morph proportion and their spatial distribution represent additional factors influencing their reproductive output and viability (Watanabe et al. 2003, Brys et al. 2004, Brys and Jacquemyn 2010).

The Primulaceae is one of the plant families in which heterostyly is most frequent. Notably, in the genus *Primula* almost 90% of the more than 400 species studied to date are heterostylous (Richards 1997). In the *Primula* species in which reproductive biology has been studied most recently, variations in reproductive success have been found both at the population and at the individual level. Population-level variations have been reported in flower number, fruit set, seed set, seed number and seed weight, and may depend on morph ratio (Ishihama et al. 2003, see however Brys et al. 2004) or population size (Kery et al. 2000, Jacquemyn et al. 2001,

Jacquemyn et al. 2002, Brys et al. 2004, Shao et al. 2008a). Within-population variations in female reproductive success have been attributed to differences among individuals in characteristics like morph type (Mazer and Hultgard 1993, Washitani et al. 1994, Larson and Barrett 1998, Nishishiro et al. 2000), number of flowers, and scape length (Ehrlen et al. 2002, Brys et al. 2004), as well as to the proportion of morphs in the population (Brys et al. 2004) or to the population structure (Watanabe et al. 2003).

Primula elatior (L.) L. subsp. *bergidensis* Kress is a strictly endemic primrose, characteristic of calcareous grassland communities, with a limited distribution in the mountains of the northwest Iberian Peninsula. In the present study we explore the causes of within- and among-population variation in female reproductive success of this subspecies by assessing the characteristics of three populations. Some traits, such as plant size or flower morph, can be responsible for increasing the reproductive performance of plants (de Jong and Klinkhamer 1994, Ollerton and Lack 1998, Kéry et al. 2000). Flowering environment also affects the behaviour and effectiveness of pollinators. Flower density or abundance of opposite-morph flowers, in particular, can be expected to result in higher reproductive success in distylic species (Ishihama et al. 2003, Watanabe et al. 2003, Brys and Jacquemyn 2010). Hence, we expected large individuals to show higher reproductive success, together with those that grow in dense patches or with a large number of opposite-morph flowers nearby. Two specific questions are dealt with: (1) What is the effect of vegetative (leaf length and width, and scape length) and reproductive (number of flowers and morph ratio) traits on female reproductive success of *Primula elatior* subsp. *bergidensis*?, and (2) how does local plant density and spatial morph distribution affect plant reproductive success in its populations?

Material and methods

Species and study area

Primula elatior subsp. *bergidensis* (hereafter *P. bergidensis*) is a perennial species with leaves in a basal rosette, and a single erect scape in an umbel inflorescence holding a variable number of flowers, frequently 4 - 12. The corolla is yellow, with 5 fused petals and the androecium is formed by five stamens. Its superior ovary gives rise to a capsule with numerous angular seeds. Plants are heterostylous, with clearly differentiated pin and thrum morphs (Darwin 1962, Ganders 1979). *Primula elatior* has been reported to be self-incompatible and pollination must occur between pin and thrum flowers to result in seed set (Jacquemyn et al. 2002, Van Rossum et al. 2002), although data on ssp. *bergidensis* is still lacking. Flowering takes place between March and June/July, partially overlapping with fruiting, which occurs largely in July. In the relative *P. elatior* ssp. *elatior*, flowers are mainly pollinated by bumble-bees and Diptera, as well as Lepidoptera (Van Rossum et al. 2002, Jacquemyn et al. 2009). This taxon is restricted to calcareous substrates of north-western Iberian Peninsula (mountains of western León province and eastern Galicia region). We studied all known populations of this subspecies but one, which only contains less than twenty scapes. After visiting all populations, we decided to discard the smallest one because we considered that strong endogamy could be altering our analyses and in order to avoid any interference with its ecological dynamics. The three populations are located in Ferradillo (Priaranza del Bierzo, León Province), Apóstoles (Ponferrada, León Province), and Couto (Folgosos do Courel, Lugo Province). The Ferradillo population grows patchily on steep-sloping calcareous meadows, among limestone outcrops (Ferradillo quadrat 1, see below, 1400 m a.s.l.) or within mixed woodland dominated by *Corylus avellana* (Ferradillo quadrat 2, 1350 m a.s.l.). The Apóstoles population (1450 m a.s.l.) is located on calcareous meadows, similar to

those of Ferradillo 1. The Couto population (1150 m a.s.l.) is located on pastures with scant outcropping rocks, with scattered individuals of *Sorbus aucuparia* and *Quercus pyrenaica*.

