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# On the function of flower number: disentangling fertility from pollinator-mediated selection

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## Abstract

In animal-pollinated angiosperms, the "male-function" hypothesis claims that male reproductive success should benefit from large floral displays, through pollinator attraction, while female reproductive success is expected to be mainly limited by resource availability. As appealing as this theory might be, studies comparing selection strength on flower number in both sexes rarely document the expected asymmetry. This discrepancy could arise because flower number impacts both pollinator attraction and overall gamete number. In this study, we artificially manipulate floral displays to disentangle the fertility versus pollinator attraction components of selection, both in terms of mating and reproductive success. In females, flower number was under strong fertility selection, as predicted in the absence of pollen limitation. In contrast, in males, flower number was mainly under sexual selection, which in turn increased male reproductive success. However, these selection patterns were not different in males with artificially increased floral displays. This suggests that sexual selection acting on flower number in males do not occur because flower number increases pollinator attraction, but rather because more pollen is available to disperse on more mates. Our study illustrates the power of disentangling various components of selection with potentially sex-specific effects for understanding the evolution of sexual dimorphism.



**Keywords:** sexual selection – pollinator attraction – gametes – sexual dimorphism – "male-function" hypothesis – phenotypic manipulation

## Introduction

Darwin himself introduced the notion of sexual selection, advocating that certain traits are selected because they enhance individual fitness through mating success, i.e. the number of sexual partners [1]. According to theory, anisogamy should result in male reproductive success being more limited by gamete acquisition (e.g. through mate acquisition) than female reproductive success [2–5]. Across the animal kingdom, this statement is widely supported by two lines of evidence. First, empirical studies measuring sex-specific Bateman's gradients, the slope of the regression of reproductive success on mating success, generally demonstrate this relation to be steeper in males than in females [6]. Second, male biased sexual dimorphism for armament and ornament is pervasive in animal taxa, particularly in reptiles, birds and mammals, suggesting stronger sexual selection on male versus female phenotypes [7,8].

The scope for such sex differences in the dependence of reproductive success on access to sexual partners has long been suggested to include all sexually reproducing anisogamous organisms, thus including plants [2,8]. In animals, during the pre-copulation phase of reproduction, sexual selection can involve (i) competition for the access to sexual partners (usually among males) and/or (ii) choice of sexual partners (usually by females [4]). In plants, during the pre-pollination phase of reproduction when pollen is dispersed from anthers to stigmas, sexual selection can operate through male intrasexual competition to access receptive stigmas [9]. Sexual selection

51 can also take the form of female intrasexual competition to receive viable pollen in
52 the common case of pollen limitation [10,11]. One might thus expect between-sex
53 differences in the intensity of sexual selection to decrease with increasing pollen
54 limitation, as observed in broadcast spawning animals when male gametes become
55 limiting [12].

56 Yet, the pre-pollination phase of plants strongly differs from sexual selection in
57 animals given that it involves a third party – the pollinators – in a vast majority of
58 angiosperm species [13]. Sexual selection mediated by pollinators may thus drive the
59 evolution of phenotypic traits linked with pollinator attraction, with obvious
60 parallels with mate attraction in animals [9,14]. In this respect, a wide range of floral
61 traits has been demonstrated to be involved in pollinator attraction, such as nectar
62 quantity [15], flower size [16,17] or flower number (reviewed in [18]). In dioecious
63 plants, i.e. species with separate sexes, sexual dimorphism in floral traits is generally
64 thought to have evolved through differential sexual selection between sexes [9,19].

65 In dioecious species, males generally carry more flowers than females, at least in
66 temperate regions, suggesting stronger directional selection on this trait in males
67 than in females [20–22]. Similarly, in hermaphroditic species, flower number has
68 been historically considered as a typical 'male' trait, as earlier work in plants argued
69 that flower number, beyond a certain threshold, served the purpose of the male
70 function only [23,24]. Indeed, hermaphroditic plants often display fruit-sets lower
71 than one (i.e. produce many flowers that do not mature into fruits), even in optimal

pollination conditions [25,26]. The rationale for this "male-function" hypothesis is based on the common observation that flower number affects pollen donation more than seed production [23,24,27,28]. One process behind this involves pollinator attraction : large number of flowers often increase the number of pollinators visits [17,18,29,30]. Thus, if pollen donation depends more on the number of pollinators visits compared to ovule fertilization, large floral displays would mainly benefit male fitness after a certain threshold [23]. Yet, empirical support for the male-function hypothesis remains ambiguous [31]. Indeed, several studies quantifying selection on flower number through both sexual functions in hermaphroditic species found either (i) weaker selection on flower number through male function compared to female function [32,33], (ii) no selection through the male function [34] or even (iii) negative selection through the male function [35,36]. Quantifying the relative effect of flower number through different components of selection in each sex may help understand the current discrepancy between verbal expectations and empirical data.

