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Sex-specific selection on plant architecture through 'budget' and 'direct' effects in experimental populations of a wind-pollinated herb

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Abstract

Sexual selection may contribute to the evolution of plant sexual dimorphism by favoring architectural traits in males that improve pollen dispersal to mates. In both sexes, larger individuals may be favored by allowing the allocation of more resources to gamete production (a ‘budget’ effect of size). In wind-pollinated plants, large size may also benefit males by allowing the liberation pollen from a greater height, fostering its dispersal (a ‘direct’ effect of size). To assess these effects and their implications for trait selection, we measured selection on plant morphology in both males and females of the wind-pollinated dioecious herb *Mercurialis annua* in two separate experimental common gardens at contrasting density. In both gardens, selection strongly favored males that disperse their pollen further. Selection for pollen production was observed in the high-density garden only and was weak. In addition, male morphologies associated with increased mean pollen dispersal differed between the two gardens, as elongated branches were favored in the high-density garden while shorter plants with longer inflorescence stalks were favored in the low-density garden. Larger females were selected in both gardens. Our results point to the importance of both a direct effect of selection on male traits that affect pollen dispersal, and, to a lesser extent, a budget effect of selection on pollen production.

Key-words: budget and direct effects, sexual selection, sexual dimorphism, male-male competition, resource allocation, mating system

Data accessibility statement: Morphological and genotype data produced in this manuscript are archived in DRYAD, (doi:10.5061/dryad.g4d7p46).

Introduction

Sexual selection through female choice (Fisher 1930) or through direct competition among males for mating (Bateman 1948) is responsible for many cases of sexual dimorphism in animals (e.g., Darwin 1871; Kuntner and Elgar 2014; Rouse et al. 2015). Although sexual dimorphism is rarely as extreme in plants as it is in animals (Lloyd and Webb 1977), transitions towards strong sexual dimorphism are nonetheless common (Tonnabel et al. 2014). Males of dioecious species often flower earlier and display smaller sizes (Delph 1999), are less well defended (Avila-Sakar and Romanow 2012), and express lower rates of gas exchange and lower water-use efficiency than conspecific females (Dawson and Geber 1999). Males and females of dioecious plants often differ also in their longevity (Delph 1999), which typically leads to biases in the sex ratio of natural populations (Field et al. 2012). The reasons for morphological sex differences in plants, analogous to secondary sexual dimorphism in animals, have attracted the attention of researchers ever since Darwin's first investigations on the subject (e.g., Darwin 1871; Delph 1999; Moore and Pannell 2011; Barrett and Hough 2013).

Some of the morphological differences observed between sexes may be the outcome of selection under the influence of sex-specific trade-offs based on the physiological needs of reproduction (e.g. Downhower 1976; Freeman et al. 1976; Harris and Pannell 2008). First, the cost of reproduction may often be higher for females than males in absolute terms, because females invest in seeds and fruits, and this burden on females might favor the evolution of vegetative traits that emphasize the harvesting and deployment of resources such as carbon and water (Obeso 2002; Burd 1994; Dawson and Geber 1999). Second, reproduction by males and females may simply draw on different limiting resources, with the evolution of sex-specific morphologies for acquiring them. Examination of sex-specific responses to resource availability have shown that males and females respond differently to resource limitation (e.g. Conn and Blum 1981; Zimmerman and Lechowicz 1982; Lovett-Doust et al. 1987; Dorken and Barrett 2004; Herlihy and Delph 2009; Harris and Pannell 2008; Teitel et al. 2016; Tonnabel et al. 2017). Such sex-specific plastic responses are consistent with the evolution of divergent morphologies in males and females that allow each sex to harvest the resource type that most limits its respective reproduction (reviewed in

Tonnabel et al. 2017). Despite the importance of sex-specific resource deployment and acquisition, however, it seems unlikely that these factors alone can explain the variation in sexual dimorphism displayed by dioecious plants.

The evolution of sexual dimorphism in plants may also be the outcome of sexual selection (Delph and Ashman 2006; Delph et al. 2011; Moore and Pannell 2011). The scope for female choice in plants is probably restricted to the short phase during which pollen grains grow down the female styles (reviewed in Pannell and Labouche 2013), but males must often compete for mating success during the transfer of pollen grains to styles. A growing number of studies indeed point to the importance of male-male competition in shaping several reproductive and floral traits in insect-pollinated species. For instance, male-male competition is hypothesized to have led to an increase in the size of flowers and floral display to attract insect pollinators (e.g. Bond and Maze 1999; Elle and Meagher 2000; Wright and Meagher 2004; Delph and Ashman 2006; Waelti et al. 2009; Schiestl and Johnson 2013; Dorken and Perry 2017), to an adjustment of the flowering phenology of males to that of females (Delph and Herlihy 2011; Forrest 2014), and to an increase in pollen-grain performance (Lankinen et al. 2017). Even a case of direct male-male competition involving physical contact has been reported in milkweeds, with the evolution of ‘horn weapons’ on pollen-bearing structures (pollinia) that allow males to prevent the successful dispersal of pollen from other males (Coccuci et al. 2014). Yet it is still largely unknown whether such instances of male-male competition could also contribute to the evolution of sexually dimorphic vegetative traits.

Components of plant architecture are thought to be shaped both through selection for gamete production (fecundity selection) and through selection for access to mates (sexual selection), especially for the male function in wind-pollinated plants (Klinkhamer et al. 1997; Harder and Prusinkiewicz 2013); these two components have been usefully referred to as ‘budget’ and ‘direct’ effects, respectively (Klinkhamer et al. 1997). Budget effects refer to selection for larger plant sizes in males, notably because large males have access to more resources and can produce more pollen (Klinkhamer et al. 1997). In important respects, the budget effect of the size of males is similar to that which favors large females, i.e., it may be interpreted as a component of fecundity selection. Size may also have a direct effect on fitness, if large plants

gain greater fitness than small plants for a given investment in reproduction. Such direct effects on male fitness are thought to occur through the physical placement of male flowers, because pollen dispersed from a greater height or a more exposed position in wind-pollinated plants are more likely to reach available ovules in the population (Klinkhamer et al. 1997; Harder and Prusinkiewicz, 2013). Increased plant height, branch flexibility and flower placement at greater height could thus enhance male siring success (Harder and Prusinkiewicz 2013). Importantly, we should expect the direct effects on fitness to be environment-dependent, because different spatial arrangements of the candidate mates can mediate the expected success of dispersing pollen.

Because budget and direct effects of size might lead to sex-specific selection on plant morphologies, they are likely to be central for the evolution of plant sexual dimorphism. On the one hand, budget effects may be aligned between sexes and select for larger plants through both male and female functions. In contrast, direct effects may lead to sex-specific selection on plant architecture. In males of wind-pollinated species, direct effects should select for larger plants that are more efficient at dispersing pollen from greater heights, therefore allowing their pollen to access more ovules and to reduce local mate competition (Klinkhamer et al. 1997). Direct effects are likely to be less important for females – unless they facilitate seed dispersal (Klinkhamer et al. 1997; Pickup & Barrett 2012), and may be diminished (or even reversed) through male function in some species, e.g., those with a spherical growth form, (e.g., *Atriplex canescens*; Freeman et al. 1993), perhaps because size may foster pollen capture by the plant itself (Bickel & Freeman 1993).