Experimental design

Due to the large number of individuals, in the Apóstoles and Ferradillo populations we established two permanent quadrats in each population, each including 36 to 69 reproductive rosettes of *P. bergidensis*. Quadrat size varied at each site in order to ensure they contained a sufficient number of scapes and individuals, being 5 x 5 m and 11 x 6 m in Ferradillo, and 5.5 x 5.5 m and 8 x 8 m in Apóstoles. To ensure the representability of the quadrats, we selected them to cover the natural variability of microhabitats within each population, as regards slope, scape density and tree cover. In the Couto population, data were recorded for every individual. We marked vegetative and reproductive individuals, which were revisited at 10- to 15-day intervals, in order to measure reproductive individuals during their flowering period (at least one flower on anthesis) and to collect one completely ripe fruit before seed releasing. For all reproductive individuals we noted floral morph (pin or thrum), width and length of the largest leaf, scape length, and number of flowers produced. At the end of the fruiting period we counted the number of fruits produced by each plant, and blind-selected one random fruit for determining the number of seeds and their weight (± 0.1 mg). Total number of seeds per plant was estimated as the product of number of fruits and number of seeds in the sampled fruit. Considering a sample of plants in the Apóstoles population, the correlation between number of seeds per plant estimated in this way and real number of seeds per plant was 0.828 ($n = 19$, $p < 0.001$).

We only studied female reproductive success which may be undoubtedly linked to the male function and to pollinator efficiency. Although deserving further research, these are out of the scope of our work. While studying only the contribution of female function to reproductive

success may limit the conclusion of our work, a close relation with that of male function has been shown to exist in natural settings (Broyles and Wyatt 1990, Dudash 1991).

We mapped the position of each plant within each quadrat or population. In the Ferradillo and Apóstoles populations we established a coordinate system and located the position of each plant on two orthogonal axes. In Couto it was not possible to use this system, as the population covers an area of 4500 m², and therefore we defined an origin point and determined the location of each plant on the basis of distance to and angle with respect to a previously located plant. In Couto, when several plants appeared densely aggregated we used a local coordinate system, with its origin referred to some other known point.

In order to capture the spatial variability in the pollination processes that may affect a plant's female reproductive success, we defined each reproductive plant environment on the basis of the aforementioned mapping procedures. Thus, we used the spatial analysis modules of the GIS software package Idrisi 32 (Clark Labs) to calculate the following variables: distance to the nearest scape of opposite morph (*DOP*), number of flowers in the nearest scape of opposite morph (*FNOP*), number of scapes of opposite morph in a radius of 1 m (*SOP*), number of flowers of opposite morph in a radius of 1 m (*FOP*), number of scapes of both morphs in a radius of 1 m (*STOT*), and number of flowers of both morphs in a radius of 1 m (*FTOT*). All these variables were measured once, during the flowering period, and number of flowers refer to total number of flowers produced by each scape. We selected a 1 m radius because most of the foraging flights of the most likely pollinators are shorter than one meter (Pyke 1978, Schmitt 1980) and previous works with similar plant species have found this to be a relevant distance to detect an effect of the proportion of opposite-morph flowers (Watanabe et al. 2003, Brys and Jacquemyn 2010). Since in Ferradillo and Apóstoles individuals were mapped within a rectangular quadrat (not the whole population) and we were thus unable to take into account

scapes located outside the quadrat, we estimated all density measurements from the area of the circle (of radius 1 m) that lay within the quadrat. Distance measurements were not adjusted. These procedures are equivalent to assuming that the relative distribution of scapes outside the quadrat is the same as that within the quadrat.