Selection on flower number may arise through two distinct but simultaneous selective processes. First, flower number can be under sexual selection if individuals with larger display benefit from increased reproductive success through enhanced pollinator attraction (i.e. pollinator-mediated sexual selection), ultimately acquiring a higher number of sexual partners. Second, flower number can be under fertility selection if individuals with larger displays benefit from higher gamete production at the plant level. Comparing the strength of selection between arrays of hand-

93 pollinated and open-pollinated plants has allowed to partition the effect of
94 pollinator-mediated and fertility selection on flower number in a number of study
95 systems [37–40]. One important caveat of this approach, however, is that it focuses on
96 female fitness exclusively, precluding any explicit test of the male-function
97 hypothesis [31]. A powerful method to alleviate this issue is the use of phenotypic
98 manipulation [41]. In animals, experiments manipulating traits thought to be
99 involved in mate acquisition has proven to be an effective method in sexual selection
100 studies [42–45]. While phenotypic manipulation has allowed to explain the evolution
101 of various floral traits through pollinator-mediated selection [46–48], this approach
102 has not yet been applied to explicitly test the male-function hypothesis.

103 In this study, we performed phenotypic manipulation in order to test whether flower
104 number benefitted more the male rather than the female reproductive success,
105 through its impact on pollinator attraction. By adding dummy flowers on half of the
106 individuals in an experimental population of the dioecious species *Silene dioica*, at
107 the population flowering peak, we amplified the visual signal displayed by some
108 plants without changing their gamete production. Such approach allows
109 disentangling pollinator-mediated from fertility components of selection on flower
110 number with a common methodology for both sexes. Within each sex, two
111 components of individual fitness will be considered: (i) the number of offspring
112 produced, referred to as reproductive success hereafter, which corresponds to the
113 number of seeds for females, and to an estimate of the number of sired offspring for

114 males, obtained through paternity analysis and (ii) the number of acquired mates,
115 referred to as mating success hereafter, which corresponds to the number of genetic
116 mates for both sexes. Because female reproduction is supposed to be limited mainly
117 by resource availability for ovule production and maturation, at least when
118 pollinators are not a limiting factor, we predict that neither female reproductive
119 success, nor its relationship with the number of real flowers, should be impacted by
120 our treatment (control vs. enhanced visual signal). We however hypothesize that
121 female mating success can be affected by our treatment if the addition of dummy
122 flowers enhances pollinator attraction and mate acquisition. In males, we predict that
123 enhancing the visual signal to pollinators should increase both mating and
124 reproductive success, and possibly strengthen the relationship between the number
125 of real flowers and mating/reproductive success assuming that the benefit of
126 displaying a showier signal increases with the number of gametes produced at the
127 plant level.

128 ## Material and methods

129 ### *Study system*

130 *Silene dioica* (L.) Clairv. is a short-lived perennial herb of the Caryophyllaceae
131 family. This dioecious plant is widespread throughout northern and central Europe
132 [49,50]. Sex determination is genetic, with males being the heterogametic sex [51,52].
133 The species is sexually dimorphic for flower number and size, with males being more

conspicuous than females [22,53]. *Silene dioica* is insect-pollinated and displays a generalist pollination system with bees and hoverflies as main pollinators [49,54].

## *Plant material and experimental design*

Plant material was generated using seeds collected in three forests from the same region where the experiment was conducted (Lille University in France, 50°36'27.9''N 3°08'36.3''E), in order to reach a sufficient sample size while avoiding the inclusion of half or full-sibs. All individuals were sown in a greenhouse and placed in separate 0.7-L pots filled with a standard soil mixture.

An array of 172 plants with 1:1 sex-ratio was kept in an insect-proof greenhouse until the beginning of the experiment. The surface area of the experimental plot was of 50 $m^2$ and local density was 3.3 individuals.$m^{-2}$, mirroring typical densities observed in nature. No wild populations of *Silene dioica* were growing in the vicinity. At the flowering peak (31th May 2019), plants were randomly assigned to our two treatments and placed in the experimental garden for ten days with individual spatial positions arranged to follow a grid pattern where sexes and treatments were alternated.

In the control group (C), flower number was left unmanipulated. It included 43 females (FC) and 43 males (MC). In the treatment group (T), which also included 43 females (FT) and 43 males (MT), the visual signal was amplified by adding enough dummy flowers to double the flower number of each individual (i.e. different

numbers of dummy flowers were thus used depending on individual flower production). Dummy flowers were created using crepe paper which was selected based on its color spectra to approach average *S. dioica* petals' reflectance, and circles of 3.5cm diameter were cut in the paper with the closest spectra and rolled to roughly mimic *S. dioica*'s flower shape and size (Figure S1). These dummy flowers were then tied on plants using an invisible nylon thread.

In addition to these open-pollinated plants, a group of 20 females (FHP) were placed in the same experimental garden over the same period and artificially hand-pollinated to quantify pollen limitation of female reproductive success. All open flowers were supplemented every other day with pollen from a pool of males from the same initial collection, isolated in a greenhouse. Each FHP female received pollen from an average of 20 different males over the course of the experiment, with two males being used for each pollination session for each FHP female. After ten days, all females (FT, FC and FHP) were placed in an insect-proof greenhouse until fruits reached maturity and could be collected, typically two to three weeks after flower opening.

### *Pollinator observations*

In order to verify our expectation that dummy flowers increased pollinator attraction, pollinator visitation patterns were reported and compared between treatments. We conducted observations on groups of ten neighbouring plants

through three observational sessions of 20 minutes spread over the course of the experiment. Accordingly, each plant was observed for a total of sixty minutes. For each pollinator entering the group under scrutiny, the identity of the visited plants and the number of visits per plant were noted. A visit was defined as a clear contact between the insect and the reproductive structures of a flower. For each plant, three variables were derived from these observations (Figure 1): (i) whether a plant was visited or not over the course of the sixty minutes, defined hereafter as visitation, (ii) the number of visiting pollinators (i.e. the number of independent insects visiting a given plant), and (iii) the total number of visits (i.e. the total number of contacts between any pollinator and the reproductive structures of a given plant). Note that we did not observe any contact between pollinators and a fake flowers.