Budget effects on selection of plant architecture via the female function have been widely studied (Younginger et al. 2017), but studies on the importance of both budget and direct effects on males are scarcer. In particular, our understanding of the effect of plant architecture on pollen dispersal remains limited. Recently, Pickup and Barrett (2012) reported that males of the wind-pollinated dioecious herb *Rumex hastatulus* were taller than females during pollen dispersal, when males presumably compete for siring success, whereas females were taller than males when seeds were dispersed. The physical placement of inflorescences on peduncles was also shown to provide an advantage in terms of pollen dispersal in the herb *Mercurialis annua* (Eppley and Pannell 2007a).

Here, we estimate budget and direct effects on fitness by analyzing the strength and direction of selection on key plant morphological traits and their relationship with both pollen production, pollen dispersal and seed production in the sexually dimorphic wind-pollinated herb *Mercurialis annua*. We aimed to evaluate: (1) the effect of pollen production and/or dispersal on male reproductive success; (2) how male vegetative morphology impacts pollen production and dispersal; and (3) the extent of sex-specific selection on plant architecture. We hypothesized that vegetative morphologies allowing an upper placement of flowers should foster pollen dispersal and therefore be favored in males, and that larger vegetative biomass should be correlated with larger gamete production in both sexes. To test these hypotheses, we modified methods developed by Klein et al. (2008) to produce a joint estimate of reproductive successes and the mean pollen dispersal distance of males, allowing an assessment of the variance in pollen dispersal.

We applied our approach to two common gardens of *M. annua* growing at low and high densities, representative of the range of plant densities found in natural populations (Hesse and Pannell 2011). In each common garden, we used classical selection-gradient analysis to estimate selection on pollen production, pollen dispersal and plant architecture. Selection gradients on females were estimated using their seed production weighted by rates of seed germination. Our study did not assess effects of traits on seed dispersal, since seeds were collected prior to their dispersal. Our experimental design consisted of pairs of males and females in the same pot, which allowed us to distinguish between selection acting at small *versus* large spatial scales. In particular, we hypothesized that direct effects should be less important in promoting fitness gains by siring seeds produced by the local partner than by more distantly positioned partners. In contrast, budget effects should be as important for mating with near neighbors and more distant partners. Differentiating between selection operating through budget and direct effects can be challenging if several morphological traits are correlated with each other and with both effects, as is probably often the case. To facilitate interpretation in the presence of multicollinearity, we therefore expressed the effect of morphology on pollen production and dispersal via principle components of the measured morphological dimensions. We included both high and low-density plots in our study to test

the reproducibility of our results under different conditions, but the effect of density itself was not of explicit interest and was not replicated.

Materials and methods

Study system

Mercurialis annua is a wind-pollinated annual European herb (Tutin et al. 1964). It displays striking variation in sexual systems, with dioecious, androdioecious and monoecious populations occurring in different parts of its range (Durand 1963; Pannell et al. 2004). In the present study, we focused on dioecious populations in which males are smaller than females (Harris and Pannell 2008). Males also possess stalk-like (pedunculate) inflorescences that are supposed to enhance pollen dispersal (Eppley and Pannell 2007a). In contrast, females hold their green dehiscent two-seeded capsules on short pedicels in their leaf axils. Flowering in both sexes begins several weeks after seeds germinate, and continues during plant growth, with new inflorescences produced in each new leaf axil over a period of three to four months (Pannell 1997a).

We sampled *M. annua* by pooling seeds from 35 populations from across northern Spain; seeds from approximately 30 females had been sampled from each population (see Tonnabel et al. 2017). *M. annua* is a colonizing species subject to frequent local extinctions and colonizations (Eppley and Pannell 2007b); genetic and phenotypic variation in our experiment thus reflects metapopulation-wide rather than demic diversity. Prior to our experiment, genotypes were grown and allowed to mate in a single common garden over three generations (from 2012 to 2014) in Lausanne, Switzerland, to eliminate any potential maternal effects and to decrease genetic correlations originating from selection of combinations of morphological traits in different populations.

Common gardens

We established two common gardens of *M. annua* at the experimental field platform of the LabEx CeMEB in Montpellier, France, which are part of a larger experiment (for further details see Tonnabel et al. 2017). Here, we investigate mating events through paternity analyses in two common gardens of this larger experiment. Each garden consisted of a square array of pots each containing one male and one female. Seeds were initially germinated in greenhouses in February 2015, using separate pots in sterile compost. After seven weeks of growth, plants could be sexed, and pairs of males and females were transplanted into two-liter pots of 20 cm of diameter containing sterile soil (1/3 of sieved clay and chalky soil, 1/3 of recycled compost and 1/3 of compost). To reduce asymmetries in competition caused by differences in the timing of germination, we paired in each pot a male and a female of similar height at the onset of the experiment. Each male-female pair was randomly assigned to one of our two gardens, each consisting of 100 pairs, as was the position of each pot within its respective garden.

Both gardens were initially established with pots 1.0 m apart. Because high density is known to elicit a shade avoidance response (Schmitt and Wulff 1993), and because we wished to minimize the plant's plastic responses to density that could possibly obscure the relationship between a plant's genotype and its morphological traits, we restricted variation in plant density between the two gardens to the last four weeks of the experiment. Specifically, we changed the position of all pots in both gardens four weeks after potting out, when plants had reached full maturity and had begun to disperse pollen and seeds (while continuing to produce new receptive flowers). For the low-density garden, we transplanted pots while maintaining them at the same density, with 1.0 m spacing; for the high-density garden, pots were similarly transplanted but moved closer to one another, with 20 cm spacing). Because fruits disperse their seeds a couple of weeks after fertilization, all seeds on the plants we harvested should have been fertilized during the second phase of the experiment at their assigned density (and not prior to pot movement). In total, the experiment lasted for 19 weeks, and the change in plant density was applied for four weeks.

Morphological traits and analysis of pollen production

At the time of harvest, we recorded the height, diameter and the length of the first two branches for all plants (i.e., the lowest ramifications down the plant whose lengths we averaged; we refer to this mean as the branch length). For males, we assessed plant height by excluding the erect pedunculate inflorescences, so that vegetative plant height could be compared directly with females. For males, the total number of pedunculate inflorescences was counted, and they were separated from the vegetative parts prior to drying. Also, we characterized male inflorescence architecture as the mean length of five peduncles sampled on the fifth highest nodes of the primary axis, which were later averaged; we refer to this average as peduncle length. We separated vegetative and reproductive parts of females after drying the plants and counted their seeds using an automatic seed counter (Elmor C3; Elmor Angewandte Elektronik, Schwyz, Switzerland). Vegetative dried parts of both sexes and dried pedunculate inflorescences were weighed. To estimate a germination rate per female, we sowed ten seeds per female in compost at the greenhouses of the University of Lausanne. When fewer than three seeds germinated, another set of ten seeds was sown, giving a mean number of 10.8 (± 2.29 SD) and 11.3 (± 2.72 SD) seeds sown per female for low and high density, respectively. This second round of seed germination was aimed at obtaining leaf material for a sufficient number of seedlings per female to allow paternity analysis (see below). The five technical assistants involved in data acquisition were randomly assigned between gardens.

Female seed production continues over the life-span of the plant as flowers and fruits are produced in new leaf axils. Plants were thus harvested at a time when female seed production was still largely ongoing. Female reproductive success (*FRS*) was estimated both with and without weighting the number of seeds by their germination rate. Since our results were robust to these two estimates of *FRS*, we present them using solely the former estimate. Female selection gradients solely reflect budget effects, because seed production was assessed prior to dispersal. We refer to the mass of male inflorescences as ‘pollen production’ and use this estimate to assess budget effects in males; pollen accounts for 60% of male flower biomass (Pannell 1997b).