Data analysis

We performed a Principal Components Analysis (PCA) where we included all variables related to both plant size and scape environment (see table S1 for a list of the variables used). We transformed data to improve normality, selecting the optimal transformation independently for each variable, after testing various alternatives. The variables *DOP*, *FOP* and *FTOT* were transformed to $\log(x + 1)$; *FNOP* to $(x)^{1/2}$; *SOP* to $(x + 1.5)^{-1/2}$; and *STOT* $(x + 1.3)^{-1}$. The variables leaf length and leaf width were combined to give a single variable "leaf size", calculated as the geometric mean of length and width (and thus an indicator of the area of the plant's largest leaf). With these variables we then performed a normalized varimax rotation and obtained four well-defined factors (Table S1). The first factor, hereafter inter-individual distance (or simply distance), explained 54.1% of total variance and showed strong positive correlations with *DOP* and strong negative correlations with the remaining density variables. The second factor —leaf size, hereafter— explained 12.7% of total variance and was correlated almost exclusively with the leaf-size variable. The third factor —abundance of opposite-morph flowers, hereafter— was almost exclusively defined by a single variable (*FNOP*). The fourth factor —scape length, hereafter— was likewise almost exclusively defined by a single variable: scape length. These four factors were used in all subsequent statistical analyses.

Despite the conceptual appeal of fruit-set for analysing the reproductive components of fitness, the use of ratios in ecology have been repeatedly discouraged on the grounds of their poor statistical robustness (Atchley et al. 1976, Albrecht 1978, Jackson and Somers 1991,

Packard and Boardman 1999). In fact, the effect of the denominator (flower number) may not be completely eliminated from the numerator (fruit number; Packard and Boardman 1999) and spurious correlations may arise (Atchley et al. 1976). Hence, instead of analysing fruit-set as a ratio of fruit on flower number, we analysed fruit number as the response variable while including flower number as a covariate, an approach that overcomes the problems of ratios (Packard and Boardman 1999). By doing so we are controlling for the variance in fruit number that is due to flower number and the relationship with the remaining factors and covariates may be regarded as effects on fruit-set. For this reason, for the sake of readability, we will be referring to “relative fruit production” when talking on the number of fruits (controlled for flower number).

We followed a similar approach as regards the number of seeds produced per fruit, by analyzing total number of seeds per scape as response variable, by including number of fruits as a covariate and by referring to “relative seed production”.

We analysed the effects of the two fixed factors (population and morph) and covariates (the four PCA factors accounting for inter-individual distance, leaf size, scape length and abundance of opposite-morph flowers) on female reproductive success variables by means of Generalized Linear Models. We treated population factor as fixed because the study considered three of the four known populations of this subspecies, so that it cannot reasonably be treated as a random factor. In analyses of this type, the large number of interactions complicates model interpretation, increasing the probability of obtaining spurious significant effects, and requiring increased sample size (Huitema 1980; Quinn and Keough 2002). Thus, in each case we selected those interactions of greatest biological relevance in the present context. For the analyses with flower number as dependent variable, we only considered possible interactions between the covariates (i.e. the four PCA factors) and population. For the analyses with fruit number or seed

number as dependent variable, we only considered interactions with population for distance and leaf size factors. We considered all second- and third-order interactions, including population, for abundance of opposite-morph flowers and scape length factors.

For each dependent variable, we first fitted various complete models on the basis of different error distributions and their corresponding canonical link functions. In each case we selected the model with the lowest Akaike information criterion (AIC), which proved to be a Poisson distribution for flower number and a negative binomial distribution for fruit and seed number, with a logarithmic link function in both cases. We then applied a model reduction procedure, eliminating from each model the effect with the highest p value, as long as it was above 0.25 (Winer et al. 1991, Underwood 1997). For all cases, the final model selected was the one exhibiting the lowest AIC value. *A posteriori* among-population comparisons and simple slope comparisons were performed on the basis of the corresponding coefficients (Aiken and West 1991) with adjustment of significance levels using Bonferroni's sequential correction.

