The effects of inbreeding, genetic dissimilarity and phenotype on male reproductive success in a dioecious plant

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Pollen fate can strongly affect the genetic structure of populations with restricted gene flow and significant inbreeding risk. We established an experimental population of inbred and outbred *Silene latifolia* plants to evaluate the effects of (i) inbreeding depression, (ii) phenotypic variation and (iii) relatedness between mates on male fitness under natural pollination. Paternity analysis revealed that outbred males sired significantly more offspring than inbred males. Independently of the effects of inbreeding, male fitness depended on several male traits, including a sexually dimorphic (flower number) and a gametophytic trait (*in vitro* pollen germination rate). In addition, full-sib matings were less frequent than randomly expected. Thus, inbreeding, phenotype and genetic dissimilarity simultaneously affect male fitness in this animal-pollinated plant. While inbreeding depression might threaten population persistence, the deficiency of effective matings between sibs and the higher fitness of outbred males will reduce its occurrence and counter genetic erosion.

Keywords: inbreeding; fitness; pollen; plant; relatedness

1. INTRODUCTION

During pollination, stigmas may receive pollen from multiple individuals [1−3] such that pollen tubes compete to fertilize ovules. This promotes the evolution of mechanisms for pollen selection in females that allow the sorting of compatible pollen and increase offspring number or quality, and for selection of male traits that increase attractiveness towards pollinators and pollen-competitive abilities [4−6]. As plants are sessile, the risk of inbreeding is high, especially when seeds disperse locally and pollination depends on insects, which tend to visit nearest neighbouring plants [7−9]. The negative effects of inbreeding on female function may be avoided through post-pollination mechanisms, such as genetic self-incompatibility, selection of pollen tubes before fertilization and/or selective abortion of seeds [10−13].

Similarly, when sires contribute to the phenotypes of their offspring for fitness-related traits (e.g. germination time, number and size of flowers [14,15]), pollen recipients may be selected to favour fertilization by pollen donors that provide genetic benefits. The benefits of both good genes and of compatible genes are fundamental to the evolution of pollen receipt and pollen/embryo selection mechanisms, as pollen recipients should favour fertilization both by sires of high genetic quality and/or genetically dissimilar sires [16−18], although extreme dissimilarity may be disadvantageous if it causes outbreeding depression [19,20]. Male reproductive success also depends on a combination of different factors, including pollen donor−recipient relatedness (either directly or through phenotypic resemblance, e.g. positive assortative mating for phenology [21]), and heritable traits affecting sporophytic vigour, pollen production and attractiveness to pollinators [22]. In addition, spatial effects, such as the number of recipient plants in close proximity and the dispersal pattern of pollen, may also affect the fecundity of competing males [23,24].

Late-acting inbreeding depression may also directly influence pollination and siring success. Such inbreeding depression can be considerable because of slightly deleterious alleles that are not easily purged [25]. Indeed, some studies found evidence for inbreeding depression (or hybrid vigour) on pollen traits such as pollen production, pollen viability, number or growth of pollen tubes, and siring success in controlled crosses [26−29]. Since pollen is haploid, dominance relationships as a genetic mechanism underlying inbreeding depression cannot directly affect the growth of the pollen tube. Nevertheless,

inbreeding depression on pollen expressed traits can result from genetic stress acting in the diploid parent during pollen formation [26].

Using controlled crosses, we have shown previously that inbreeding depression affects many vegetative and reproductive traits in the dioecious white campion, *Silene latifolia*, and that genetic dissimilarity between mates affected siring success in hand pollination competition with two donors [15]. However, to our knowledge there is no study that has assessed whether male reproductive success in plants is directly affected by the levels of inbreeding, in particular, under conditions of natural interaction with pollinators and mixed pollen loads [1]. In animals with multiple mating, sometimes the effects of inbreeding depression on siring success are only revealed under competition for fertilization [30,31], and few studies have investigated whether inbreeding directly affects male fitness (e.g. [18,32]).The investigation of inbreeding effects on male fitness remains relatively unexplored across taxonomic boundaries.

In this study, we address the determinants of male reproductive success under natural pollination in *S. latifolia*, using five previously unpublished microsatellite markers to assess paternity. By exposing inbred and outbred plants generated previously [15] to natural pollinators, we specifically ask whether siring success is affected by (i) levels of inbreeding in males, (ii) male–female relatedness, and (iii) male phenotypic traits, including sexually dimorphic and gametophytic traits.