Genotype data and paternity analysis

To estimate male reproductive success (*MRS*) and mean pollen dispersal, we generated genotype data on adults and their progeny resulting from the germination assay (see above). We collected fresh leaves from the 100 females and 100 males of each of our two gardens at the time of sampling, storing them in silica gel for later DNA extraction. The germination assay resulted in an average of 7.36 (\pm 2.35 SD) and 7.13 (\pm 2.34 SD) germinations per mother, in the low- and high-density gardens, respectively, for which leaf material was also stored in silica gel. To assess paternity, we genotyped all parents and a total of 651 and 621 seedlings in the low- and high-density gardens, respectively, at eight polymorphic microsatellite loci, using standard molecular protocols described in Machado et al. (2017). Four males in the low-density and three males and two females in the high-density garden were excluded from the analysis because of a failure in genotyping.

To assess the intensity of male-male competition in each garden, we performed paternity analyses on our two gardens separately using CERVUS version 2.0 (Marshall et al. 1998), allowing for a maximum of four mismatches and accounting for a 0.7% genotyping error rate (calculated as the average across markers of the proportion of offspring with a genotype inconsistent with that of their mother). We assigned paternity based on a (strict) 95% confidence criterion, as defined by Marshall et al. (1998). To perform a mixed-effect logistic regression, we further transformed the output of the paternity analysis into a binary variable describing whether paternity is assigned to an intra-pair mating or not, treating pairs as random. We calculated the number of effective fathers, f_e , using the estimator of Nielsen et al. (2003), which is unbiased for small sample sizes. The difference between the two gardens in the number of effective fathers was evaluated using a linear model of $\ln(f_e)$. We used likelihood ratio tests to test for differences between gardens (1) on the probability of an extra-pair mating and (2) on f_e . These analyses allowed us to describe the relative degree of effective polyandry established in each of our two common gardens.

Individual male reproductive success and kernels of pollen dispersal

Extending the spatially explicit mating models of Klein *et al.* (2008), we developed a method that jointly estimates for each male its *MRS* and the mean dispersal distance of its pollen.

Compared to classical methods estimating *MRS*, spatially explicit models allow correcting for spatial distribution of plants, including the elimination of border effects, and provide an estimate of the pollen dispersal kernel, which was one of the objective of our study (see Oddou-Muratorio et al. 2018 for a description of the methodological advantage of spatially explicit mating models). Our method combines genotyping data and the spatial distributions for both adults and offspring to explicitly model mating events and pollen dispersal kernels using exponential functions. Compared to Klein et al. (2008), our extension (1) considers a dioecious population, (2) estimates an individual random effect for mean pollen dispersal distance (instead of a single value common to all males) and (3) computes a *MRS* for each male as the expected number of seeds that should be sired by this male among all seeds collected across all mothers sampled.

To investigate whether selection on male traits occurred through siring seeds locally on the paired female or globally on extra-paired females, our model provides a decomposition of *MRS* into intra-pot and extra-pot *MRS*. It also estimates parameter values common to all individuals in the population: (1) f_0 , a parameter controlling the pollen dispersal at the position zero (i.e., intra-pot scale) and (2) m , the rate at which pollen could not be assigned to any male in the population. Note that m accounts for both genotyping errors, manipulation errors, and pollen immigration into the garden, and should be interpreted accordingly. For each garden separately, model parameters were inferred in a Bayesian framework using two MCMC chains of 300,000 steps with a burn-in of 10,000 steps, and by thinning one over 20 steps. After evaluating the convergence of our MCMC chains, we calculated the credibility intervals of our model parameters (see Table S1). We estimated punctual estimates of *MRS* and mean dispersal distance for each male by computing their posterior modes. To compare our spatially-explicit method to results of the paternity analyses, we also computed the number of mating events assigned by the paternity analysis (see above) across our common gardens as a function of distance. This allowed us to represent the frequency of mating events with distance. The number of mating events was corrected by dividing by the number of male-female pairs present at each distance classes to eliminate border effects. The resulting distribution of mating events was similar to our estimated pollen dispersal kernel which validates our Bayesian methodology.

Sex-specific selection gradients, and analysis of pollen production and dispersal distances

To assess budget and direct effects and their implications for trait selection in each sex, we analyzed the two sexes separately, because *FRS* was estimated as the product of the number of seeds multiplied by their germination probability, whereas *MRS* was estimated on the basis of Bayesian posterior modes. In males, we examined (1) the relationship between *MRS* and both individual pollen production (the budget effect) and individual mean dispersal distance (the direct effect); (2) how pollen production and dispersal distance were correlated with morphological traits; and (3) the relationship between *MRS* and morphological traits. In females, we examined (4) the relationship between *FRS* and morphological traits (accounting solely for budget effects, as *FRS* was estimated prior to seed dispersal). The analyses were performed separately for each common garden. Finally, we compared the estimates of selection gradients between our two common gardens, which varied in their location at the experimental site and likely associated factors, as well as in their plant density. The comparison between plots is indicative of differences that might occur among populations under different conditions. We discuss, in particular, the differences that are likely attributable to density in a wind-pollinated plants. For males, the estimation of selection gradients described below was repeated for *MRS* as well as for intra-pot and extra-pot *MRS* To disentangle these two fitness components. We expected stronger direct effects of pollen dispersal on extra-pair *MRS* than on intra-pair *MRS*.

We calculated both *MRS* and *FRS* as relative values (with mean of 1.0). Pollen production and mean dispersal distance were also standardized to have a mean of zero and unit variance. All quantitative morphological characters were first standardized to have a mean of zero and unit variance. Prior to gradient selection estimation, we performed a principal component analysis (PCA) on the standardized morphological traits for each sex separately. Our estimation of selection gradients was undertaken both on the individual traits measured (see Table S2, S3, S4), and on PC scores. We performed our selection-gradient analysis on PC scores to circumvent multicollinearity among our morphological traits revealed by pairwise Spearman correlation coefficients (Table S5), as well as because patterns of selection were simpler to interpret using PC scores than individual traits (see Chong et al. 2018); the significant correlational selection on individual traits (Table S3) was included in linear selection on PC scores, but both analyses provided congruent signal. We performed separate

PCA on males and females because they did not share all measured traits (e.g., peduncle length was only present for males), but also because Spearman correlation coefficients calculated between all morphological traits differed between sexes (Table S5). Within each sex, we performed a single common PCA for the two common gardens to allow a comparison between gardens (see below). This choice was justified by the finding of similar correlations between morphological traits in the two gardens for both sexes. The use of PCs in the estimation of selection gradients (see below) has the advantage of providing independent axes that can be included in a single common regression. Both for males and females, all PCs accounted for substantial variation in morphological traits, and we therefore chose to keep all PCs in subsequent analyses (i.e., four and five PCs for females and males, respectively). Keeping all PCs also allowed us to avoid statistical bias that occurs when PCs that explain a small part of the variance are omitted (Chong et al. 2018). Finally, estimating selection gradients on PCs allowed us to visualize correlational selection on individual morphological traits, through directional selection on a linear combination of morphological traits.