Results

Plant traits and female reproductive success

Morph ratio did not differ significantly from 1:1 in any of the populations (Apóstoles: $\chi^2 = 0.87$, $p = 0.349$; Ferradillo: $\chi^2 = 0.10$, $df = 1$, $p = 0.753$; Couto: $\chi^2 < 0.005$, $df = 1$, $p = 1.000$), or considering all three populations together ($\chi^2 = 0.52$, $df = 1$, $p = 0.471$); nor was there significant heterogeneity in morph ratio among the three populations ($\chi^2 = 0.46$, $df = 2$, $p = 0.499$).

Number of flowers per plant varied significantly among the three populations (Table 1a), being significantly higher in Couto (Fig. 1; Table S2). There were marginal differences between morphs in the number of flowers (difference on mean flower number between both morphs = 0.066). Individuals with larger scapes and larger leaves produced more flowers (Table 1a). The

effect of scape length didn't vary among populations but that of leaf size showed a marginally significant difference.

Relative fruit production varied between populations (Table 1b), being significantly higher in Apóstoles and Couto than in Ferradillo (Fig. 1). Plants with larger leaves and longer scapes produced more fruits (relative to the number of flowers), although in the former case the relationship was only marginally significant (Table 1b). However, the effect of scape length differed among populations (Table 1b; Fig. 2).

Overall, relative seed production per plant did not differ between morphs (morph: $\chi^2 = 0.6783$, $df = 2$, $p > 0.05$; morph*population: $\chi^2 = 0.7835$, $df = 2$, $p > 0.05$) but did differ among populations, being highest for Couto (260 seeds) and lowest for Ferradillo (96) and Apóstoles (146; $\chi^2 = 25.54$, $df = 2$, $p < 0.0001$). However this effect was due solely to the number of fruits, as population effects disappeared when we included number of fruits as a covariate. Scapes that produced a larger number of fruits likewise produced a larger number of seeds. None of the other variables considered in the model showed a significant relationship with relative seed production (Table 1c).

Spatial distribution and female reproductive success

The effect of spatial structure differed among populations: while in Apóstoles lower local densities resulted in a lower relative fruit production, we found the opposite trend in Ferradillo and a non-significant slope in Couto (Fig. 2). Likewise, we found a significant effect of the third order interaction between population, morph and abundance of opposite-morph flowers, meaning that the slope of the relation between relative fruit production and abundance of opposite-morph flowers differed among populations and morphs (Fig. 3; Table 1b). Significant differences between morphs were only found in Ferradillo (single effects test; Apóstoles: $\chi^2 = 2.1$, $df = 1$, $p = 0.1477$; Couto: $\chi^2 = 0.00$, $df = 1$, $p = 0.9665$; Ferradillo: $\chi^2 = 8.88$, $df = 1$, $p = 0.0029$).

Discussion

Plant traits and reproductive investment

Our results show that mean number of flowers per plant varied among populations, and was highest in the Couto population. We found that the two variables representative of plant size (scape length and leaf length) showed positive relationships with flower number. Kery et al. (2000) found a similar result in *P. veris*, showing that flower number was positively related to plant size.

In other taxa of this genus flower number per plant has likewise been reported to vary between populations, with higher flower numbers in larger populations, either in the same species (Jacquemyn et al. 2001) or in other species of the genus (Brys et al. 2004). While we found higher flower numbers at the smallest population, extreme caution is needed when interpreting these relation, as the low number of populations available to study (and included in this work) prevent any statistical analysis and robust conclusion.

No relevant differences on flower number were detected between morphs in either population. In other taxa of *Primula* studied, rather disparate results have been obtained. For some populations of *P. vulgaris* no between-morph differences in flower number have been reported (Brys et al. 2004), while studies in other populations have found higher flower number either in thrum (Boyd et al. 1990) or in pin plants (Fisogni et al. 2011). Studies on *P. farinosa* (Ehrlen et al. 2002), *P. mistassinica* (Larson and Barret 1998) and *P. sieboldii* (Nishihiro et al. 2000) have not detected between-morph differences in flower number .

