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1 **Sex-specific selection patterns in a dioecious insect-pollinated plant**

2 **Running title:** Sex-specific selection in a dioecious plant

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13 **Author contributions**

14 E.B, M.D and I.D.C conceived and planned the experiments. E.B carried out the experiments,  
15 analyzed the data, and took the lead in writing the manuscript. M.D and I.D.C provided help  
16 to analyze the data, and contributed to the interpretation of the results and to the final  
17 manuscript.

18

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29 **Data and materials availability**

30 All data are available in Dryad (<https://doi.org/10.5061/dryad.j6q573nh1>).

31 **Conflict of interest**

32 The authors declare no conflict of interest.

### 33 **Abstract**

34 Competition for mate acquisition is the hallmark of any sexual organism. In insect-pollinated  
35 plants, competition to attract pollinators is expected to result in pollinator-mediated selection  
36 on attractive floral traits. This could overlap with sexual selection if the number of mating  
37 partners increases with pollinator attraction, resulting in an improved reproductive success.  
38 In this study, we measured a set of floral traits and estimated individual fitness in male and  
39 female *Silene dioica* in an experimental population. Results align with the predictions of  
40 Bateman's principles, in the absence of pollen limitation. In females, natural selection acted  
41 on traits that are typically linked with fertility (number of flowers and number of gametes),  
42 and selection strength was similar in open- and hand-pollinated females, suggesting a  
43 limited role of pollinator-mediated selection. In males, flowering duration and corolla width  
44 were positively associated with both reproductive success and number of mates, suggesting  
45 that sexual selection has played a role in the evolution of these traits. The use of Bateman's  
46 metrics further confirmed stronger sexual selection in males than in females. Taken together,  
47 our results shed light on the occurrence of sex-specific patterns of selection in an insect-  
48 pollinated plant population.

## 49 **Introduction**

50 Sexual selection (selection arising from competition for mating partners and/or their  
51 gametes), along with viability selection (selection for traits that increase survival) and  
52 fertility selection (selection for traits that increase the number of offspring per reproductive  
53 episode, e.g. via gamete production), constitute the three routes through which natural  
54 selection shapes phenotypic traits (Arnold 1994*a*; Delph and Herlihy 2012). In animals, pre-  
55 copulatory sexual selection has often been proposed as the ultimate cause for the evolution  
56 of extravagant phenotypes in males, in a large array of traits including ornaments,  
57 armaments and behaviors (Darwin 1871; Lande 1981; Jennions and Kokko 2010). In this  
58 matter, Bateman's principles predict that the sex experiencing the stronger sexual selection  
59 should display three features. First, it should have the highest variance in reproductive  
60 success (i.e. the opportunity for selection, measured as the variance in offspring number).  
61 Second, it should display the highest variance in mating success (i.e. the opportunity for  
62 sexual selection, measured as the variance in the number of reproductive partners). Finally,  
63 it should exhibit the strongest dependency of reproductive success on mating success (i.e. the  
64 Bateman gradient, which quantifies the reproductive benefit of acquiring more mates by  
65 regressing reproductive success on mating success, Bateman 1948; Arnold 1994*a*). Using  
66 metrics of sexual selection (Jones 2009), a recent meta-analysis on animal species showed that  
67 sexual selection is often stronger in males than in females (Janicke et al. 2016), which is  
68 generally interpreted as a direct consequence of anisogamy (Bateman 1948).

69 Although animal species with sexual dimorphism in secondary sexual characters have been  
70 the primary focus of sexual selection theory, there is now a wide acceptance that sexual

71 selection should occur in plants as well (Arnold 1994*b*; Delph and Ashman 2006; Moore and  
72 Pannell 2011). In plants, competition for access to reproductive partners is expected to take  
73 place both before pollination (e.g. male-male competition for access to stigmas or female-  
74 female competition for pollen delivery; Minnaar et al. 2019) and after pollination (e.g. pollen  
75 competition for access to ovules; Murphy 1998; Moore and Pannell 2011). Any plant trait that  
76 would impact the outcome of competition for mates should thus be under sexual selection.  
77 Because a wide majority of angiosperms rely on insects for mate acquisition (Ollerton et al.  
78 2011), some of the traits under sexual selection in plants are likely to be directly involved in  
79 pollinator attraction, but not necessarily all of them (e.g. some traits can be involved in post-  
80 pollination sexual selection with no link to pollinator attraction, Tonnabel et al. 2021).  
81 Selection on attractive floral traits, arising from competition among plants for pollinators, is  
82 usually referred to as pollinator-mediated selection and this should thus at least partially  
83 overlap with sexual selection (Willson 1994; Delph and Ashman 2006; Waelti et al. 2009; Dai  
84 and Galloway 2013; Paterno et al. 2020).

85 If one applies the framework of sexual selection to animal-pollinated plants, male  
86 reproductive success should be more dependent on pollinator attraction than female  
87 reproductive success, at least in situations where female reproduction is not limited by  
88 pollen availability (Stephenson and Bertin 1983). First, plants are anisogamous organisms,  
89 and male reproductive success should thus be more limited by mate acquisition than female  
90 reproductive success, as is the case in animals without reversed sex-roles (Bateman 1948;  
91 Willson and Burley 1983; Janicke et al. 2016). Second, a recent study demonstrated that,  
92 across hermaphroditic angiosperms, the allometric relationship between flower biomass and  
93 sexual organ biomass was steeper for the male than for the female organs, a result that can be

94 explained by a widespread role of male-male competition in the evolution of floral  
95 morphology (Paterno et al. 2020). Third, selection on floral traits has been documented to be  
96 stronger through male than female function in some hermaphroditic species. This is not a  
97 general trend and it may depend on the availability of pollinators (see below) but when  
98 selection seems stronger through male function, this may be because these traits promote  
99 pollinator attraction, although sexual selection was not directly measured in these studies  
100 (e.g. Hodgins and Barrett 2008 for corolla width; Briscoe Runquist et al. 2017 for petal area).  
101 These observations thus seem consistent with sexual selection occurring in males, and  
102 potentially being stronger than in females, as observed in the animal kingdom (Janicke et al.  
103 2016). Nonetheless, contrary to what is typically seen in female animals, which are rarely  
104 sperm limited provided they acquire at least one mating partner (with the exception of  
105 animals with external fertilization; Levitan & Petersen 1995), female reproductive success is  
106 often pollen limited in angiosperms (Burd 1994; Knight et al. 2005). This means that both  
107 sexual functions can be limited in their access to reproductive partners and that both sexual  
108 functions can display a positive Bateman gradient. In other words, under pollen limitation,  
109 pollinator-mediated selection should shape attractive traits through both sexual functions in  
110 hermaphroditic species, or in both sexes in dioecious species (Burd 1994). On the contrary in  
111 the absence of pollen limitation, one could expect that attractive traits would be under  
112 stronger selection through the male function, or in males in dioecious species (Arnold 1994*a*).  
113 From the female perspective, one way to estimate the strength of pollinator-mediated  
114 selection is to use pollen supplementations: by comparing selection gradients on floral traits  
115 in open-pollinated versus hand-pollinated females, one can assess the magnitude of  
116 pollinator-mediated selection on these traits (Sletvold and Ågren 2010; Trunschke et al. 2017).