### *Trait measurement*

**Floral traits –** A total of twelve floral traits were measured on all plants. Three traits were measured three times during the course of the experiment and averaged across dates: (1) the number of open flowers, (2) corolla width and (3) calyx height. The nine remaining traits were measured once, just before the beginning of the experiment: (4) nectar volume, (5) sugar concentration, (6) petal colour luminance, (7) petal colour saturation, (8) petal reflectance in UV wavelengths, (9) petal length, (10) petal largest width, (11) petal area and (12) petal cleft length. Details about floral trait measurements are available in appendix (Text S1; Figure S4).

**Gamete production -** The number of gametes per flower was assessed for both sexes. For females, ovule number was assessed by imaging the contents of a random sample of fruits. For males, pollen quantity was estimated on two anthers using a particle counter. Details about estimation of gamete production in each sex are available in appendix (Text S1).

### *Female reproductive success*

Seed production was assessed on half of the mature fruits (one to six fruits per plant) using image processing, as described in Text S1. Germination rates were estimated on a sub-sample of 60 seeds per female that were sown in petri dishes filled with 40mL of 10g/L agar in sterile water (photoperiod 14:10 and temperature 21-15°C) and surveyed during 15 days. For each female, reproductive success over the course of the experiment was then obtained by multiplying three terms: total number of fruits, mean seed number per fruit, and germination rate.

### *Male reproductive success*

Male reproductive success was estimated by genotyping a subsample of offspring. The number of seedlings genotyped for each female reflected its contribution to the total seed pool (23.6 ± 11.4 seeds genotyped per female). Within each female, seedlings were randomly sampled across fruits. Total genomic DNA from adults (86 females and 86 males) and seedlings (2030 individuals) was extracted, and PCR assays were used to amplify five nuclear microsatellites (Table S1, Text S2). Using

genotypes and individual spatial data, we then used a spatially explicit mixed mating model (MEMM model, [55]) to assess variation in male reproductive success and in pollen dispersal kernels, as described in Text S3.

### *Estimation of mating success*

We performed categorical paternity assignments using CERVUS 3.07, allowing for a 2% genotyping error and using an 80% confidence criterion [56,57]. To assess the number of effective mating partners for each female and male (i.e. genetic mating success), we used a bootstrapping procedure that sampled 8 seeds per mother 1000 times. Fixing the number of offspring per mother allows avoiding the establishment of an artificially positive correlation between reproductive and mating success that would solely reflect variation in seed production across females [58]. The mean number of effective mating partners by individual across bootstraps was used in the analysis. All data (pollinator visits, floral traits, male and female reproductive and mating success, as well as pollen dispersal distances) is available on Dryad (doi:10.5061/dryad.0rxwdbs4d).

### *Statistical analysis*

**Phenotypic homogeneity between groups –** Prior to all analyses, we removed one male from the treatment with manipulated flower number (MT) because it produced over one standard-deviation more flowers than the second most-floriferous male individual. One female was also removed from dataset because it did not produce

any seeds (FC). For each sex, a multivariate analysis of variance (MANOVA) was then performed to ascertain that values for the thirteen measured traits (twelve floral traits and gamete number, see above) were homogeneous within sex and among treatments, using Wilks' Lambda statistic.

**Assessing pollen limitation in the experimental plot** – The occurrence of pollen limitation was tested by assessing whether the treatment (control FC, manipulated FT or hand-pollinated FHP) affected seed set. Seed set was calculated for each dissected fruit as seed number divided by ovule number. Comparisons were performed using a generalized linear model with a binomial distribution. A random individual effect was added because several fruits were sampled for each female (*lme4* package, [59]), and a likelihood ratio test was used to test the effect of pollination treatment, by comparing models without vs. with pollination treatment as a fixed effect.

**Testing the effect of phenotypic manipulation on pollinator visits, pollen dispersal distances, reproductive success and mating success –** In the following models, we included as fixed effects (1) the first axis of a PCA on all morphological floral traits, (2) the number of real flowers, and (3) gamete number per flower. The latter two were explicitly included in models as fixed effects to account for fertility variation between plants while the former described the general floral phenotype of individuals. PCAs were performed on males and females separately. The first axis accounted for 27.6% of floral traits' variance in females and 29.8% in males. We only

retained the first axis of the PCA on basis of the broken-stick method ([60]; Figure S2). In both sexes, this first axis mainly represents flower size variation (Figure S2).

***Pollinator visits*** – Three groups of generalized linear models were built to explain pollinator attraction, one for each estimate (visitation, number of pollinators visits, total visits). For each model, likelihood ratio tests were used to test the significance of the following fixed effects: (i) the number of real flowers, (ii) the first axis of PCA defined above, (iii) treatment, (iv) sex and (v) the interaction effect between treatment and sex, by comparing the likelihood of models including or not those effects. Because pollen production can also play a role in pollinator attraction [61], additional models testing for the effect of gamete production and its interaction with sex were also built. Visitation was modelled using a GLM with a binomial distribution. For models explaining either the number of pollinators or the total number of visits, GLMs with negative binomial distributions were retained based on their greater AIC values compared to hurdle or zero-inflated models (i.e. accounting for overdispersion and/or excess of zeros).