In *P. bergidensis* there is no evidence of any trade-off between reproductive investment and growth, since there was a positive relationship between flower number and leaf area. As in *P. veris*, *P. elatior* and *P. vulgaris*, larger plants have more flowers (Kery et al. 2000,

Jacquemyn et al. 2002, Brys et al. 2004), suggesting that plants may be producing flowers using resources obtained by photosynthesis during that season.

Plant traits and female reproductive success

Almost all studies of species of the genus *Primula*, have reported both among-individual and among-population variations in different components of the female reproductive success, such as fruit set, seed set, seed number or seed weight (e.g. Jacquemyn et al. 2001 and 2002 and van Rossum et al. 2002 for *P. elatior*, and Kéry et al. 2000, Brys et al. 2003 for other species). These variations have been related to population size (Jacquemy et al. 2001 and 2002 for *P. elatior*, Kéry et al. 2000 and Brys et al. 2003 and 2004 for other species) and density (van Rossum et al. 2002, for *P. elatior*), and to morph ratio (Jacquemyn et al. 2001 and 2002, for *P. elatior*; Brys et al. 2003 for *P. veris*) and morph distribution within populations (Ishihama et al. 2003 for *P. sieboldii*). In the present study a longer scape length was associated with a higher relative fruit production in all populations, although with a stronger effect in Ferradillo, as has been reported for the close relative *P. elatior* ssp. *elatior* (Jacquemyn et al. 2001). Scape length may influence reproductive success, not only because of the effect of plant size already mentioned, but also because it may directly affect pollination processes by altering plant attractiveness for interacting animals (see Ehrlén et al. 2002). This might explain the effect observed in the present study for scape length (independent of leaf size), which differs among populations. A similar effect has been reported for *P. farinosa*, where the effect of longer scapes depended on the micro-environment of the population (Ehrlén et al. 2002).

In *P. bergidensis* the relative seed production of plants varied among the three populations. The smallest population (Couto) showed the highest relative seed production, as a consequence of the higher production of fruits and flowers per plant; however, this effect was reduced after eliminating the effect of number of fruits. This indicates that the relative seed

production of a plant depends basically on its number of developed fruits, meaning seed production per fruit does not differ among populations.

Jacquemyn et al. (2001, 2002,) found differences among populations of *P. elatior* in fruit set, number of seeds per plant, number of seeds per fruit, and total seed mass; while van Rossum et al. (2002) only found differences for the number of seeds produced (see also Taylor and Woodell 2008 for a review). In this taxon there is a positive relationship between population size and all of these indicators of female reproductive success, but a negative relationship between population size and mean seed weight. They also show that population size affects the presence/absence of a trade-off between seed number and seed size, with a trade-off only seen in small populations. A similar conclusion was reported for *P. veris*, in which female reproductive success was lower in small populations (Brys et al. 2003). Kery et al. (2000) likewise detected a marked trade-off between seed number and seed size, without any indication of an effect of population size. Nishihiro et al. (2000) reported an interactive effect of plant size and morph on seed set in *P. sieboldii*, with only pin plants showing a positive relationship between plant size and seed set. In *P. bergidensis* the smallest population (Couto) showed the highest relative seed production, as a consequence of the higher production of fruits and flowers per plant. However, any relationship to population size in our data must be considered very cautiously, as the small number of extant populations of this taxon prevents robust conclusions on the issue. Considering all three populations together, larger plants showed a higher relative fruit production. However, the relative seed production did not depend on plant size.

Population structure and female reproductive success

In *P. bergidensis* the relationship between plant density and relative fruit production varied among the three populations studied. Distance to scapes of opposing morphs appears to be

closely related to total scape density, as shown by the results of principal components analysis, and its effect on female reproductive success varies greatly from one population to another.

In the smallest population (Couto), no significant relationship between local density and relative fruit production was observed, while in Apóstoles there was a positive relationship, and in Ferradillo a negative one. In other words, in Apóstoles plants growing in areas of higher plant density showed higher relative fruit production, while in Ferradillo the reverse was true. However, we did not detect any effect of density on relative seed production.