The white campion is ideally suited to address these questions. First, in *S. latifolia*, biparental inbreeding is likely because of gravity-dispersed seeds and restricted pollen flow [33–35], while the species frequently occurs in metapopulations with small and isolated subpopulations [34]. Founder populations appear as a consequence of occasional long-distance seed dispersal and consist of sibships from few or single fruits. This creates a unit of plants sufficiently large to be visible to pollinators [36], but increases the risk of inbreeding [15,34]. However, it is unknown whether inbred *S. latifolia* males suffer reduced fecundity under natural pollination. Second, there is evidence that post-pollination pollen competition or embryo selection may reduce inbreeding by favouring unrelated males, and therefore reduce the risk of local deme extinction ('genetic rescue' [34,37,38]). This has been tested through hand pollinations where male–female relatedness affected the proportion of seeds sired by competing pollen donors [15], and also indirectly, as pollen flow was greater into experimental patches of full-sibs compared with patches of unrelated individuals [34]. However, an experimental design that combines natural pollination with variation in male–female relatedness and that is independent of inbreeding or allelic variation is required to demonstrate that genetic rescue occurs under natural pollination.

Moreover, *S. latifolia* is a challenging species in which to address phenotypic selection on male traits, being sexually dimorphic for several traits, such as flower number [39], which may be under sexually antagonistic selection. Males are likely to produce many flowers as a result of sexual selection to attract more pollinators [40], whereas fecundity selection may favour females with larger and hence fewer flowers because of a negative genetic correlation between flower size and number, and a positive genetic correlation between flower size and ovule number [39,41,42]. Positive selection on the number of flowers in males has been suggested, but never, to our knowledge, tested with direct methods. Finally, it has been shown that pollen germination is heritable in this species [43], suggesting that selection can also occur at the gametophytic phase. Therefore, in this study, we consider both the effects of sporophytic and gametophytic traits on fitness.

2. MATERIAL AND METHODS

(a) *Study species*

Silene latifolia (Poiret) (Caryophyllaceae) is a short-lived perennial, entomophilous species, native to Eurasia [44]. The species is dioecious with chromosomal sex determination [45]. Pollinators are mainly moths [46], including *Hadena bicruris* [47,48].

(b) *Structure of experimental population*

As described in Teixeira *et al.* [15], we conducted controlled crosses to generate inbred and outbred plants from seeds collected from fruits in a natural population in Village-Neuf (France, 47°36′25″ N; 7°33′31 E; 245 m a.s.l.). Twenty female plants were pollinated by a brother (from the same field-collected fruit as the female plant, i.e. a full- or half-brother) or by a male from a different field-collected fruit (36 males in total). In the field, each fruit was sampled from a different female, and female plants were at least 2 m apart. For simplicity, we refer to the latter treatment as a cross with an 'unrelated male', although the female and male, even when stemming from different maternal plants, may be related in some cases (e.g. as paternal half-sibs). A previous estimate of relatedness based on three microsatellite loci confirmed that females were significantly more closely related to brothers (mean relatedness $r = 0.45 \pm 0.38$) than to the 'unrelated' males ($r = -0.12 \pm 0.28$) [15]. This crossing design allowed us to remove confounding effects other than the level of inbreeding, such as possible genetic maternal effects, which was controlled by applying pollen of both types to different flowers on individual maternal plants, and/or environmental effects that could be removed by raising all the plants under controlled greenhouse conditions.