***Pollen dispersal distance*** **–** Variation in mean pollen dispersal distances among males was assessed using multiple linear regressions including the following explanatory variables: (i) the number of real flowers, (ii) the number of gametes per flower and (iii) the first axis of PCA defined above. Separate analyses were first performed on control males (MC) and on manipulated males (MT). Then, a second analysis on both groups of males was performed to test differences between groups.

276 T-tests were conducted on estimates to assess their significance in separate models,
277 and likelihood ratio tests were used to test the effect of interactions between
278 treatment and each of the three explanatory variables in the complete model. Prior
279 weights were set up to take into account uncertainty of a posteriori individual mean
280 pollen dispersal distance estimated using the MEMM model (Text S3).

281 ***Patterns of selection on flower number*** – We used structural equation modelling
282 (SEM, [62]) which allows to jointly assess the direct and indirect effects of floral traits
283 (number of real flowers, gamete number and first PCA axis) on both mating and
284 reproductive success. Specifically, we evaluated how floral traits affected mating
285 success (Figure 2) while testing the relationship between mating success and
286 reproductive success (i.e. indirect effect of floral traits on reproductive success
287 through mating success; Figure 2). We also evaluated the direct relationship between
288 floral traits and reproductive success, independently of an indirect effect through
289 mating success (Figure 2). This was done on two separate datasets, one for each sex,
290 using *piecewiseSEM* package in R [63]. We then performed a multigroup analysis
291 using local estimation of the parameters for each sex, which is carried out in two
292 steps. The first step provides statistics for each treatment × variable interactions using
293 type III ANOVA to assess which selection patterns were similar between groups (i.e.
294 treatment × variable interaction effects that are not significant). The second step
295 provides correlation estimates, and significant treatment × variable interaction are

296 free to vary between each group. Estimates were then plotted on the network using
297 *semPlot* package in R [64].

298 Another aim of the study was to evaluate if enhanced pollinator attraction through
299 phenotypic manipulation resulted in higher mating success (MS) and/or
300 reproductive success (RS). To that aim and in addition to the SEM analyses, we
301 tested for a main effect of the treatment on MS and/or RS, which cannot be achieved
302 using SEMs. We thus built multiple linear models, predicting either reproductive or
303 mating success, again considering males and females separately. Apart from the
304 treatment variable, we tested for the same independent variables than those included
305 in the SEM analyses, i.e. number of real flowers, number of gametes per flower and
306 the first PCA axis. In models predicting reproductive success, mating success was
307 added as an additional explanatory variable. Significance of estimates were assessed
308 using T-tests for separate models, and likelihood ratio tests were used to test effects
309 of (i) treatment as main effect, (ii) treatment × trait interactions, as well as (iii)
310 treatment × mating success interaction in reproductive success models. Uncertainties
311 in the estimation of male reproductive success were again accounted for by
312 modelling prior weights using a posteriori distributions of the MEMM model. The
313 analyses were performed using as response variables either relative reproductive or
314 mating success (i.e. estimates were divided by the mean), and used standardized trait
315 values as explanatory variables (i.e. traits were centred and scaled), following
316 standard recommendations [65,66]. Relativization and standardization were

317 performed per sex and across treatments, since the methods used to estimate fitness
318 differed between sexes.

319 **Results**

320 *No evidence for pollen limitation*

321 We found no evidence of pollen limitation in our experimental garden. Hand-
322 pollinated females (FHP) did not show higher seed sets than open-pollinated females
323 (FC: 0.62 ± SE 0.012; FT: 0.64 ± SE 0.011; FHP: 0.59 ± SE 0.018; $\chi^2_{2,556}=1.45, P=.48$ ).

324 *Phenotypic manipulation increased pollinator visitation*

325 A total of 280 pollinators were observed, including Syrphidae (60.7% of visitors),
326 *Bombus* species (28.6%), Halictidae (6.8%) and Lepidopterae (3.9%). Pollinators were
327 more attracted by males than females as revealed by a significant sex effect for all
328 three descriptors of pollinator visitation patterns: visitation ( $\chi^2_{1,160}=14.68, P<.001$
329 ),number of pollinators ( $\chi^2_{1,160}=63.19, P<.001$ ) and total number of visits (
330 $\chi^2_{1,160}=92.36, P<.001$ ; Figure 1). MANOVAs on the thirteen measured traits did not
331 indicate phenotypic differences between treatments (females: $F_{15,67}=1.34, P=.20$ ;
332 males : $F_{15,62}=0.71, P=.77$ ), so any effect of treatment on visitation patterns should
333 be ascribable to the phenotypic manipulation. Results showed that dummy flowers
334 did not increase visitation ( $\chi^2_{1,160}=1.82, P=.17$ ), but increased both the number of
335 visiting pollinators and the total number of visits ( $\chi^2_{1,160}=6.04, P=.014$ and
336 $\chi^2_{1,160}=6.91, P<.01$ respectively; Figure 1). Although the effect of the treatment

appeared stronger in females compared to males, none of sex × treatment interactions were significant (visitation: $\chi^2_{1,160}=1.98, P=.16$; pollinator number: $\chi^2_{1,160}=2.43, P=.12$; total visits: $\chi^2_{1,160}=2.58, P=.11$). Pollinators were also attracted by higher numbers of real flowers (pollinator number: $\chi^2_{1,160}=25.37, P<.001$; total visits: $\chi^2_{1,160}=35.87, P<.001$), but this effect was weaker for visitations ($\chi^2_{1,160}=3.25, P=.71$). Finally, larger values along PC1 (representing larger flower sizes) did not increase visitation ($\chi^2_{1,160}=0.0021, P=.96$) nor the number of pollinators ($\chi^2_{1,160}=2.088, P=.15$) but increased the total number of visits ($\chi^2_{1,160}=6.44, P=.11$). When included in the models, gamete number had no effect on pollinator attraction, and did not modify the results described above.