We first used linear models to regress *MRS* against pollen production and mean pollen dispersal distance to account for correlation between these two quantities, which we examined using Pearson's correlation tests. The effects of both pollen production and pollen dispersal were examined using likelihood ratio tests. We further analyzed the relationship between both pollen production and mean pollen dispersal distance (separately) and plant morphology by regressing them against all PCs at the same time, again for each garden separately. This analysis was repeated using standardized morphological traits prior to PCA analysis. We used likelihood ratio tests to investigate the effect of each PC (and of each morphological trait) on pollen production and dispersal distance.

We estimated β , the vector of standardized linear selection gradients, from the partial regression coefficients of a multiple regression of relative reproductive success against the standardized PCs (Lande and Arnold 1983). We estimated γ , the matrix of standardized nonlinear selection gradients, using a full second-order polynomial regression, including linear, quadratic and correlational coefficients (Lande and Arnold 1983). The γ matrix informs on the potential signal for stabilizing selection (significant negative quadratic coefficient), disruptive selection (significant positive quadratic coefficient), and correlational

selection when particular trait combinations are favored (i.e., with significant cross-product coefficient; Mitchell-Olds and Shaw 1987). Following the recommendation of Stinchcombe et al. (2008), we doubled the quadratic coefficients of the partial regression. Because values for reproductive success were calculated as relative, we estimated selection gradients using classical linear models. However, our results were robust to the use of quasi-Poisson distributions. We used likelihood ratios to test the effect of each PCs on reproductive success (i.e., to test the significance of selection coefficients). We also used likelihood ratio tests to compare models including strictly linear terms and models including quadratic and correlational terms. Values of percentage of variance explained by our statistical models (R^2) were also calculated. We also performed this analysis on standardized morphological trait values prior to PCA analysis, for which results were congruent with the analysis on PCs and are reported in Table S2, S3 and S4. All statistical analyses were performed using the `lm` and `glmer` functions in the `lme4` package (Bates et al. 2015) in R version 3.2.2 (R Core Team, 2015).

Results

Direct and budget effects on male fitness

Pollen dispersal distance was a strong determinant of male reproductive success in both gardens, reflecting a direct effect of architecture and size, whereas the effect of pollen production on male reproductive success (a budget effect) was much weaker. In the low-density garden, pollen dispersal distance was strongly positively related to male reproductive success, whereas pollen production was not (bivariate regression, $R^2=26\%$: pollen dispersal distance : $\beta=0.27$, $X^2=7.02$, $df=1$, $p<0.001$; pollen production: $\beta=-0.02$, $X^2=0.05$, $df=1$, $p=0.61$; Fig. 1). In the low-density garden, pollen production and its mean dispersal distance were not correlated ($r=0.11$, $t=1.13$, $df=98$, $p=0.26$). In the high-density garden, pollen dispersal distance was also highly positively related to male reproductive success, whereas pollen production was marginally significantly associated with male reproductive success (bivariate regression, $R^2=72\%$: pollen dispersal distance: $\beta=0.62$, $X^2=33.2$, $df=1$, $p<0.001$; pollen production: $\beta=0.08$, $X^2=0.6$, $df=1$, $p=0.06$; Fig. 1). By contrast, pollen production and

its mean dispersal distance were positively correlated in the high-density garden ($r=0.31$, $t=3.13$, $df=93$, $p=0.002$).

Selection on male morphology in the high-density garden

Our analysis in the high-density garden revealed selection for females with greater biomass, taller stature, longer branches and wider diameters, as revealed by a significant positive selection gradient on the first PC (Table 1; Fig. 2). Quadratic and correlational selection on PCs were not significant in this garden, as revealed by comparing models that included or excluded quadratic and correlational terms ($X^2=0.69$, $df=10$, $p=0.73$). In males, we detected significant linear selection gradients on PC scores, and no quadratic or correlational selection, whether we compared models that included or excluded quadratic and correlational terms on global reproductive success (*MRS* at the high-density garden: $X^2=2.79$, $df=15$, $p=0.92$), or when we distinguished the intra-pot versus extra-pot components (*MRS* intra-pot at the high-density garden: $X^2=10.30$, $df=15$, $p=0.65$; *MRS* extra-pot at the high-density garden: $X^2=4.63$, $df=15$, $p=0.84$; Table S6).

In the high-density garden, selection favored males that displayed longer branches, wider diameters and greater biomass, as revealed by a significant negative selection gradient of *MRS* on PC1 (Table 2; Fig. 3a). This pattern was maintained at both the intra-pair and extra-pair scales (i.e., significant negative selection gradients on *MRS* intra-pair and *MRS* extra-pair mating of PC1, Table 2). This male morphology with longer branches, wider diameter and greater biomass was associated to both a greater ability to produce pollen and an ability to disperse it further (i.e., significant negative association with PC1, Table 2; Fig. 3d, g). The mean dispersal distance of pollen in the high-density garden was of 0.78 m across all males (Table S1; Fig. S1).

Still in the high-density garden, males with longer branches and thinner diameters (i.e., erect branches) were favored over those with shorter branches and wider diameters (i.e., flattened branches), as revealed by a significant negative selection gradient of *MRS* on PC5 (Table 2; Fig. 3c). This pattern of selection was maintained at both intra-pair and extra-pair scales (i.e.,

significant negative selection gradients on *MRS* intra-pair and *MRS* extra-pair of PC5, Table 2). The advantage of males with erect long branches was not related to the amount of pollen produced but to its longer dispersal distance (Table 2; Fig. 3f, e). Significant selection gradients on individually measured traits revealed a similar pattern of selection (Table S2, S3 and S4), highlighting selection for females with greater biomass (Table S2) and for males with longer branches (Table S3).

Males combining greater stature and greater biomass, and males combining greater biomass and thinner diameters, produced more pollen, as revealed by a significant association of pollen production with PC2 and PC4, respectively (Table 2). However, this greater production of pollen only translated into significant selection for greater stature and greater biomass at the extra-pair scale (i.e., PC2, Table 2), whereas selection on PC2 was only marginally significant on global *MRS* (i.e., Table 2).

Selection gradients at the low-density garden

Selection-gradient analysis in the low-density garden revealed selection for females with greater biomass, taller stature, longer branches and wider diameters, as revealed by a significant positive selection gradient on the first PC (Table 1; Fig. 2). In females, quadratic and correlational selection were significant in the low-density garden as revealed by a comparison between models including or excluding quadratic and correlational terms ($X^2 = 2.16$, $df = 10$, $p = 0.03$). The significant pattern of correlational selection detected in the low-density garden indicated that two combinations of female morphologies were selected: plants with lower biomass and long erect branches, or plants with greater biomass and shorter flattened branches (Fig. S2a; Table S7). In the low-density garden, we did not detect quadratic and correlational selection on males as revealed by comparing models including or excluding those components (*MRS* at the low-density garden: $X^2 = 3.49$, $df = 15$, $p = 0.73$; *MRS* intra-pot at the low-density garden: $X^2 = 9.74$, $df = 15$, $p = 0.39$; *MRS* extra-pot at the low-density garden: $X^2 = 4.30$, $df = 15$, $p = 0.14$; Table S6).

However significant linear selection gradients were found in males. Males with shorter stature and longer peduncles were favored over taller males displaying short peduncles, as revealed by a significant negative selection gradient of *MRS* on PC3 (Table 2; Fig. 3b). This pattern of selection was observed only at the extra-pair scale (i.e., significant negative selection gradients on *MRS* extra-pair of PC3, Table 2), and was associated with a greater efficiency to disperse pollen over longer distances rather than to pollen production (Table 2; Fig. 3e, h). The mean dispersal distance of pollen in the high-density garden was of 3.95 m across all males (Table S1; Fig. S1).