Kery et al. (2000) studied fecundity taking into account population density, and found that density had a positive effect on the number of fruits per plant, but a negative effect on the number of seeds per fruit and per plant. In *Primula merrilliana* (Shao et al. 2008a) the four main female reproductive success parameters (fruits per plant, seeds per fruit, seeds per plant, and the proportion of flowers setting fruit) were all positively correlated with floral density. On the contrary, in the close relative *P. elatior*, population density was found to affect positively the percent of aborted fruits (van Rossum 2002). Together with patch size (Ishihama and Washitani 2007, Shao et al. 2008b), floral density may be one of the main factors of attractiveness for pollinators (van Treuren et al. 1994, Kunin 1997, Roll et al. 1997, Shao et al. 2008b), and is likely to be responsible for these patterns. Alternatively, proximity to other plants of opposing morph may increase the female reproductive success (Watanabe et al. 2003, see also Brys and Jacquemyn 2010 with *Pulmonaria officinalis*). In the present case, proximity to scapes of opposing morphs appears to be closely related to total scape density and its effect on female reproductive success varies greatly from one population to another. However, we found the number of flowers of the nearest scape of opposite morph to affect relative fruit production, although this effect varies between morphs and populations. This variable effect deserves further research, but agrees with our results for other reproductive success variables, as regards the

pattern of the interaction: while Couto shows no effect, the effect is of opposite sign in Apóstoles as compared to Ferradillo. Anyway, this result highlights the complex role of the pollination environment of the plant in determining its female reproductive success.

In conclusion, the present study provides data on the variation in female reproductive success in *P. bergidensis*. Population spatial structure and plant size traits govern the reproductive output in this taxon, while morph type does not seem to play an important role. However, the effect of spatial structure and scape length (probably affecting the pollination environment of the scape) differ notably among populations.

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Table 1. GLM results showing the effect of population and different plant traits on (a) the number of flowers; (b) the relative fruit production; and (c) the relative seed production.

<i>(a) Number of flowers</i>	d.f.	χ^2	<i>p</i>
Population	2	17.19	0.0002
Morph	1	2.75	0.0972
Population * Distance	3	5.12	0.1634
Leaf size	1	28.27	< 0.0001
Population * Leaf size	2	5.02	0.0813
Scape length	1	133.47	< 0.0001
<i>(b) Relative fruit production</i>			
Population	2	15.3	0.0005
Number of flowers (sqrt)	1	47.60	< 0.0001
Leaf size	1	3.35	0.0670
Scape length	1	43.88	< 0.0001
Population * Scape length	2	18.97	< 0.0001
Morph * Scape length	1	0.70	0.4025
Inter-individual distance	1	2.08	0.1495
Population * Inter-individual distance	2	26.97	< 0.0001
Population * Morph * <i>FNOP</i>	6	17.42	0.0079
<i>(c) Relative seed production</i>			
Population	2	3.11	0.2116
Morph	1	2.19	0.1387
Number of flowers (sqrt)	1	1.66	0.1982
Number of fruits (sqrt)	1	62.49	< 0.0001
Inter-individual distance	1	1.68	0.1951

FIGURE LEGENDS

Fig. 1. Estimated mean number of flowers per plant (upper panel) and relative fruit production (lower panel; both of them log-transformed) in the three populations, after controlling for the effects of the covariates. Bars indicate standard errors of the mean. Populations with the same letter do not differ significantly at the 5% level after application of Bonferroni's sequential correction.

Fig. 2. Plot of simple linear regressions of *Scape length* (upper panel) and *Inter-individual distance* (lower panel) against relative fruit production for each population: (+) and dotted line, Apóstoles; (▲) and solid line, Ferradillo; (○) and dashed line, Couto. Slopes with the same letter do not differ significantly at the 5% level after applying Bonferroni's sequential correction. The three slopes in the upper panel were significantly different from zero.

Fig. 3. Plot of simple linear regressions of factor *Abundance of opposite-morph flowers* against relative fruit production for each population and morph: (▲) and solid line, pin morph; (○) and dashed line, thrum morph. Differences between morphs were significant only in Ferradillo.

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Fig. 1. Estimated mean number of flowers (upper panel) and fruits (lower panel) per plant (log-transformed) in the three populations, after controlling for the effects of the covariates. Bars indicate standard errors of the mean. Populations with the same letter do not differ significantly at the 5% level after application of Bonferroni's sequential correction.