117 However, because these methods focus on the female reproductive success, they do not  
118 allow any comparison between male and female reproductive functions (Barrett & Harder,  
119 2017). One solution to compare sexes would be to estimate the access to reproduction  
120 through a measurement of the number of different genetic sexual partners (Tomaszewski et  
121 al. 2018), an approach that is commonly used in animal studies. Although the number of  
122 genetic sexual partners may underestimate the actual number of mates, because it depends  
123 on both access to mates and post-fertilization processes (cryptic female choice and  
124 competition among male gametes; Anthes et al. 2016), this estimate has the clear advantage  
125 of providing an equivalent measurement of mating success in both males and females. Only  
126 two studies estimated the Bateman gradient using the number of genetic sexual partners in  
127 angiosperm species and both found that while mating and reproductive success were  
128 independent in females, they were strongly positively correlated in males (Tonnabel et al.  
129 2019; Kwok and Dorken 2022). Noteworthy, this measurement might not perfectly reflect  
130 the number of mating opportunities in plants, in particular in animal-pollinated taxa. Indeed,  
131 because each flower constitutes a reproductive tract, mating events can be quite independent  
132 among flowers, depending on pollinators' visitation sequences. One could thus expect the  
133 number of genetic mating partners to increase with the number of flowers (and thus with the  
134 number of ovules for the female function or the number of pollen grains for the male  
135 function) and to be an indirect by-product of individual fertility. In such a context, it  
136 becomes difficult to distinguish between sexual and fertility selection, a situation which has  
137 been referred to as "the grey zone of sexual selection" by Alonzo and Servedio (2019). By  
138 correcting mating success estimates by individual fertility, one should be able to compare the



139 strength of sexual selection between males and females while minimizing this bias (Henshaw  
140 et al. 2018).

141 In this study, we measured a set of floral traits and estimated male and female reproductive  
142 success in an experimental population of the dioecious insect-pollinated species *Silene*  
143 *dioica*. Our objectives were fourfold. First, by regressing reproductive success on floral  
144 phenotypes in both sexes, we explored sex-specific patterns of natural selection during one  
145 flowering season. In our study, this includes both sexual and fertility selection, but not  
146 viability selection since no deaths were observed. Second, by comparing the relationship  
147 between reproductive success and floral phenotypes between open-pollinated and hand-  
148 pollinated females, we quantified the relative importance of pollinator-mediated selection in  
149 females. Third, we quantified the importance of floral phenotypes for mate acquisition in  
150 both sexes by regressing genetic mating success (i.e. the number of genetic mating partners)  
151 on floral phenotypes in males and females. In females, this allowed us to compare patterns of  
152 pollinator-mediated selection (objective 2) and sexual selection (objective 3), a useful  
153 approach to determine whether sexual selection arises from competition to attract pollinators  
154 or not. Finally, overall sexual selection was compared between sexes using the metrics  
155 derived from Bateman's principles: the opportunity for selection  $I$ , the opportunity for  
156 sexual selection  $I_s$ , and a partial Bateman gradient (regression of reproductive success on  
157 mating success controlling for flower and gamete production). Studying sexual selection in  
158 plants combining several approaches will provide an integrative framework to understand  
159 how sexual selection on floral traits parallels with other selection components in plants.

## 160 **Material and methods**

### 161 *Study system*

162 *Silene dioica* (L.) Clairv. is a herbaceous short-lived perennial species of the Caryophyllaceae  
163 family that is widely distributed throughout most of northern and central Europe (Baker  
164 1947; Jalas and Suhominen 1986). It is a dioecious species with XY sex determination  
165 (Warmke 1946; Ming et al. 2011). The species flowers during late spring, but is known to  
166 sometimes exhibit a second flowering peak at the end of summer (Jürgens et al. 1996; Bopp  
167 and Gottsberger 2004). In Lille (France), this second peak is generally brief and presents  
168 extremely male-biased sex ratios, so most of the reproduction effectively occurs during the  
169 first peak, between end-April and mid-July (pers. obs). This species presents a generalist  
170 insect-pollinated system, with *Bombus* species, Syrphidae, diurnal butterflies and  
171 Sphingidae as pollinators (Baker 1947; Westerbergh and Saura 1994). *Silene dioica* has been  
172 documented as sexually dimorphic for flower size and number (males > females; Kay et al.  
173 1984; Baena-Díaz et al. 2019; Moquet et al. 2020), total nectar production at the flower level  
174 (females > males; Hemborg and Bond 2005) and flowering duration (males > females;  
175 Hemborg 1998).

### 176 *Experimental population*

177 Experimental plants were derived from seeds harvested in three forests during 2015, 2016  
178 and 2017 (site A 50°12'05.4''N 3°44'95.7''E, site B 50°26'06.0''N 3°18'39.5''E and site C  
179 50°23'19.3''N 3°25'24.9''E). Our experimental population thus included three cohorts (one,  
180 two and three-year-old plants). Sampling sites were geographically close to each other (i.e.

181 maximum distance of 40 km between sites) and to our experimental garden (<65 km). Seeds  
182 were collected at different study sites to reach a sufficient sample size while minimizing the  
183 probability of including half or full-sibs. Plants from all three populations and from all  
184 cohorts were repotted every year in new potting mix and kept in the exact same conditions in  
185 the greenhouse until the experiment.

### 186 *Experimental design*

187 Three groups of plants were established in the same common garden. The three source  
188 forests and the three cohorts were evenly distributed among these groups. The two first  
189 groups were open-pollinated females (OP females hereafter, 98 individuals) and open-  
190 pollinated males (OP males, 102 individuals) that were left to be naturally pollinated by  
191 insects present in the experimental garden. In the third group, composed of hand-pollinated  
192 females (HP females, 65 individuals), all open flowers were supplemented three times a  
193 week with pollen from a pool of 65 males from the same collection that were kept in a  
194 greenhouse. For a given HP female and a given supplementation day, dehiscent anthers  
195 were collected on two randomly selected pollen donors and were brushed on the stigmas.

### 196 *Common garden*

197 The experimental population was set up in a common garden in April 2018 on the campus of  
198 Lille University in France (50°36'32.5''N 3°08'40.3''E) during spring 2018. No wild  
199 populations of *Silene dioica* were growing in the vicinity. All individuals were in separate  
200 0.7-L pots filled with a standard soil mixture. Plants were watered every other day, or every  
201 day on the hottest days. OP and HP plants were placed separately in two experimental plots

202 1.5 meter away from each other. Because of inter-individual variance in flowering  
203 phenology, the density of flowering plants changed across time (from 2 to 12 individuals per  
204 m<sup>2</sup>). OP females and males were spatially alternated following a grid pattern. Each week, the  
205 spatial position of individuals within each plot was randomized while keeping the male-  
206 female alternation.

### 207 *Plant traits*

208 A total of 18 traits were measured on all plants from the three experimental groups. A  
209 summary of the number of observations per trait and experimental group is available in  
210 appendix (Table S3).

211 **Flowering phenology** - Flowering phenology was described with two traits: the date of first  
212 flowering and flowering duration. Since plants were surveyed once a week, flowering  
213 duration is the number of weeks during which at least one flower was open for a given  
214 individual.

215 **Number of gametes per flower** - In females, the number of ovules per flower was estimated  
216 at the end of the survey, by dissecting a subsample of fruits per plant. Fruits were collected  
217 at maturity, typically two to three weeks after flower opening. At the end of the flowering  
218 season, 1004 fruits (one fifth of total fruit production) were randomly chosen and dissected to  
219 estimate ovule and seed production for each female (6 to 44 fruit per female, with the  
220 number reflecting the females' contribution to the overall fruit production at the population  
221 level). Unfertilized ovules and seeds were counted by imaging the fruit content using a high  
222 resolution scanner (Epson Perfection V700 Photo, Seiko Epson, Suwa, Japan) and using

223 digital image processing (*EImage* package in R; Pau et al. 2010). Unfertilized ovules and  
224 seeds were distinguished on the basis of size ( $0.062 \pm 0.031 \text{ mm}^2$  for unfertilized ovules  
225 versus  $0.86 \pm 0.12 \text{ mm}^2$  for seeds). The number of unfertilized ovules and seeds were then  
226 summed within each fruit and averaged over all fruits imaged for each OP and HP female,  
227 yielding an estimate of female gamete production per flower.