In the low-density garden, selection operating at the extra-pair scale favored males with longer branches, wider diameters and greater biomass, as revealed by a significant negative selection gradients of *MRS* extra-pair on PC1 (Table 2). Such pattern of selection was not present at the intra-pair scale or on the global scale (Table 2; Fig. 3a). Such male morphology was associated both to a greater ability to produce pollen and to disperse it further (i.e., significant negative association with PC1, Table 2; Fig. 4d, g). Significant selection gradients on individual traits revealed similar patterns of selection (Table S2, S3 and S4), highlighting significant, or marginally significant, selection for females with greater biomass (Table S2) and for shorter and larger males (Table S3).

Males combining greater stature and greater biomass, and males combining greater biomass and thinner diameters, produced more pollen, as revealed by a significant association of pollen production with PC2 and PC4, respectively (Table 2). However, this greater production of pollen did not translate into significant selection on these male morphologies.

Differences in male-male competition between the two gardens

Paternity analysis revealed substantial differences in the intensity of male-male competition between the two gardens. The proportion of extra-pair mating was significantly higher in the high- compared to the low-density garden ($\mu_{high}=0.77$ vs. $\mu_{low}=0.60$, $df=1$, $\chi^2=22.27$; $p<10^{-5}$; Fig. 4a). The number of effective fathers (reflecting the diversity of fathers among seeds of a given mother) was also consistently higher in the high-density garden ($\mu_{high}=10.10$ vs.

$\mu_{low}=7.24$, $df=1$, $\chi^2=6.85$; $p<10^{-2}$; Fig. 4b). Additionally, the variance in standardized male reproductive success was much higher in the high- than the low-density garden (33.36 vs. 20.18, respectively; Fig. S3).

Discussion

Our study examined selection on vegetative morphologies through a budget effect (larger resource pools in larger individuals) and/or a direct effect (more effective pollen dispersal by larger males; Klinkhamer et al. 1997). We found that females displaying greater biomass, taller stature, longer branches and wider diameters had greater fitness in both common gardens. The association between size and fitness has been a common observation for annual plants (Harper 1977; Younginger et al. 2017; Hesse and Pannell 2011), where biomass reflects both general vigor and resource availability for allocation to reproduction. In males, we additionally found that direct effects acting through pollen dispersal resulted in larger fitness. Through the use of a novel method for estimating male reproductive success and pollen dispersal, our study establishes a direct link between particular vegetative architectures, greater pollen dispersal abilities, and higher siring success. It therefore supports the view that several vegetative traits in wind-pollinated plants may be targets of sexual selection by promoting access to more ovules through the male function (cf. Arnold 1994; Stanton 1994; Delph & Ashman 2006).

Male fitness may often depend on both budget and direct effects, especially in highly polyandrous populations of wind-pollinated plants (Klinkhamer et al. 1997). The selection gradients we measured in the high-density garden, in particular, provide strong support for direct effects, whereas the signal for budget effects here was weak. Indeed, males dispersing pollen further achieved greater fitness, whereas their fitness depended only marginally on pollen production. Importantly, male morphology affected both pollen dispersal and pollen production. First, males with longer branches and greater biomass (negative PC1) enjoyed a siring advantage; this combination of traits was associated predominantly with pollen dispersal over greater distances (a direct effect) and, secondarily, with higher pollen production (a budget effect). Pollen dispersal distance and pollen production were correlated in the high-density garden, so that both direct and budget effects may coincide in the

selection of males with longer branches and greater biomass (negative PC1). Second, although we found no siring advantage for taller males *per se*, males with long erect branches (i.e., negative PC5) dispersed their dispersal further had greater fitness in the absence of greater pollen production, pointing unambiguously to a direct effect. In *M. annua*, pollen is likely dispersed poorly from inflorescences on lower branches, unless they can be elevated by greater length or by adopting a more erect stature. Interestingly, direct effects appeared to favor access to both extra-pair and intra-pair partners in the high-density garden, in contrast with our expectation that they should mostly be involved in accessing partners located far away. Our results point to a budget effect only on extra-pair male reproductive success through selection of taller plants with greater biomass, which produced more pollen (though notably not further away; negative PC2).

Despite the lower level of polyandry in the low-density garden, there was nevertheless selection for male morphologies that promoted pollen dispersal over greater distances. In this garden, pollen dispersal distance, but not pollen production, favored greater fitness, pointing to a direct but not a budget effect. Shorter males with longer peduncles dispersed their pollen further and sired more offspring (negative PC3). In contrast with results from the high-density garden, the siring advantage of males dispersing pollen further in the low-density garden occurred only through promotion of access to non-local mates. The differences between gardens at the scale at which direct effects operate may thus lie in the higher male-male competition for siring success on local females in the high-density garden. Another probable example of a direct effect occurred in the low-density garden, where larger plants with longer branches dispersed their pollen further and sired more offspring on extra-paired females (negative PC1).

The above results suggest that selection may often shape male morphology through its direct effects over a wide range of plant densities, whereas its budget effects may be important primarily at higher density. Previous work on the variance in male reproductive success has provided some support for the existence of both direct and budget effects in male plants. For instance, Schoen & Stewarts (1986) found a positive relationship between male cone production and fitness in white spruce. In *Plantago lanceolata*, while Tonsor (1985a,b) recorded no effect of the height of flowers in the canopy on the distance of gene flow, Young

& Schmitt (1995) documented a positive effect of the release height of pollen on the amount of pollen dispersed to traps. A budget effect was found for *Ambrosia artemisiifolia*, but there was no direct effects of plant height (Nakahara et al. 2018). Our study contributes to this body of work both by examining the relationship between fitness components (pollen production and dispersal) and morphological components that tend to have been examined separately (biomass and dimensions of plant architecture), as well as by providing a novel way to estimate variance in pollen dispersal distance in a spatially explicit context.

It is also noteworthy that our two common gardens differed in which component of plant architecture was most strongly associated with the distance of pollen dispersal. While our experimental design does not allow us to attribute this difference to density with any confidence, an effect of density seems most likely, given what is known about sensitivity of mating to density in wind-pollinated plants in general (Compagnoni et al. 2017), including *M. annua* (Eppley and Pannell 2007a; Hesse and Pannell 2011; Labouche et al. 2017). At low density, canopies are sparser, with correspondingly fewer obstacles to pollen dispersal. We might thus anticipate strong direct effects of male plant architecture on dispersal. In contrast, dense canopies are likely to affect wind dispersal of pollen by slowing air flow and/or increasing humidity (Whitehead, 1969; Freeman *et al.* 1993). Supporting these ideas, the average mean dispersal distance of pollen was greater in the low- compared to the high-density garden, even after correcting for spatial configuration effects by using a SEMM. Whereas morphologies that promote effective pollen dispersal were characterized by long erect branches (PC5) in the high-density garden, they were characterized by shorter main stems, but longer apical pedunculate inflorescences (negative PC3) in the low-density garden. This suggests that the optimal plant architecture and placement of male flowers may vary according to the conditions of pollen transport. The sparsity of physical obstacles and longer interplant distances at low density may emphasize the importance of flowers positioned at the plant apex, which in *M. annua* typically develop on erect inflorescences held above the plant canopy (Eppley and Pannell 2007a).