244x340mm (300 x 300 DPI)

Fig. 2. Plot of simple linear regressions of Scape length (upper panel) and Inter-individual distance (lower panel) against number of fruits for each population: (+) and dotted line, Apóstoles; (▲) and solid line, Ferradillo; (○) and dashed line, Couto. Slopes with the same letter do not differ significantly at the 5% level after applying Bonferroni's sequential correction. The three slopes were significantly different from zero

Fig. 3. Plot of simple linear regressions of factor Abundance of opposite-morph flowers against number of fruits for each population and morph: ($\blacktriangle$) and solid line, pin morph; ($\circ$) and dashed line, thrum morph. Differences between morphs were significant only in Ferradillo.
318x629mm (300 x 300 DPI)

SUPPLEMENTARY MATERIAL

Table S1. Results of the Principal Components Analysis showing the variance explained by each of the four factors extracted and variable loadings on each factor. DOP: distance to nearest scape of opposite morph; FNOP: number of flowers in nearest scape of opposite morph; SOP: number of scapes of opposite morph in radius of 1 m; FOP: number of flowers of opposing morph in radius of 1 m; STOT: number of scapes of both morphs in a radius of 1 m; FTOT: number of flowers of both morphs in a radius of 1 m; scape length; leaf size.

Variable	Factor 1	Factor 2	Factor 3	Factor 4
<i>SOP</i>	0.951	0.077	-0.001	0.087
<i>FOP</i>	0.950	0.037	0.100	0.110
<i>STOT</i>	-0.952	-0.074	0.120	-0.104
<i>FTOT</i>	-0.946	0.010	-0.012	0.028
<i>DOP</i>	0.831	0.031	-0.008	0.188
Leaf Size	0.059	0.967	0.028	0.246
<i>FNOP</i>	-0.005	0.026	0.998	0.005
Scape Length	0.166	0.263	0.005	0.946
Explained variance	4.331	1.018	1.021	1.021
% of total variance	54.1	12.7	12.8	12.8

Table S2. Basic statistics for the different variables considered, in the three study populations. DOP, leaf size and scape length values are expressed in centimeters. In the lower section we show the statistics for the four principal components used. Untransformed values for DOP, FNOP, SOP, STOT, FOP and FTOT are shown.

PLANT TRAIT	POPULATION								
	Apóstoles			Couto			Ferradillo		
	N	Mean	S.d.	N	Mean	S.d.	N	Mean	S.d.
Number of flowers	114	7.34	3.11	91	10.30	4.78	120	7.43	2.75
Number of fruits	112	3.93	2.99	84	7.36	4.625	120	2.90	3.00
Number of seeds per fruit	91	27.74	16.10	71	30.45	15.78	69	20.01	15.32
Number of seeds	91	145.77	118.88	71	260.03	246.10	69	96.26	92.64
DOP	118	58.21	48.91	91	321.48	367.70	100	56.27	48.97
FNOP	118	7.43	3.00	91	8.75	4.61	100	6.57	2.69
SOP	118	1.52	1.28	91	0.27	0.365	100	1.71	1.66
STOT	119	3.47	2.33	98	0.913	0.582	100	4.22	2.98
FOP	118	11.21	10.50	91	2.92	4.64	100	12.22	11.72
FTOT	119	25.68	19.12	98	9.67	7.72	100	29.59	20.92
Leaf size	117	7.98	1.34	100	9.03	1.89	102	8.81	1.39
Scape length	108	18.93	5.48	100	22.96	6.31	99	18.93	5.29
‘Inter-individual distance’ factor	101	-0.281	0.853	86	0.801	0.742	95	-0.426	0.929
‘Leaf size’ factor	101	-0.258	0.918	86	-0.033	1.065	95	0.304	0.950
‘Abundance of opposite-morph flowers’ factor	101	-0.046	0.853	86	0.338	1.215	95	-0.256	0.841
‘Scape length’ factor	101	-0.207	0.962	86	0.376	1.072	95	-0.120	0.880