228 In males, the number of viable pollen grains per flower was assessed on one nearly open bud  
229 at the flowering peak. This measurement involved multiplying the proportion of viable  
230 pollen grains by the pollen quantity per flower. To do so, each bud was dissected to keep  
231 three of the ten anthers. Pollen was removed from the first one and placed on a glass slide  
232 with one drop of staining solution to assess the proportion of viable pollen grains under x100  
233 microscope (Peterson et al. 2010). The two remaining anthers were used to estimate pollen  
234 quantity per flower using a particle counter (CASY® Model TT, Roche Innovatis AG,  
235 Bielefeld, Germany) and following the protocol described in Dufayé et al. (2008).

236 **Floral traits** - The remaining 15 traits fall in three categories: (i) non-destructive  
237 measurements, performed weekly, (ii) measurements involving the removal and dissection  
238 of a flower that were only conducted once, at the flowering peak and (iii) measurements  
239 performed at the end of the flowering season.

240 *Weekly measurements* - Daily flower display, stem number (i.e. number of branches with at  
241 least one open flower), height of the highest open flower (cm), as well as corolla width (mm)  
242 and calyx height (mm, measured on two randomly chosen flowers using a digital calliper  
243 precise to 0.01 mm and averaged), were surveyed once a week. All the weekly measured  
244 traits showed higher variation among than within individuals, as well as a Gaussian

245 distributions across repeated measures, and were thus summarized using an individual  
246 mean.

247 *Measurements at the flowering peak* - On the evening before measurements, all mature  
248 flower buds were marked using watercolor paint, and individuals were isolated under an  
249 insect-proof tent in order to exclude nectar feeding pollinators. The next day, one newly  
250 opened flower per individual was randomly selected among the marked ones and collected.  
251 On this flower, we estimated the total volume of nectar using 2 $\mu$ L microcapillary tubes.  
252 Nectar sugar content ( $\mu$ g of sucrose equivalent per flower) was then determined using a  
253 refractometer (Abbe NAR-1T LIQUID, Atago Co., Ltd., Tokyo, Japan). On the same flower,  
254 one freshly dissected petal was used to measure reflected light on average every 0.6 nm  
255 within 200-700 nm using a probe connected to an AvaLight-XE ULS2048 spectrometer and to  
256 an AvaLight-XE xenon pulsed light source (Avantes, Apeldoorn, the Netherlands). Spectra  
257 were calibrated against a fully absorbing black standard and a fully reflecting barium sulfate  
258 white standard. Two measures were performed for each individual, one in the center of each  
259 petal lobe (*i.e.* on both sides of the axis of symmetry of the petal). Averaged reflectance  
260 spectra were analyzed using the *pavo* package in R (Maia et al. 2019). Using *Apis mellifera*  
261 visual systems, which should be representative of bumblebee's perceptions (Peitsch et al.  
262 1992) we extracted cartesian X-Y coordinates from the polygon color space, resulting in two  
263 traits for petal color. Finally, to characterize petal size, area and shape, another petal was  
264 dissected on each collected flower, taped flat and imaged using a high resolution scanner  
265 (Epson Perfection V700 Photo, Seiko Epson, Suwa, Japan). Using ImageJ (Schneider et al.  
266 2012), we then measured petal length from the paracorolla to the apical part (mm), largest  
267 width (mm), cleft length (mm) and petal area (mm<sup>2</sup>). Two indexes of petal shape were then

268 calculated: elongation (i.e. length divided by width) and bifidity (i.e. cleft length divided by  
269 petal length).

270 *Measurements at the end of the flowering peak* - At the end of the flowering season, total  
271 flower production was estimated on both sexes by counting all pedicels attached on the  
272 stems.

### 273 *Reproductive and mating success*

274 **Females** – Seed number per fruit was estimated jointly with ovule number (see above).  
275 Female reproductive success was then estimated by multiplying mean seed number per fruit,  
276 total number of fruits and germination rate. Germination rates were estimated on a sub-  
277 sample of seeds (15 to 90 seeds per female according to its relative production) that were  
278 sown in petri dishes filled with 40mL of 10g/L agar in sterile water (photoperiod 14:10 and  
279 temperature 15-21°C).

280 **Males** – Reproductive success was estimated as the total number of seeds sired on a sample  
281 of genotyped seedlings, which was determined using paternity analyses. Genotyped  
282 seedlings were selected so as to reflect each female's contribution to the total seed pool (for  
283 any given female, the proportion of genotyped seeds relative to the total number of  
284 genotyped seeds is the same as the proportion of seeds produced relative to the total seed  
285 production at the population level) and were sampled across all fruits the female produced  
286 during the flowering season. On average, 23 seedlings per mother were genotyped, and this  
287 number ranged from a minimum of 6 to a maximum of 56 seedlings per mother.

288 Total genomic DNA of all adults (200 OP plants) and 2916 seedlings was extracted and  
289 single-step multiplex PCR assays were used to amplify five nuclear microsatellites (Text S1  
290 and Table S1). Paternity assignments were conducted using the maximum-likelihood  
291 procedure implemented in the software CERVUS v.3.0.7 (Marshall et al. 1998; Kalinowski et  
292 al. 2007). Likelihood scores, based on allele frequencies in the experimental population, were  
293 calculated for each seedling / potential father couple. To determine whether the paternity of  
294 each offspring could be assigned to the father with the highest likelihood, we used the  
295 difference in likelihood scores between the two most likely pollen donors ( $\Delta_{\text{LOD}}$ ). The critical  
296 value ( $\Delta_c$ ) of  $\Delta_{\text{LOD}}$  below which paternity could not be assigned at 80% was determined using  
297 a distribution of  $\Delta$  obtained from 10 000 simulated pollination events. This distribution was  
298 generated using the following simulation parameters: no unsampled pollen donors and 2%  
299 of genotyping error. The reproductive success of each male was estimated as the number of  
300 genotyped seedlings fathered. Three males did not sire any seeds in the sample of genotyped  
301 offspring. We also used the results from the paternity analysis to estimate mating success of  
302 each OP female and male as the number of genetic mating partners per individual.

### 303 *Statistical analyses*

304 The dataset with the 18 floral traits and reproductive and mating success for each  
305 experimental group is available on Dryad (<https://doi.org/10.5061/dryad.j6q573nh1>).  
306 Statistical analyses are organized as follows. First, we evaluated the correlations between all  
307 pairs of traits, and then we selected a subsample of traits covering different functional  
308 categories and weakly correlated with each other to estimate natural and sexual selection  
309 gradients. All linear models verified (i) normal distribution of residuals, (ii) homoscedasticity



310 and (iii) low levels of multicollinearity (i.e. variance inflation factor smaller than 4;  
311 Thompson et al. 2017).

312 All analyses were conducted in R (R Development Core Team 2019).

313 **Trait variation** – Within each of the three experimental groups (HP females and OP females  
314 and males), correlations between all 18 measured traits were investigated to explore the  
315 phenotypic architecture and to identify trade-offs between traits. Correlations were  
316 estimated using the Pearson correlation coefficient and significance was assessed using a *t*-  
317 test with Bonferroni correction. Additionally, phenotypic variation between groups was  
318 tested for all floral traits using Likelihood Ratio Tests (LRT) to assess group effect, in order to  
319 (i) verify trait homogeneity between OP and HP females and (ii) assess the occurrence of  
320 sexual dimorphism between OP females and males.