### *Pollen dispersal distance was influenced by the number of real flowers*

Across treatments, average pollen dispersal distance was 1.50 meters (± 0.32 SD) within our experimental garden. Mean pollen dispersal distance did not differ between control (MC) and manipulated (MT) males ($\chi^2_{1,77}=0.44, P=.51$; Figure S3). Mean pollen dispersal distance increased with the number of real flowers in the manipulated group. This effect was weaker in the control group, although the effect of the interaction between the number of real flowers and treatment was not significant (Table 1). Neither the number of gametes per flower nor PC1 (representing variation in flower size) affected pollen dispersal distances in both groups.

357 *Selection patterns differed between sexes*

358 Results from SEM analyses and linear models were globally congruent. Estimates
359 and statistics from linear models are thus reported in supplementary (Table S2). SEM
360 analyses showed the same selection patterns in control and manipulated females
361 (Table 2). In both groups, we detected a significantly positive direct effect of the
362 number of real flowers on both reproductive and, to a lesser degree, mating success
363 (Table 2, Figure 2A), as well as a significant positive effect of the number of gametes
364 per flower on reproductive success. In both groups of females, MS and RS were not
365 significantly correlated. In the linear model, treatment did not increase reproductive
366 success (i.e. treatment as main effect was not significant; $\chi^2_{1,74}=1.23, P=.27$ ), but
367 significantly increased mating success ( $\chi^2_{1,76}=4.97, P=.26$ ).

368 In both control and manipulated males, the number of real flowers was significantly
369 positively correlated with plant mating success (Table 2; Figure 2B). Because the SEM
370 analyses also detected a strong correlation between MS and RS in males, this
371 suggests an indirect effect of real flower number on reproductive success. The only
372 difference detected between manipulated and control males concerned the
373 correlation between the number of gametes per flower and reproductive success:
374 whereas this correlation was marginally positive in manipulated plants, it was
375 negative in the control group. In the linear model, neither the average reproductive
376 nor mating success were affected by the treatment (respectively $\chi^2_{1,74}=0.87, P=.35$
377 and $\chi^2_{1,76}=0.10, P=.75$ ).

## Discussion

The current study aimed at exploring the male-function hypothesis [23,27], by measuring the relationship between flower number and fitness in both sexes, through its potential impact on pollinator attraction. Specifically, our aims were (i) to investigate the impact of flower production on reproductive and mating success in male and female plants of *S. dioica*, and (ii) to explore whether an experimental enhancement of the floral signal would mainly impact selection patterns in males, highlighting the role of flower number in pollinator attraction and pollen export. Adding dummy flowers indeed increased pollinator attraction, enabling us to test our predictions. As expected, we found that neither female reproductive success, nor its relationship with flower number were impacted by this enhanced attraction. We also found that this manipulation increased female mating success (in terms of mate acquisition), but with no detected impact on seed production. In males, contrarily to our hypotheses, we found no effect of enhanced attraction to pollinators on either reproductive or mating success. Whereas large flower numbers seem to be associated with a higher mating success, we found no difference in this selection pattern between control and manipulated males. The reach of these results for the historical discussion on the male function hypothesis are discussed below.

### *Pollination context*

Seed production was not statistically different between open- and hand-pollinated plants, suggesting that female reproductive success was not limited by pollen receipt in the experimental garden. In such conditions, according to sexual selection theory, one expects fitness of male plants to depend more on their ability to attract pollinators compared to that of females [9,10], provided that pollinator attraction is associated with the probability to reach sexual partners.

The strongest determinant of pollinator attraction was clearly sex (males received on average twelve-fold more visits than females), confirming previous observations in *S. dioica* [53,67]. It should be noted that this preference for male *S. dioica* plants is known to be at least partly driven by the high degree of sexual dimorphism for flower number [68]. This effect of flower number on pollinator attraction is confirmed in our study: adding dummy flowers resulted in an overall increase in the number of pollinators attracted, as well as in the total number of visits.

### *Female reproductive success was mainly driven by gamete production*

In females, the number of real flowers was shown to positively affect reproductive success, and this effect was similar in both manipulated and control plants. This observation, along with the fact that no pollen limitation was detected, suggests that the benefit for the female function of carrying more flowers is only linked to a higher ovule production at the plant level, and not to any effect on pollinator attraction. In other words, our results support the sole effect of fertility selection on the number of

417 flowers, in alignment with general expectations from sexual selection theory. Our
418 analysis also revealed positive selection on the number of ovules per flower, but no
419 selection on flower size (a trait often associated with pollinator attraction [16,17]).
420 Taken together, these findings support that, as long as the pollination context is
421 favourable, traits that increase female reproductive success are associated with
422 fertility, as found in several other plant species [37,39,69].