The common differences in morphology between male and female inflorescences of wind-pollinated plants have prompted much speculation about their functional significance (Klinkhamer et al. 1997; Eppley and Pannell 2007a; Pickup and Barrett 2012; Harder and

Prusinkiewicz 2013). Whereas inflorescences that extend beyond the plant foliage must be exposed to the wind better than those held within the canopy, experimental manipulations have shown that the advantage of such inflorescences extends to their tendency to liberate more pollen, because they flex when blown (Friedman and Harder 2005). We have not evaluated the fitness consequences of inflorescence flexibility in *M. annua*, but our study advances earlier work by establishing a clear link between male morphological architecture, including a common sexually dimorphic inflorescence, and pollen dispersal ability.

Importantly, not only did the morphology promoting effective pollen dispersal differ between our two gardens, but so did selection gradients. As anticipated, the degree of polyandry was lower at low density, which puts more weight on the component of selection mediated by the local female for each male. In this situation males may benefit more by dispersing their pollen further to pollinate external mates, as well as by not suppressing the growth of their local mate that may bear a large proportion of their offspring. Recall that the reproductive success of females was subject to a budget effect, and that their reproductive success ought to be affected by increased competition with neighbors for light (Tonnabel et al. 2017). On this basis, morphologies positively selected at the extra-pair level could be negatively selected at the intra-pair level, especially in the low-density garden, where male-male competition is less strong. Limited growth could reduce competitive interference in low-polyandry conditions, allowing the neighboring female to harvest light, just as relaxed sexual selection under monogamy leads to reduced harm by males to their mates in animals, e.g., *Drosophila* (Holland and Rice 1999; Hollis et al. 2016).

In both common gardens, selection favored males with longer branches and greater biomass (negative PC1), although this selection was significant only at the extra-pair scale in the low-density garden, while at high density the advantage was expressed also in terms of siring success on the local female. Thus, although the differences between the two gardens conform to the idea of decreased competitive interference with the local female, opposite selection at intra- and extra-pot level was not observed, even at low density. Further experiments exploring even lower densities, lower male-male competition and greater morphological differences within a male-female pair, might be necessary to detect possible antagonistic selection between intra- and inter-pot levels. Our results are nonetheless consistent with the

idea that male morphologies that promote pollen production through a budget effect may be less effective at securing paternity on local mates when male competitors are more distant and exert less competition.

Of course, our estimates of intra-pair male reproductive success probably suffer from a lack of statistical power, as they rely on a smaller number of reproductive events than our estimate of extra-paired matings. Such a possibility is foreshadowed by the observation that male traits explained a much larger proportion of the variance in extra-pot than intra-pot male fitness, especially in the low-density garden. In this garden, males may ultimately have experienced less selection locally, notably if a larger proportion of their pollen is carried away than at high density, as suggested by our estimates of pollen dispersal distance.

Further investigation on the effect of increased plant density and polyandry on selection gradients would be valuable. Nevertheless, by disentangling the effects of pollen production and pollen dispersal on fitness, our study provides empirical support for the effect of direct selection on plant morphology, likely through male-male competition over ovules, and it underscores the complexities of selection on plant architecture in different ecological contexts. These complexities may arise because males must compete with other plants not only for resources to grow, but also for access to mates. Plants ought to be sensitive to the mating opportunities afforded by their neighbors, just as they are to the threat of competition posed by those same neighbors (Sleeman et al. 2002; Tonnabel et al. 2017). In this context, it is interesting that the gametophytes of certain homosporous ferns indeed switch gender in response to the composition of their local mating neighborhood (DeSoto et al. 2008). Given the growing evidence that flowering plants can be sensitive to the identity and status of their neighbors (Belter and Cahill 2015; Ninkovic et al. 2016; Torices et al. 2018), we would expect them to deploy such sensitivity to optimize their mating opportunities, too.

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Figure 1. Effect of morphological variables (through PC1 scores) on female reproductive success of *M. annua* at low and high plant density (empty dots and solid line and filled dots and dashed line, respectively). The drawings of plants illustrate morphological trait values on extreme values of PC1 (-2.5 and 2.5); the different parts of the drawings are proportional to the plant height, the branch length, the plant diameter and the rectangle below the plant is proportional to the biomass.

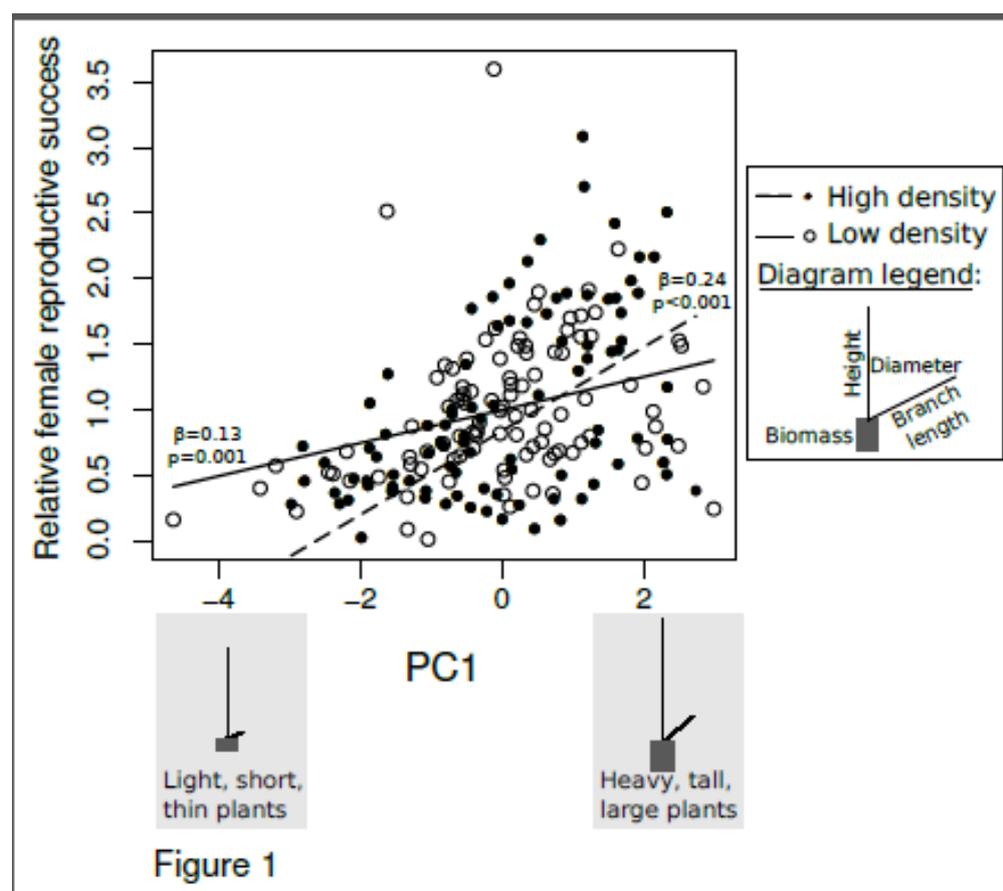


Figure 1

Figure 2. Effect of mean pollen dispersal distance (a) and pollen production (b) on male reproductive success in *M. annua* at low and high plant density (empty dots and solid line and filled dots and dashed line, respectively). Bivariate regressions were used to account for the correlation in pollen dispersal distance and production, which were standardized. Abbreviations: st. : standardized.