321 **Standardization** – Prior to estimating selection gradients, all floral traits were centered-  
322 scaled within groups to allow comparison by removing dimension (i.e. variance(SD)-  
323 standardized selection gradient; Lande and Arnold 1983; Matsumura et al. 2012). Female and  
324 male reproductive success were relativized within groups by dividing the absolute  
325 reproductive success for each individual by group mean. Mating success was estimated in  
326 OP females and males only and were relativized within sex.

327 **Natural selection gradients** – Cohort and source forest were included as random effects in  
328 the following models and their effect was tested using LRT. Because source forest had weak  
329 effects ( $P > 0.05$ ), this factor was not kept in final models.

330 The strength and direction of selection was estimated following Lande and Arnold (1983)  
331 using multiple regression analysis with relative reproductive success (i.e. produced / sired

332 seeds) as dependent variable and standardized trait values as independent variables. The  
333 data set was simplified by reducing the number of traits because (i) our statistical power was  
334 limited due to sample size and (ii) multicollinearity is known to produce unstable selection  
335 gradient estimates (Thompson et al. 2017). We selected six traits in order to keep a data set  
336 with limited correlations and to cover different functional categories: one trait linked with  
337 phenology (flowering duration, i.e. the number of weeks the individual produced flowers),  
338 one trait that is presumably involved in long distance attraction (plant height, which has  
339 been found to positively affect pollinator attraction in other systems; Zu and Schiestl 2017;  
340 Brunet et al. 2015), two flower morphology traits (corolla width and calyx height , which are  
341 expected to impact both attraction and various aspects of plant-pollinator fit, see for instance;  
342 Glaetli and Barrett 2008), and two descriptors of plant fertility (number of gametes per  
343 flower and number of flowers produced over the whole flowering season). 64 HP females, 98  
344 OP females and 100 males were measured on all six traits. Of these, three males and one HP  
345 female were removed from the analyses because they did not sire / produce any seeds.

346 Multiple linear regressions of reproductive success on the six selected floral traits were  
347 performed (i) group by group, (ii) on a dataset grouping HP and OP females, allowing us to  
348 test for the occurrence of pollinator-mediated selection and (iii) on a dataset grouping OP  
349 females and males, allowing us to test for sex-specific selection. Significance of selection  
350 gradients was assessed using t-tests for group by group models, and LRT were used to test  
351 the effect of the group  $\times$  trait interaction. Since multicollinearity precluded us from fitting all  
352 traits in the same analysis, we also estimated selection differentials on each character one by  
353 one. Differentials correspond to the slope of simple regressions of reproductive or mating  
354 success on each trait taken separately. It thus covers both direct and indirect selection (i.e.

355 selection indirectly acting on a trait due to the correlation between this trait and another one  
356 that would be the direct target of selection, see Figure S1).

357 **Sexual selection gradients and indexes** – We estimated sexual selection gradients (i.e. direct  
358 sexual selection), by regressing relative mating success (i.e. number of genetic sexual  
359 partners) in OP females and males on the six standardized traits, using the same method as  
360 for natural selection gradients.

361 Additionally, we used Bateman's three metrics to quantify the overall strength of sexual  
362 selection in each sex. First, we estimated the standardized variance in reproductive success,  
363 which corresponds to the opportunity for selection  $I$  (Wade 1979). Second, we estimated the  
364 standardized variance in mating success in each sex, which corresponds to the opportunity  
365 for sexual selection  $I_S$  (Wade 1979; Wade and Arnold 1980). We tested for differences in  
366 reproductive and mating success variances between sexes using Levene tests. Third, we  
367 estimated a standardized partial Bateman gradient in each sex, by assessing the slope of the  
368 linear regression of relative reproductive success on relative mating success, while  
369 controlling for total flower number and mean number of gametes per flower (Henshaw et al.  
370 2018).

371 To estimate Bateman gradients, we used two alternative sampling strategies for estimating  
372 female mating success. In a first set of analyses, we used the same estimate of male and  
373 female mating success as before, based on the genotyping of a number of seedlings per  
374 female that would reflect each female's contribution to the total seed pool (hereafter pro-rata  
375 method). Whereas this method has the advantage of not heavily truncating the variance in  
376 female MS (see below), it can however create an artificial relationship between mating and

377 reproductive success for females (Anthes et al. 2016). To estimate Bateman gradients without  
378 this possible bias, we produced a second estimate of mating success, by using a  
379 bootstrapping procedure where we sampled ten genotyped offspring for each female 1000  
380 times (hereafter fixed method). The possible disadvantage of this method is that it sets an  
381 upper limit of ten reproductive partners per female. Male mating success was directly  
382 deduced from female MS and averaged over the bootstrapping procedure. Finally, to take  
383 into account that the variance in female RS can translate into a variance in male RS, we  
384 calculated individual RS of each male  $p$  ( $RS_{m,p}$ ) as the sum of its contribution to each female  $i$   
385  $\Pi_p(\chi_i)$  weighted by individual RS of each female ( $RS_{f,i}$ ), as follows:

$$386 \quad RS_{m,p} = \sum_{i=1}^I \Pi_p(\chi_i) \times RS_{f,i}$$

387 For each sampling method, we then tested for significance of the Bateman's gradient in each  
388 sex using t-tests. We also tested for the differences in the Bateman gradient between sexes by  
389 using LRT to test for mating success  $\times$  sex interaction.

390 **Pollen limitation** – The occurrence of pollen limitation was tested on three components of  
391 overall female reproductive success by assessing differences between OP and HP females in  
392 (i) seed-set (i.e. seed number divided by ovule number over all dissected fruits), (ii) fruit-set  
393 (i.e. total fruit number divided by total flower number) and (iii) total number of seeds.  
394 Differences in seed-set and fruit-set between OP and HP females were assessed using  
395 generalized linear models with binomial distribution and an individual random effect for  
396 seed-set as several fruits were sampled per individual. Differences in total seed production  
397 were assessed by using a generalized linear model with negative binomial distribution to

398 account for overdispersion (package glmmTMB; Brooks et al. 2017). LRT were used to test  
399 the effect of pollination treatment.

400

## 401 **Results**

### 402 *Trait variation*

403 **Correlations between traits in open-pollinated plants** - Within each sex, correlations  
404 between traits were explored for all possible pairs (153 pairs). Overall, males appeared to be  
405 more phenotypically integrated than females (32.0% versus 13.7% of all correlations were  
406 significant after Bonferroni correction, Figure 1 and Table S2). In females, significant  
407 correlations were mostly positive (22 out of 26 significant correlations), while nearly half of  
408 them were negative in males (23 out of 49 significant correlations). Some obvious correlations  
409 were observed in both males and females, such as positive correlations between various  
410 measurements of flower size (corolla width, calyx height, petal length, width and area) or  
411 positive correlations between stem number, flower number (both daily and total) and height  
412 (Figure 1 and Table S2). The most notable differences between males and females included (i)  
413 a positive correlation between estimates of flower number and flowering duration in males  
414 only, (ii) a trade-off between flower size and total flower number, as well as between flower  
415 size and flowering duration in males only, (iii) a negative correlation between the number of  
416 gametes per flower and total flower number in females and (iv) a negative correlation  
417 between viable pollen production per flower and flowering duration in males.