423 In our experiment, female mating success increased both when plants received more
424 pollinator visits (treatment effect) and when they carried more real flowers. These
425 two effects are likely to emerge from mechanistic aspects of pollen dispersal. On the
426 one hand, an increase in the number of independent pollinator visits is expected to
427 increase the diversity of pollen arriving on the stigmas. On the other hand, because
428 producing more flowers implies that more ovules are available for fertilization, an
429 increase in flower number should result in a parallel increase in the number of mates.
430 However, because female mating and reproductive success were uncorrelated, a
431 result equivalent to non-significant Bateman gradients found in females of many
432 animal species ([6] but see exceptions within) and in another plant species [70], the
433 detected positive effects on mating success did not translate in an increase in
434 reproductive success. In other words, females did not benefit from an increased seed
435 number because they accessed more sexual partners, confirming that only fertility
436 selection occurred in females of our experiment.

### *Males do not seem to benefit from enhanced pollinator attraction*

Contrary to our expectations, artificially enhanced floral display did no impact male reproductive success or patterns of selection on floral traits. The only difference between control and manipulated male plants concerned selection on the number of pollen grains per flower, with control plants experiencing negative selection on this trait, whereas manipulated plants seemed to experience positive selection. Although we could not find any satisfactory explanation for this difference between control and manipulated plants, this effect is unlikely to be driven by treatment differences in pollinator attraction, since the quantity of gamete per flower had no significant impact on pollinators visits in our analyses. While a negative trade-off between pollen quantity and quality could contribute to the observed change in selective patterns between control and manipulated males, our previous evaluation of pollen phenotypes suggests that this correlation is rather positive [22].

In both categories of males, the number of real flowers positively impacted mating success. In strong contrast with the patterns observed in females, however, mating and reproductive success were strongly associated, reflecting the positive Bateman gradient that is often observed in male animals [6] and in a couple of plant species [70,71]. Thus, although flower number did not have any detectable direct effect on male reproductive success, our results support an indirect effect through mating success: male plants with more (real) flowers tended to have a better access to mates, which in turn significantly increased the number of seeds they sired. This part of our

458 results thus seems consistent with the expectation of flower number being under
459 sexual selection in males. Whereas observational studies cannot assess whether such
460 selective pattern is mediated by an increased pollinator attraction or an increased
461 gamete production, our manipulation of floral display -that increased pollinator
462 attraction only- allowed us going further. Under the hypothesis of a male function
463 through pollinator attraction, we were expecting differences between manipulated
464 and control males. Our results did not fit our expectations. Manipulated males did
465 not benefit from increased mating or reproductive success, and they appeared to
466 experience the same selection patterns on real flower number compared to control
467 males. This suggests that the possible benefit of exhibiting a high number of flowers
468 in males is not mediated by pollinator attraction per se. One likely explanation for
469 our results is that producing many flowers results in larger pollen loads on
470 pollinators (e.g. through increased visit time, [72]) and, ultimately, in reaching a
471 larger number of females. The fact that the number of real flowers was positively
472 associated with longer dispersal distance in both control and manipulated males
473 indirectly supports this hypothesis. Pollen production at the plant level is a trait that
474 typically falls in the realm of what is sometimes referred to as "the grey zone" of
475 sexual selection [73], since it can both be argued to be mainly under fertility selection
476 or under sexual selection.

477 This study documented extremely restricted pollen dispersal distances (typical for
478 entomogamous species, [74-76]), which might weaken the opportunity for sexual

selection, since male-male competition would typically occur among directly neighbouring individuals. Nevertheless, our result support that males producing more flowers gain higher fitness at through enhanced access to females. It thus seemed that sexual selection played a part in the evolution of flower number in males of *Silene dioica*, whereas selection on the same trait in females was undoubtedly based on fertility selection. Nonetheless, this difference between males and females could not be attributable to a differential in the relationships between male vs. female fitness and pollinator attraction. Taken together, our results are thus not congruent with the male-function hypothesis, when interpreted as an advantage of producing many flowers to the male function through an increased capacity of attracting pollinators [22,26].

## Data accessibility

Data are available on Dryad: https://dx.doi.org/10.5061/dryad.0rxwdbs4d [77].

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# Tables

**Table 1:** Effect of floral traits on pollen dispersal distances and differences between groups.

| | Control males (MC) | *P* | Manipulated males (MT) | *P* | Treatment × trait | *P* |
|---|---|---|---|---|---|---|
| | $R^2_S = -0.014$ | | $R^2_S = 0.11$ | | $R^2_S = -0.048$ | |
| $\beta$ Number of real flowers | $0.0045 \pm 0.0038$ | .24 | $0.012 \pm 0.0045$ | **.014** | $\chi^2_{1,77} = 1.60$ | .21 |
| $\beta$ Gametes number per flower | $8.4 \times 10^{-7} \pm 1.7 \times 10^{-6}$ | .62 | $4.4 \times 10^{-7} \pm 1.8 \times 10^{-6}$ | .80 | $\chi^2_{1,77} = 0.029$ | .87 |
| $\beta$ PC1 | $0.016 \pm 0.024$ | .51 | $0.031 \pm 0.025$ | .22 | $\chi^2_{1,77} = 0.65$ | .65 |

Note: Estimates (± SE) from linear models are reported for each effect in both groups (MC and MT). are reported from likelihood ratio tests to assess the interaction between treatment and each trait. *P*-value in bold are significant. The proportion of variance explained by each model is reported using adjusted R-squared ( $R^2_S$ ).