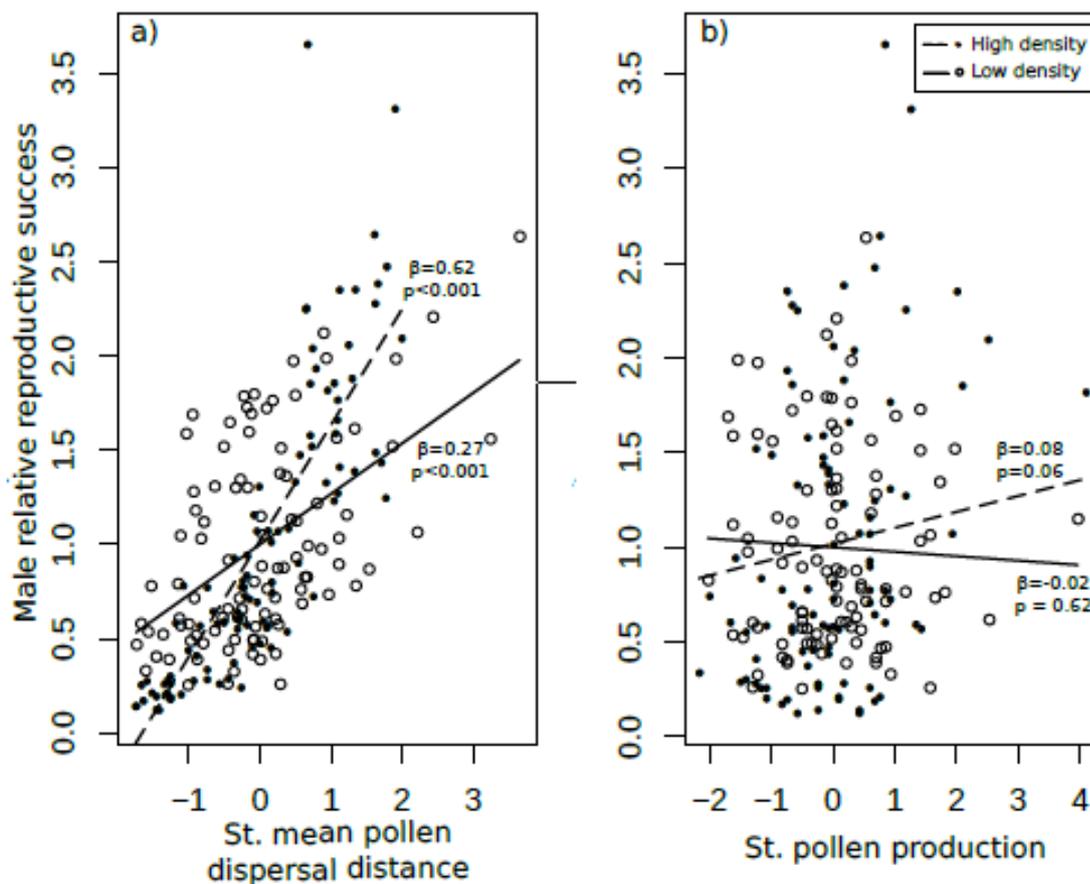


Figure 2

Figure 3. Effect of morphological variables (through three PCs scores) on male reproductive success (a), (b), (c), on pollen dispersal distance (d), (e), (f) and pollen production (g), (h) and (i) of *M. annua* at low and high plant density (non-filled dots and solid line and filled dots and dashed line, respectively). Selection gradients were estimated thanks to multiple regressions including all standardized PCs and the relative male reproductive success. Multiple regressions were also performed on standardized PCs and on either the standardized mean pollen dispersal or pollen production. The drawings of plants illustrate morphological trait values on extreme values of PC1, PC3 and PC5 (-2.5 and 2.5); the different parts of the drawings are proportional to the plant height, the branch length, the plant diameter, and the peduncle length, and the rectangle below the plant is proportional to the biomass.

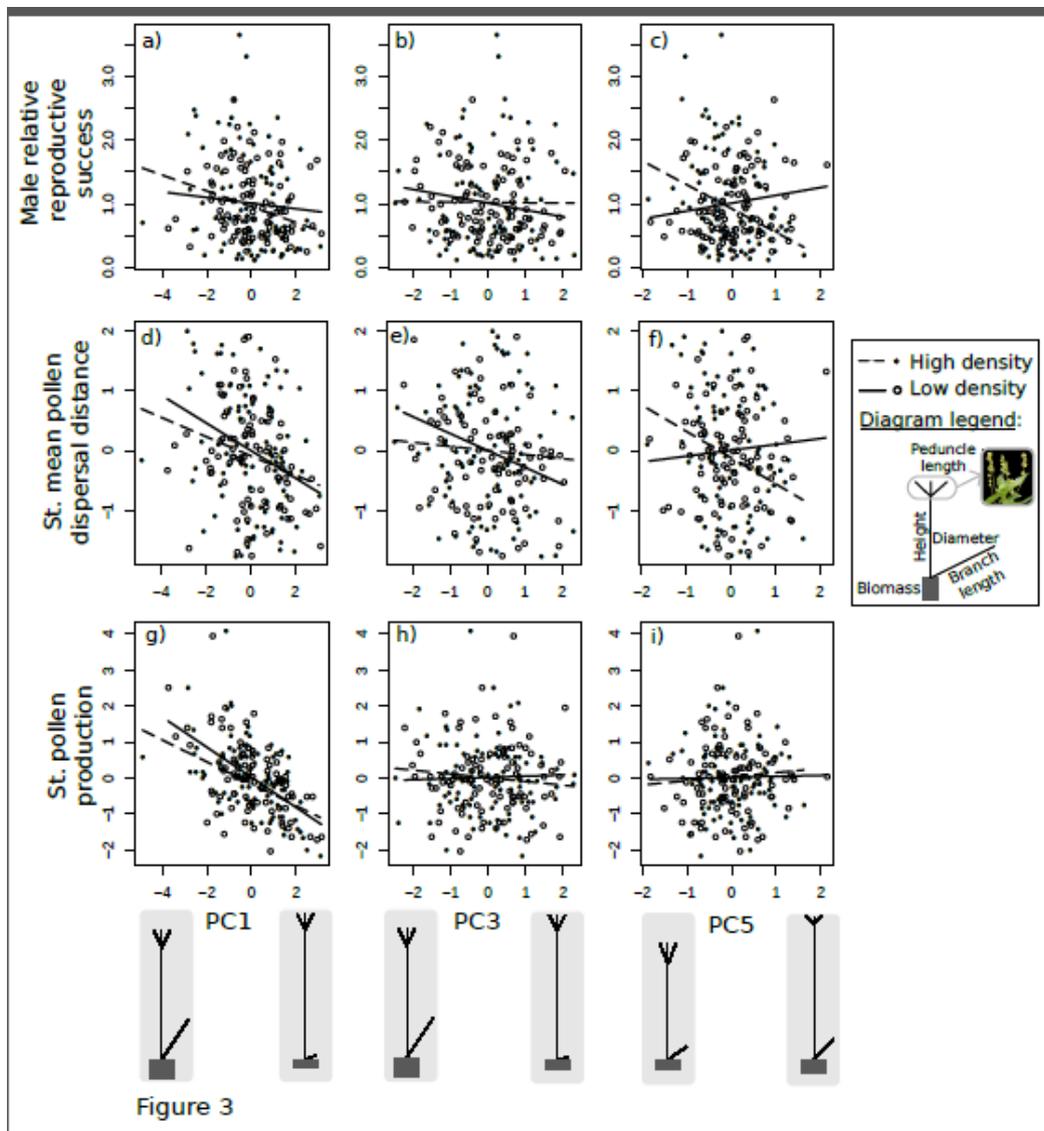


Figure 4. Mating patterns in two experimental common gardens of *M. annua* established at low and high density. Paternity analysis allowed inferring the probability of (a) extra-pair mating and (b) the effective number of fathers for the progeny of each female. The effective number of fathers was calculated using an unbiased estimator of paternal diversity for small sample sizes (Nielsen et al. 2003). Different letters designate a significant difference between gardens, as provided by likelihood ratio tests.