418 **Homogeneity between open-pollinated and hand-pollinated females** - We detected no  
419 significant differences between the two groups of females, except for three traits. HP females  
420 displayed fewer flowers per day ( $\chi^2_{1,159}=4.56, P=.033$ ), fewer gametes per flower (  
421  $\chi^2_{1,159}=4.73, P=.030$ ) and shorter calyxes ( $\chi^2_{1,159}=3.91, P=.048$ ) than OP females.

422 **Sexual dimorphism** – All measured traits were sexually dimorphic, except petal color as  
423 perceived by bees (Table 1). Males produced more flowers, both daily and total, flowered  
424 longer, were taller and produced larger flowers, both in terms of corolla width and calyx  
425 height, than females. The same was true for all petal measurements. Males also had more  
426 elongated and bifid petals. The only trait showing female-biased sexual dimorphism was  
427 nectar sugar content, with females offering significantly more reward per flower than males.  
428 However, because of the difference in flower production between sexes, nectar reward at the  
429 plant level was still overwhelmingly more abundant in males than in females.

### 430 *Natural selection gradients*

431 In females, natural selection gradients were similar in HP and OP plants: in both groups, we  
432 detected a significantly positive relation between reproductive success and two traits linked  
433 with fertility, total flower number and number of gametes per flower (Table 2). Accordingly,  
434 we did not observe any pollinator-mediated selection (i.e. none of the group  $\times$  trait  
435 interactions were significant; Table 2). Regarding selection differentials, we detected  
436 significant total selection on the same traits as well as on traits that were strongly correlated  
437 with them (Figure S1 and Table S2).

438 In males we detected significant selection for larger corolla width and longer flowering  
439 duration (Table 2). As in females, the patterns detected using selection differentials mirrored  
440 those observed with selection gradients (Figure S1)

441 When fitting both sexes in the same model, the selection gradient for flowering duration was  
442 stronger in OP males than in OP females (i.e. significant effect of the sex  $\times$  trait interaction;  
443 Table 2 and Figure S2). On the contrary, selection on total flower number and number of  
444 gametes per flower was stronger in females than in males. Although selection on corolla  
445 width was detected in males but not in females, the sex  $\times$  trait interaction was only  
446 marginally significant (Table 2 and Figure S2).

#### 447 *Sexual selection gradients*

448 We detected sexual selection for more gametes per flower and more flowers in OP females,  
449 along with positive sexual selection on both corolla width and flowering duration in OP  
450 males (Table 3). The sex  $\times$  group interaction was significant for flowering duration, as well as  
451 for flower number and number of gametes per flower (Table 3 and Figure S3).

#### 452 *Sexual selection indexes*

453 The opportunity for selection I (i.e. standardized variance in reproductive success), as well as  
454 the opportunity for sexual selection  $I_s$  (i.e. standardized variance in mating success), were  
455 significantly stronger for OP males than for OP females (0.410 versus 0.168 and 0.286 versus  
456 0.111, respectively). Levene's test on the variances in reproductive and mating success  
457 showed significantly stronger variance in males. In OP males, the partial Bateman gradient

458 was significantly positive for both methods (pro-rata method:  $1.08 \text{ SE} \pm 0.055$ ,  $P < .001$ ; fixed  
459 method:  $1.05 \text{ SE} \pm 0.036$ ,  $P < .001$ ). In OP females, it was marginally positive for the fixed  
460 number method ( $0.45 \text{ SE} \pm 0.24$ ,  $P = .067$ ) but significantly positive for the pro-rata method  
461 ( $0.72 \text{ SE} \pm 0.084$ ,  $P < .001$ ). LRT showed that Bateman's gradients were significantly different  
462 between sexes for both methods (pro-rata method:  $\chi^2_{1,183} = 8.56$ ,  $P < .01$ ; fixed method:  
463  $\chi^2_{1,183} = 7.24$ ,  $P < .01$ ; Figure 2), with reproductive success being more correlated with mating  
464 success in males than in females.

### 465 *Pollen limitation*

466 None of the components of female reproductive success indicated pollen limitation (GLMs,  
467 seed-set:  $\chi^2_{1,2676} = 2.67$ ,  $P = .10$ ; fruit-set:  $\chi^2_{1,159} = 0.074$ ,  $P = .79$ ; total production of viable  
468 seeds:  $\chi^2_{1,159} = 1.35$ ,  $P = .25$ ). OP females produced an average of 29.4 (SD  $\pm 11.8$ ) fruits and  
469 4762.6 (SD  $\pm 1835.2$ ) seeds, and HP females produced 26.3 (SD  $\pm 9.6$ ) fruits and 4336.1 (SD  $\pm$   
470 1502.2) seeds.

## 471 **Discussion**

472 Our study aimed at quantifying the strength of sexual and natural selection on an array of  
473 traits in both males and females of a dioecious plant, in which sexual selection is expected to  
474 partly occur through the action of pollinating insects. Most traits investigated in the current  
475 work displayed male-biased sexual dimorphism. Similar results have been reported in some  
476 dioecious plant species, and this has sometimes been interpreted as the possible outcome of  
477 sexual selection (Delph 1999; Eckhart 1999; Ashman 2009). Indeed, the expression of more  
478 conspicuous phenotypes in males may result from stronger selection on traits that increase



479 access to mating partners through pollinator attraction (Moore and Pannell 2011; Barrett and  
480 Hough 2012), an expected difference between sexes when pollen is not a limiting factor for  
481 female reproductive success. In *Silene dioica*, at least one previous study performed in  
482 natural populations found no evidence for pollen limitation (Carlsson-Granér et al. 1998) and  
483 this was also the case in our experimental garden. Consistent with our expectations, we  
484 found that: (i) selection acts on traits associated with fertility in females and on traits  
485 associated with mate acquisition in males and (ii) reproductive and mating success are more  
486 variable and show a stronger correlation in males than in females.

#### 487 *Patterns of selection on floral traits*

488 ***Fertility selection acts on flower and ovule number in females*** - Selection differentials and  
489 gradients for total flower number and number of gametes per flower indicated a positive  
490 effect of these traits on female fitness. Selection for more flowers through female function is a  
491 common pattern in flowering plants (Harder and Johnson 2009; Caruso et al. 2019) and ovule  
492 number per flower was also found to be under positive selection in the sister species *Silene*  
493 *latifolia* (Delph and Herlihy 2012).

494 Comparing selection patterns between OP and HP females allows inference of the nature  
495 of selection on these two traits. Indeed, flower number could affect both pollinator attraction  
496 or gamete production at the individual level, and could thus be subjected to both pollinator-  
497 mediated and fertility selection. In our experimental garden, seed production was not pollen  
498 limited, and selection was found to act on the same traits, and with the same intensity in the  
499 two groups. In other words, no pollinator-mediated selection was detected and the

500 significant selection on total flower number and gamete number could be entirely attributed  
501 to the variance in fertility among females, rather than variance in the ability to attract  
502 pollinators. This pattern is however expected to vary widely with ecological conditions.  
503 While individual density in our experimental population was within the range of values  
504 observed *in natura* (Barbot et al. pers. obs., Carlsson-Granér et al. 1998), pollinator  
505 availability is expected to vary among sites, in particular in the current context of pollinator  
506 decline (Biesmeijer 2006; Potts et al. 2010). Three fifths of published studies quantifying  
507 pollen limitation in angiosperms report significant increases in female reproductive output in  
508 hand-pollinated plants (Burd 1994) and there is evidence that the intensity of selection on  
509 attractive traits increases with the magnitude of pollen limitation (Ashman & Morgan 2004;  
510 Sletvold & Ågren 2016).