728 **Table 2:** *F*-stats (type III ANOVA) for multigroup SEM analyses

| | | Females | *P* | Males | *P* |
|---|---|---|---|---|---|
| Reproductive success | Treatment × Mating success | $F_{1,77}=0.3$ | .25 | $F_{1,77}=12.4$ | .44 |
| | Treatment × Number of real flowers | $F_{1,77}=0.3$ | .52 | $F_{1,77}=12.4$ | .75 |
| | Treatment × Gametes number per flower | $F_{1,77}=0.3$ | .33 | $F_{1,77}=12.4$ | **.030** |
| | Treatment × PC1 | $F_{1,77}=0.3$ | .29 | $F_{1,77}=12.4$ | .50 |
| Mating success | Treatment × Number of real flowers | $F_{1,77}=0.3$ | .079 | $F_{1,79}=5.2$ | .50 |
| | Treatment × Gametes number per flower | $F_{1,77}=0.3$ | .86 | $F_{1,79}=5.2$ | .51 |
| | Treatment × PC1 | $F_{1,77}=0.7$ | .22 | $F_{1,79}=5.2$ | .99 |

729

730 **Figures**

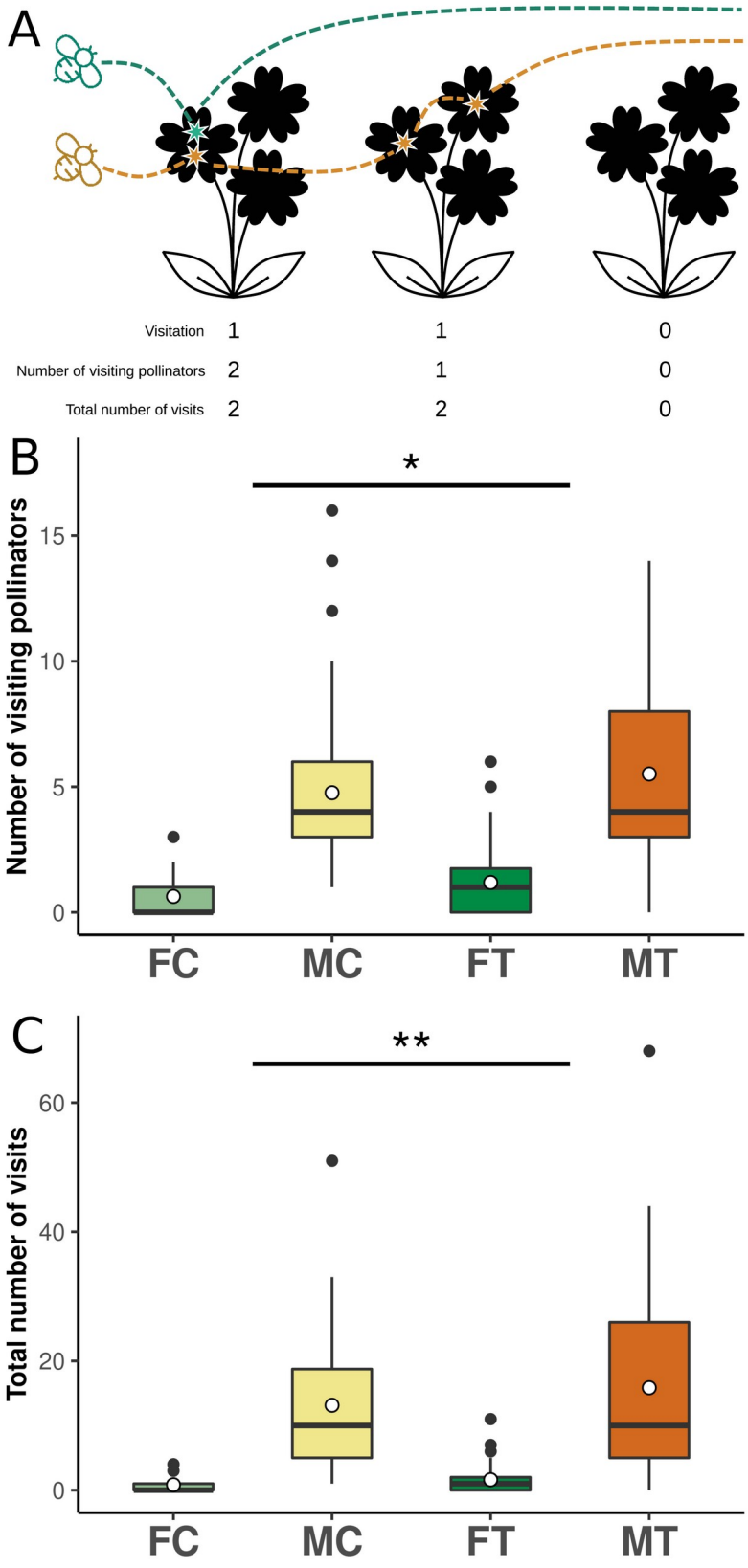
A
Visitation 1 1 0
Number of visiting pollinators 2 1 0
Total number of visits 2 2 0
B
*
Number of visiting pollinators
0
5
10
15
FC
MC
FT
MT
C
**
Total number of visits
0
20
40
60
FC
MC
FT
MT

**Figure 1:** Illustration of the three proxies used to characterized pollinator visits (A) : visitation, the number of visiting pollinators and the total number of visits. Boxplots representing the number of visiting pollinators (B) and the total number of visits (C), according to sex and treatment. P-values for the treatment effect are represented with * < 0.05 and ** < 0.01. Males (MC: yellow, MT: orange) were more visited than females (FC: light green, FT: dark green), and both sexes were more visited with the addition of dummy flowers. Means are represented with white points.