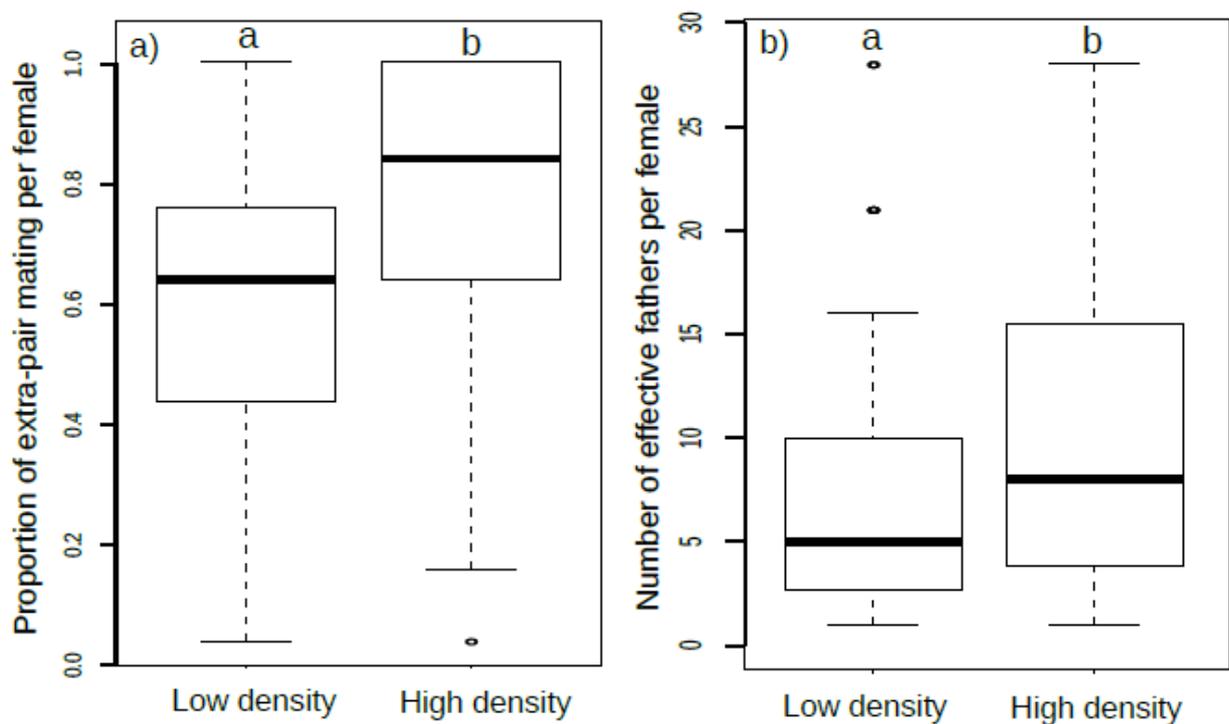


Figure 4

Table 1: Linear selection gradients and description of the principal component analysis axes in females. The vectors of standardized linear selection gradients (β) in females of *M. annua* grown in a common garden at low density and high density are displayed in the upper part of the table; the contributions of each morphological trait to each PCA axis, along with the proportion of variance explained by each PCA axis (PC1-4), are displayed in the lower part of the table. Selection gradients were performed using the female reproductive success (i.e., *FRS*, the number of seeds weighted by their germination probability), which was regressed against all four PCA axes in a multiple regression. The proportion of variance explained by each model (R^2) is given. Significance was evaluated using likelihood ratio tests: ** $p < 0.01$, *** $p < 0.001$. Results with associated p-values below 0.05 are highlighted in bold.

	PC1	PC2	PC3	PC4
Low density				
$\beta - FRS$ ($R^2 = 12\%$)	0.13**	0.03	0.02	0.11
High density				
$\beta - FRS$ ($R^2 = 26\%$)	0.24***	0.02	-0.02	0.14
Plant height	0.42	-0.72	0.19	-0.52
Branch length	0.42	0.66	0.48	-0.39
Plant diameter	0.52	0.18	-0.82	-0.14
Plant biomass	0.61	-0.12	0.24	0.75
Proportion of variance	0.48	0.25	0.16	0.10

Table 2: Linear selection gradients, analysis of pollen dispersal distance and production and description of the principal component analysis axes in males. The vectors of standardized linear selection gradients (β) on male reproductive success (*MRS*), intra-pair *MRS* and extra-pair *MRS* against PCA axes summarizing morphological characters in males of *M. annua* grown in a common garden at low density and high density are displayed in the upper part of the table; also in the upper part of the table are results of the regression of mean dispersal distance of pollen and pollen production against PCA axes PC1-5; the contribution of each morphological trait to each PCA axis, along with the proportion of variance explained by each PCA axis, are displayed in the lower part of the table. All statistical results reported correspond to multiple regressions including all four PCA axes. The proportion of variance explained by each model (R^2) is given. Significance was evaluated using likelihood ratio tests : $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Results with associated p-value are below 0.05 are highlighted in bold.

	PC1	PC2	PC3	PC4	PC5
Low density					
β – <i>MRS</i> ($R^2=9\%$)	-0.04	0.04	-0.11*	-0.05	0.12
β – <i>MRS</i> intra-pair ($R^2=5\%$)	-0.01	0.05	-0.07	-0.14	0.17
β – <i>MRS</i> extra-pair ($R^2=19\%$)	-0.10**	0.02	-0.15**	0.07	0.05
Mean dispersal distance ($R^2=18\%$)	-0.23**	-0.04	-0.28**	0.02	0.10
Pollen production ($R^2=50\%$)	-0.42***	-0.35***	0.03	0.28**	0.03
High density					
β – <i>MRS</i> ($R^2=18\%$)	-0.14**	-0.12*	-0.01	0.03	-0.33**
β – <i>MRS</i> intra-pair ($R^2=14\%$)	-0.17*	-0.10	0.02	0.15	-0.38*
β – <i>MRS</i> extra-pair ($R^2=17\%$)	-0.13*	-0.14*	-0.02	-0.02	-0.32**
Mean dispersal distance ($R^2=15\%$)	-0.18*	-0.05	-0.08	0.15	-0.38*
Pollen production ($R^2=49\%$)	-0.35***	-0.40***	-0.12	0.38***	0.10
Plant height					
	0.19	-0.76	0.40	-0.42	-0.24
Branch length					
	-0.61	0.14	0.11	0.02	-0.78
Plant diameter					
	-0.60	0.08	0.03	-0.65	0.47
Plant biomass					
	-0.48	-0.49	0.16	0.63	0.33
Peduncle length					
	-0.10	-0.40	-0.90	-0.10	-0.13
Proportion of variance					
	0.38	0.23	0.20	0.10	0.09