511 Because competition to attract pollinators is one possible component of sexual selection, but  
512 not the only one (for instance, sexual selection can also occur through post-pollination  
513 processes), comparing the results obtained with pollen supplementations to those obtained  
514 with sexual selection gradients should provide some insight on whether and how pollinator  
515 mediated selection and sexual selection overlap. We also detected positive sexual selection  
516 gradients on both flower number and ovule number. In that case, this link between traits and  
517 mating success cannot be attributed to pollinator-mediated selection. In other words, females  
518 with low values of mating success did not suffer from a shortage of pollen. Two factors could  
519 explain this discrepancy between pollinator-mediated and sexual selection patterns. First, we  
520 cannot exclude the action of post-pollination sexual selection, receiving gametes from more  
521 diverse partners could promote fecundation and zygote development, for example by  
522 enhancing male-male competition (Aizen and Harder 2007; Pannell and Labouche 2013).

523 Second, a part of this result is likely to emerge from the mechanistic relationship between the  
524 total number of available ovules and the number of reproductive partners. This constraint  
525 motivated our use of partial Bateman gradients (see below).

526 Finally, as mentioned earlier, this study provides a picture of sexual selection patterns in  
527 females in a non pollen-limited situation. Setting-up similar studies in other situations and/or  
528 species will allow covering the wide diversity of ecological situations encountered by insect  
529 pollinated plants. In particular, this should allow to investigate whether pollinator-mediated  
530 and sexual selection are detected on the same traits when pollen becomes limiting.

531 ***Sexual selection acts on corolla width and flowering duration in males*** – In males, patterns  
532 of natural selection were consistent with those of sexual selection, based on both selection  
533 differentials and gradients. Contrary to what was seen in females, we detected no association  
534 between the male mating success and components of fertility (i.e. total flower number and  
535 gamete number). Moreover, traits that appeared beneficial in terms of mating success were  
536 also likely to increase the access of males to their female sexual partners. First, the number of  
537 sexual partners increased with corolla width, a trait that has been found to increase  
538 pollinator attraction in several other species (Ashman and Stanton 1991; Stanton et al. 1991;  
539 Conner and Rush 1996). In some species, corolla width was also shown to affect flower-  
540 pollinator fit and impact the efficiency of pollen removal (Conner and Rush 1996; Ashman  
541 and Diefenderfer 2001). It thus seems likely that sexual selection on this trait arises from  
542 some pollinator-mediated selection, although this cannot be directly demonstrated in males.  
543 Second, flowering phenology played a major role in access to reproduction in males. While  
544 the strong phenotypic correlation between flowering start and flowering duration prevented

545 us of disentangling the effect of these two traits, males that started early (and thus flowered  
546 longer) clearly accessed more partners and reached higher siring success than males starting  
547 later on in the flowering season. This set of traits increased the flowering overlap with  
548 females, a trend that was already documented in *Silene latifolia* by Delph and Herlihy (2012).  
549 The advantage of an early flowering start in terms of siring success has been reported in  
550 several species (O'Connell and Johnston 1998; Munguía-Rosas et al. 2011; Forrest 2014;  
551 Austen and Weis 2016). Here, the possible overlap between pollinator-mediated and sexual  
552 selection is less clear. Selection for earlier (and thus longer) flowering may reflect the fidelity  
553 of pollinators to individuals on which they had originally been foraging or, since this pattern  
554 was also documented in a wind-pollinated species (De Cauwer et al. 2012), the fact that  
555 males that flower early have larger floral displays when females start flowering, giving them  
556 an edge over competitors starting later during the flowering season.

557 Contrary to females, we found no trace of selection acting on flower number in males. This is  
558 an unexpected result, given the common role of flower number in pollinator attraction in  
559 many plant species (Conner and Rush 1996; Vaughton and Ramsey 1998), and given the  
560 positive impact of flower number on the overall pollen production at the plant level. Flower  
561 number has historically been considered as a typical 'male trait', based on the fact that many  
562 hermaphroditic species exhibit more flowers than the number of fruits they produce, leading  
563 to the idea that these excess flowers would have a male, rather than a female function  
564 (reviewed in Burd & Callahan 2000). This view has however been challenged by several  
565 experimental studies that found no or even negative selection through male function on this  
566 trait (Elle and Meagher 2000; Hodgins and Barrett 2008; Lau et al. 2008; van Kleunen and  
567 Burczyk 2008).

568 In *Silene dioica*, the absence of selection could reflect that flower number is, for the observed  
569 range of trait values, more or less neutral for male fitness. Moquet et al. (2022) showed that  
570 one major factor explaining why males attract much more pollinators than females is their  
571 larger floral displays. However, another study showed that artificially increasing the number  
572 of flowers increased pollinator attraction in both sexes without impacting plant reproductive  
573 success (Barbot et al. 2022). One possibility is thus that the relationship between this trait and  
574 male fitness is a saturating one: after a certain threshold producing more flowers does not  
575 result in any increase in male fitness. In addition, Barbot et al. (2022) found significant  
576 selection on flower number in males, when surveying reproductive success over a period of  
577 10 days, suggesting that at this short time scale flower number may indeed increase male  
578 reproductive success. When considering the whole flowering season, as we did in the current  
579 work, this effect no longer holds and flowering phenology appeared to be the most  
580 impacting trait for male fitness.

### 581 *Estimates of sexual selection*

582 In addition to testing the dependency of mating success on floral phenotypes, sexual  
583 selection was also quantified using several indexes that are typically used in animals (Klug et  
584 al. 2010; Janicke et al. 2016). We found a greater opportunity for sexual selection in males,  
585 consistent with the results recently reported in the wind-pollinated *Mercurialis annua*  
586 (Tonnabel et al. 2019) and insect-pollinated *Sagittaria latifolia* (Kwok and Dorken 2022).  
587 Regarding the Bateman gradients, when mating success was estimated based on the pro-rata  
588 sampling method, we detected a significant positive relation between mating and  
589 reproductive success in females. This result is at least partly explained by the sampling

590 method (i.e. we sampled more seed in females with high reproductive success). When we  
591 correct for this possible bias using the fixed sampling method, the slope was not significant.  
592 In both cases we found a significantly steeper Bateman gradient for males than for females, as  
593 predicted by theory (Arnold 1994a), and as found in three plant species (Johnson and Shaw  
594 2017; Tonnabel et al. 2019; Kwok and Dorken 2022) . Again, a stimulating perspective of this  
595 work will be to replicate similar experiments in situations where pollen availability limits  
596 female reproductive success, in order to test whether these male-female differences in sexual  
597 selection decrease in less favorable situations.

### 598 *Sexual dimorphism evolution*

599 Interestingly, the selection patterns detected in our study are only partly consistent with the  
600 sexual dimorphism observed in our model species. On the one hand, our findings of males  
601 having wider corollas and flowering for a longer period of time than females are consistent  
602 with sexual selection acting on these traits in male plants. On the other hand, the stronger  
603 selection on flower number observed in females appears contradictory with the much higher  
604 number of flowers displayed by males in this species. In males, and as mentioned previously,  
605 maybe flower number is not under selection because this trait does not increase pollen export  
606 at least in the range of values that we observe. In females, the fact that positive selection on  
607 flower number does not translate into evolution towards larger floral display may be that,  
608 due to higher costs of reproduction, females are subjected to stronger trade-offs between  
609 components of their fitness. For instance, although not quantified in our study, a trade-off  
610 between flower number and survival could maintain the marked sexual dimorphism for this

611 traits even in the face of a strong selection for larger number of flowers in females (Obeso  
612 2002).