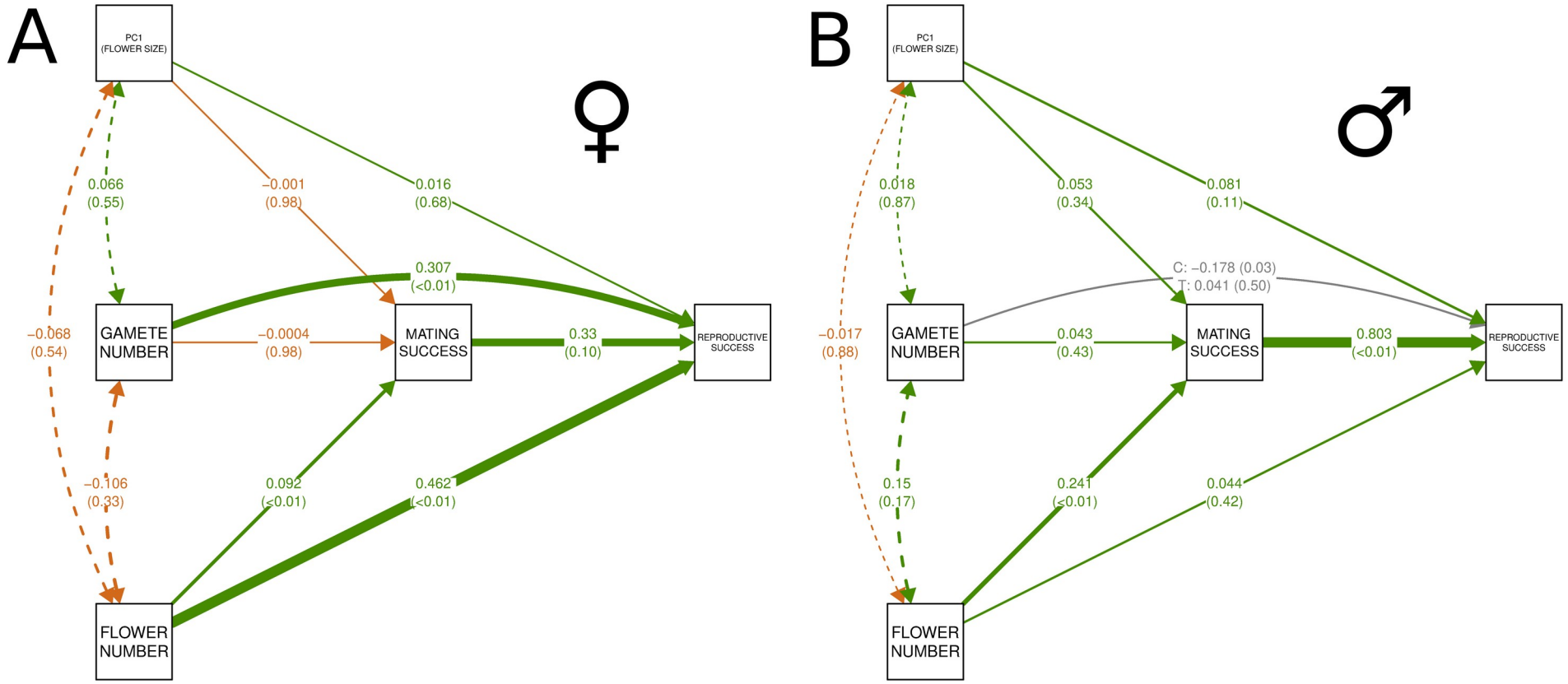
A
PC1
(FLOWER SIZE)
♀
0.066
(0.55)
−0.001
(0.98)
0.016
(0.68)
0.307
(<0.01)
−0.068
(0.54)
GAMETE
NUMBER
−0.0004
(0.98)
MATING
SUCCESS
0.33
(0.10)
REPRODUCTIVE
SUCCESS
−0.106
(0.33)
0.092
(<0.01)
0.462
(<0.01)
FLOWER
NUMBER
B
PC1
(FLOWER SIZE)
♂
0.018
(0.87)
0.053
(0.34)
0.081
(0.11)
C: −0.178 (0.03)
T: 0.041 (0.50)
−0.017
(0.88)
GAMETE
NUMBER
0.043
(0.43)
MATING
SUCCESS
0.803
(<0.01)
REPRODUCTIVE
SUCCESS
0.15
(0.17)
0.241
(<0.01)
0.044
(0.42)
FLOWER
NUMBER

**Figure 2:** Results from multigroup SEM analyses in females (A) and males (B). Each trait can have (I) a direct effet on MS and/or RS, as well as (ii) an indirect effect on RS, depending of the strenght of the relationship between RS and MS (i.e. Bateman gradient), through a direct effect on MS. Estimates for each correlations are plotted with associated *P*-values in brackets, with green arrows for positive values and orange arrows for negative values. Covariances between traits are represented with double headed dotted arrows. In the SEM analyses, only one correlation differed between control (C) and manipulated (T) group, with estimates plotted in grey for the correlation between gamete number per flower and reproductive success in males.