### 613 *Conclusion*

614 Overall, our findings show that some predictions from sexual selection theory corpus seem  
615 to apply well to plants when pollen is not limiting. However, it seems crucial to explicitly  
616 consider the specificity of plant reproduction when estimating mating success as mate  
617 diversity and interpreting its relationship with reproductive success. First, the fact that each  
618 individual typically carries several independent reproductive tracts (i.e. flowers), opens the  
619 question of the transferability of classical sexual selection metrics from animals to plants.  
620 Because a high genetic mating success may be an indirect byproduct of fertility in females,  
621 one should be cautious when interpreting sexual selection gradients. From the male  
622 perspective, one may also question whether genetic mating success accurately reflects access  
623 to reproduction in plants. Indeed, multiple pollen dispersal events to several flowers carried  
624 by the same female plant can constitute an efficient access to reproduction that would  
625 translate in a low genetic mating success. The strong positive Bateman gradient documented  
626 on male *S. dioica* yet suggests that this metrics may satisfactorily capture access to female  
627 mates in our study system. The strong correlation between male reproductive and mating  
628 successes probably stems in part from the observed variance in flowering duration: long-  
629 flowering males appear to have maximized their access to reproduction through a greater  
630 overlap with flowering females. Studying sex-specific selection on attractive traits through  
631 another angle, that of sexual partners acquisition, thus seems to constitute a promising  
632 avenue.

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886 **Table 1:** Trait means  $\pm$  SD in open-pollinated females and males.

Traits	OP females	OP males	LRT	$P_{sex}$
Stem number (mean)	1.09 $\pm$ 0.45	5.81 $\pm$ 3.07	$\chi_{1,197} = 193.76$	< .001
Height (cm, max)	47.03 $\pm$ 14.68	53.31 $\pm$ 12.86	$\chi_{1,197} = 13.07$	< .001
Corolla width (mm, mean)	17.93 $\pm$ 2.65	20.05 $\pm$ 1.87	$\chi_{1,197} = 39.62$	< .001
Calyx height (mm, mean)	13.44 $\pm$ 1.01	15.66 $\pm$ 1.01	$\chi_{1,197} = 160.18$	< .001
Nectar sugar content ( $\mu$ g sucrose/flower)	0.71 $\pm$ 0.57	0.46 $\pm$ 0.3	$\chi_{1,162} = 12.71$	< .001
Bees X-coordinate	0.10 $\pm$ 0.02	0.095 $\pm$ 0.02	$\chi_{1,162} = 0.87$	.35
Bees Y-coordinate	0.145 $\pm$ 0.02	0.143 $\pm$ 0.02	$\chi_{1,162} = 0.19$	.66
Petal length (mm)	8.81 $\pm$ 1.24	9.23 $\pm$ 1.21	$\chi_{1,168} = 6.36$	.012
Petal width (mm)	9.02 $\pm$ 1.38	9.94 $\pm$ 1.51	$\chi_{1,168} = 18.08$	< .001
Petal cleft length (mm)	4.1 $\pm$ 0.67	4.71 $\pm$ 1.13	$\chi_{1,168} = 17.34$	< .001
Petal area (mm <sup>2</sup> )	56.72 $\pm$ 15.4	63.52 $\pm$ 15.85	$\chi_{1,168} = 9.39$	< .01
Petal shape (elongation)	0.98 $\pm$ 0.1	0.94 $\pm$ 0.11	$\chi_{1,168} = 8.21$	< .01
Petal shape (bifidity)	0.47 $\pm$ 0.06	0.51 $\pm$ 0.09	$\chi_{1,168} = 9.31$	< .01
Total number of flowers	30.32 $\pm$ 11.84	316.32 $\pm$ 150	$\chi_{1,197} = 224.71$	< 0.001
Daily flower display (mean)	2.51 $\pm$ 1.12	18.59 $\pm$ 8.52	$\chi_{1,197} = 240.54$	< 0.001
Number of gametes per flower	257.71 $\pm$ 46.02	37152.45 $\pm$ 17472.81	$\chi_{1,195} = 229.49$	< 0.001
Flowering start	4.77 $\pm$ 1.79	5.16 $\pm$ 1.64	$\chi_{1,197} = 6.04$	<b>0.014</b>
Flowering duration (number of weeks)	4.3 $\pm$ 1.47	6.79 $\pm$ 1.56	$\chi_{1,197} = 104.29$	< 0.001

887 Note: Statistics from LRT are reported as well as associated  $P$ -values ( $P_{sex}$ ) for differences888 between OP females and OP males. Values in bold are significant at  $P < .05$ .



889 **Table 2:** Natural selection gradients estimates  $\pm$  SE on variance-standardized traits for hand-pollinated females, open-pollinated females and  
 890 males.

	$\beta$ HP females	<i>P</i>	$\beta$ OP females	<i>P</i>	$\beta$ OP males	<i>P</i>	$\gamma_{pol}$	<i>P</i> <sub>pol</sub>	$\gamma_{sex}$	<i>P</i> <sub>sex</sub>
	$R_M^2=0.268$	56 d.f	$R_M^2=0.157$	90 d.f	$R_M^2=0.242$	92 d.f	$R_M^2=0.412$		$R_M^2=0.372$	
Height (mean)	-0.083 $\pm$ 0.053	.12	0.008 $\pm$ 0.036	.81	0.007 $\pm$ 0.073	.92	$\chi_{1,147}^2=0.01$	.93	$\chi_{1,183}^2=0.15$	.70
Corolla width (mean)	0.004 $\pm$ 0.037	.22	-0.001 $\pm$ 0.99	.99	0.13 $\pm$ 0.058	<b>.027</b>	$\chi_{1,147}^2=0.52$	.52	$\chi_{1,183}^2=3.72$	.054
Calyx height (mean)	-0.056 $\pm$ 0.037	.14	-0.034 $\pm$ 0.037	.36	-0.059 $\pm$ 0.055	.28	$\chi_{1,147}^2=0.33$	.57	$\chi_{1,183}^2=0.30$	.59
Total number of flowers	0.23 $\pm$ 0.044	<b>&lt; 0.001</b>	0.26 $\pm$ 0.036	<b>&lt; .001</b>	-0.078 $\pm$ 0.066	.24	$\chi_{1,147}^2=0.02$	.90	$\chi_{1,183}^2=21.87$	<b>&lt;.001</b>
Number of gametes per flower	0.16 $\pm$ 0.038	<b>&lt; 0.001</b>	0.22 $\pm$ 0.035	<b>&lt;.001</b>	0.023 $\pm$ 0.052	.65	$\chi_{1,147}^2=1.07$	.30	$\chi_{1,183}^2=9.08$	<b>&lt;.001</b>
Flowering duration	-0.045 $\pm$ 0.040	.27	0.029 $\pm$ 0.036	.42	0.35 $\pm$ 0.064	<b>&lt;.001</b>	$\chi_{1,147}^2=1.17$	.28	$\chi_{1,183}^2=20.70$	<b>&lt; .001</b>

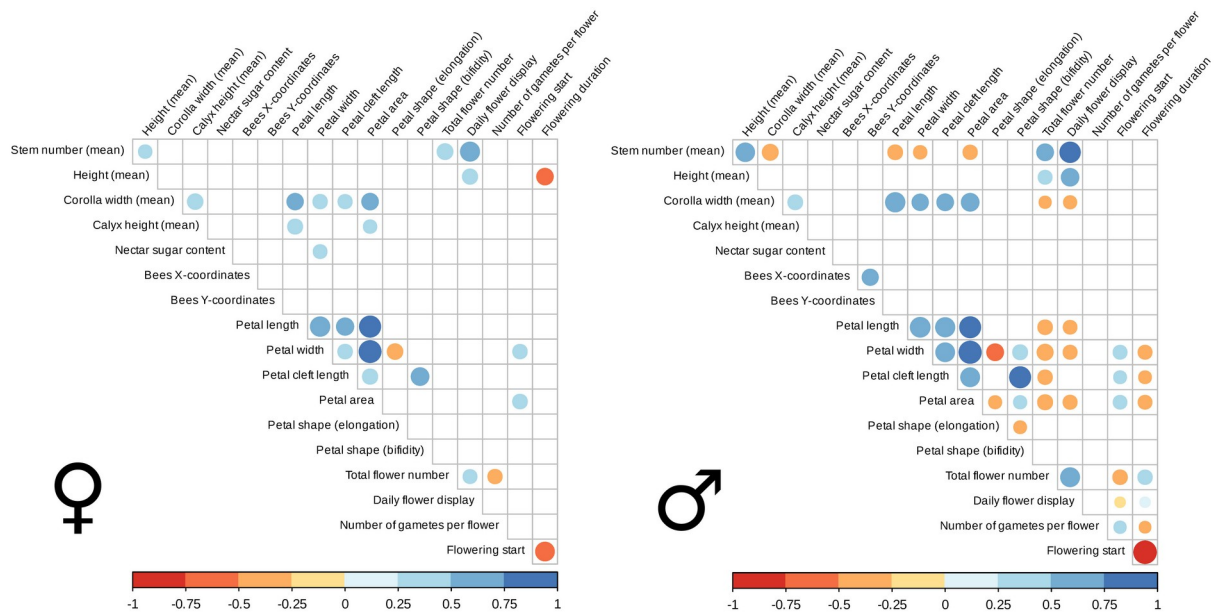
891 Note: Degrees of freedom (d.f.) and *P*-values from t-tests are reported for the significance of sexual selection gradients. Statistics from LRT  
 892 are reported for interaction terms : (i) between pollination group and trait  $\gamma_{pol}$  (OP versus HP females) to investigate pollinator-mediated  
 893 selection and (ii) between sex and trait  $\gamma_{sex}$  (OP females versus OP males) to investigate sex-specific selection. The proportion of variance  
 894 explained by fixed effects in each model is reported using marginal R-squared (  $R_M^2$  ). Values in bold are significant at  $P < .05$ .

895 **Table 3:** Sexual selection gradients estimates  $\pm$  SE on variance-standardized traits for open-pollinated females and males.

	$\beta_{MS}$ OP females	<i>P</i>	$\beta_{MS}$ OP males	<i>P</i>	$\gamma_{sex}$	$P_{sex}$
	$R_M^2=0.280$	90 d.f	$R_M^2=0.201$	92 d.f	$R_M^2=0.353$	
Height (mean)	-0.019 $\pm$ 0.033	.55	-0.068 $\pm$ 0.060	.26	$\chi_{1,183}=0.22$	.64
Corolla width (mean)	0.011 $\pm$ 0.034	.74	0.12 $\pm$ 0.048	<b>.011</b>	$\chi_{1,183}=3.75$	.053
Calyx height (mean)	-0.009 $\pm$ 0.034	.78	-0.041 $\pm$ 0.045	.36	$\chi_{1,183}=0.34$	.56
Total number of flowers	0.18 $\pm$ 0.033	<b>&lt;.001</b>	-0.062 $\pm$ 0.054	.25	$\chi_{1,183}=15.70$	<b>&lt;.001</b>
Number of gametes per flower	0.12 $\pm$ 0.032	<b>&lt;.001</b>	0.010 $\pm$ 0.042	.81	$\chi_{1,183}=4.15$	<b>.042</b>
Flowering duration	0.031 $\pm$ 0.033	.35	0.26 $\pm$ 0.053	<b>&lt;.001</b>	$\chi_{1,183}=14.19$	<b>&lt;.001</b>

896 Note: Degrees of freedom (d.f.) and *P*-values from t-tests are reported for the significance of sexual selection gradients. Statistics from LRT are reported for  
897  $\gamma_{sex}$  the interaction between sex  $\gamma_{sex}$  (OP females versus OP males) and trait, highlighting sex-specific sexual selection patterns. The proportion of variance  
898 explained by fixed effects in each model is reported using marginal R-squared ( $R_M^2$ ). Values in bold are significant at  $P < .05$ .

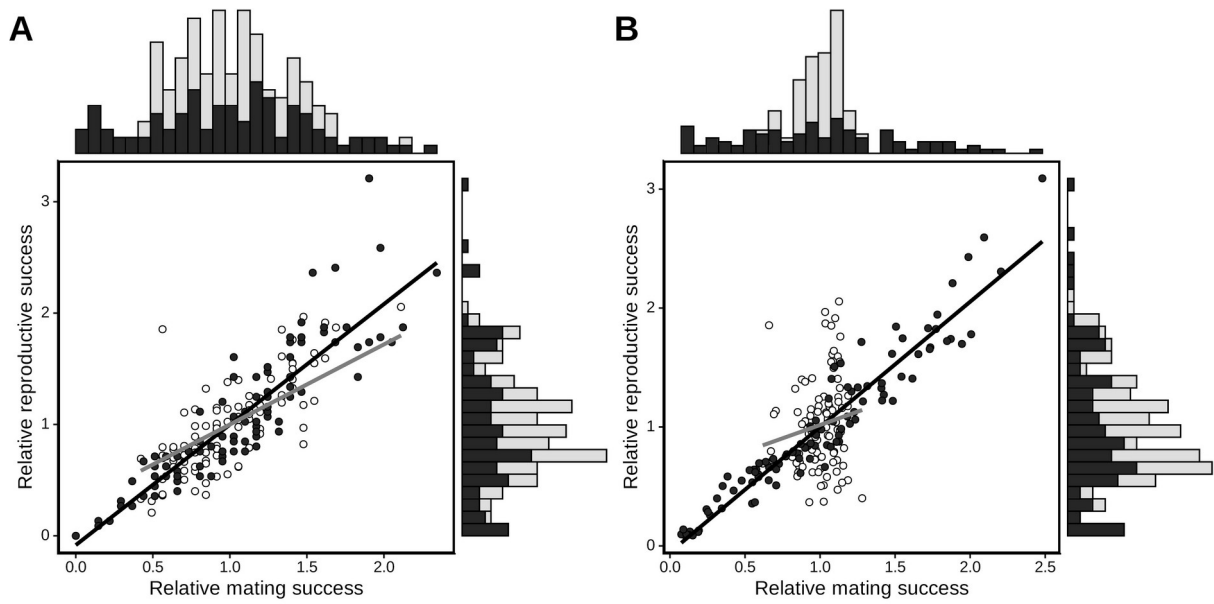
899 **Figures**



901 **Figure 1:** Correlations between traits for OP females (left) and OP males (right). Correlation  
 902 coefficients that were statistically significant after Bonferroni correction are colored, with  
 903 positive correlations in blue and negative correlations in red. The size of the dot is  
 904 proportional to the correlation coefficient value. Note that shape indexes were calculated as  
 905 follows: petal length divided by width for elongation and cleft length divided by petal length  
 906 for bifidity. Exact values for the coefficients are available in supplementary materials (Table  
 907 S2).

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909



910 **Figure 2:** Bateman's gradients (i.e. relation between reproductive and mating success)  
 911 according to the sampling method (A) pro-rata method and (B) fixed method. The  
 912 distributions of reproductive success and mating success in both sexes are represented  
 913 respectively along the right and top margins (females in white and males in black). Females  
 914 are represented with gray lines and empty circles and OP males are represented with black  
 915 lines and filled circles.

916