We sowed a random subset of 20 seeds from each cross in Jiffy peat pellets, and recorded the time from sowing to germination and the day when the first flower opened. Sixty days after germination, we measured stem length and placed a subsample of females and males arising from both outbred and inbred crossings in a common garden, so as to expose them to natural pollinators during June and July. The sex ratio was 2 : 1 (females : males) for a final sample of 342 plants exposed to pollinators (see the electronic supplementary material, table S1). This sex ratio is typical for natural *S. latifolia* populations [33] but since male plants produce on average 8–8.5 times more flowers than females [15], a female-biased sex ratio may still provide conditions of pollen competition (i.e. an excess of pollen compared with the number of ovules available for fertilization). The spatial arrangement was randomized for gender, inbreeding level and maternal seed family (see the electronic supplementary material, table S1). The experimental population was not isolated from natural populations of *S. latifolia*. We placed the pots at 75 cm inter-plant distances on a mown, flat area. In natural populations, plants can occur at distances
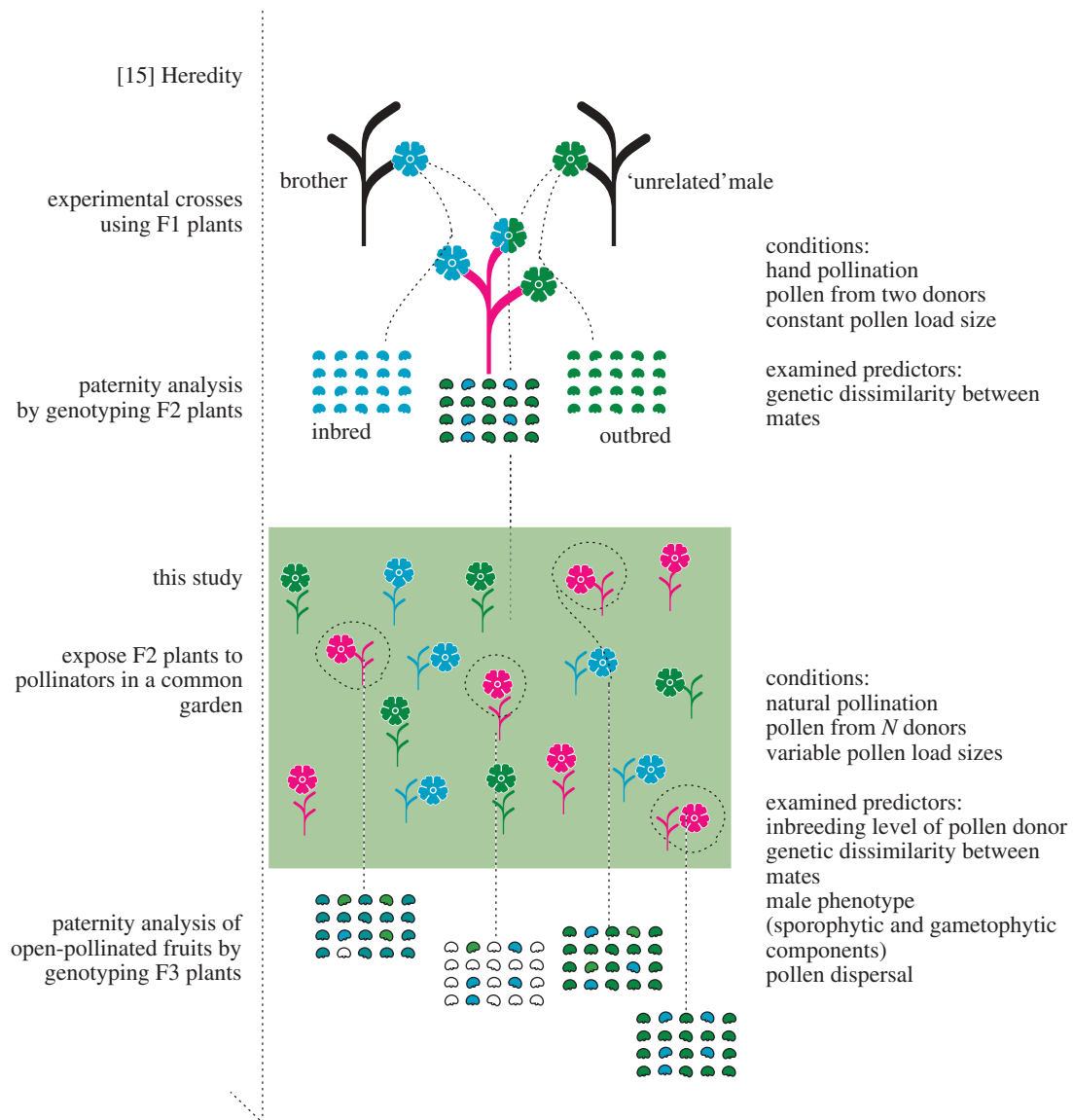
Figure 1. Experimental design comparing the emergent predictors between the present study and the previous one [15]. Here, inbred and outbred plants obtained previously [15] were exposed to natural pollination in a common garden in order to address inbreeding depression for siring success and the effects of genetic dissimilarity between mates, donor phenotype and spatial proximity on paternity success under realistic conditions of natural pollination with variable size and composition of pollen loads.

of up to 12 m; however, denser patches and inter-plant distance of 75 cm or less also occur naturally, consistent with seed dispersal by gravity [33]. At weekly intervals for six weeks (i.e. until day 100 from sowing), we counted flowers on all plants to estimate total flower production.

In addition, we assessed *in vitro* pollen germination of the experimental males. We rubbed three dehiscent anthers per male in Petri dishes containing a solid-enriched medium. This medium was prepared one day before sampling from a solution containing 0.5 g of agar, 10 g of sucrose, 10 ml of a nutrient solution (1 g l^{-1} boric acid, 3 g l^{-1} calcium nitrate, 2 g l^{-1} magnesium sulphate and 1 g l^{-1} potassium nitrate) and 90 ml of deionized water. This solution was heated, poured and stored at 4°C to allow solidification. Pollen was incubated for 3 h at 27°C, and the number of germinated pollen grains counted under ×50 magnification (see [49] for a detailed protocol).

Finally, on day 100, we collected one ripe fruit from each of 29 outbred female plants (see the electronic supplementary material, table S1) and sowed a subsample of the seeds to determine paternity of the seedlings (see below).

This study is based on the same experimental array used by Teixeira *et al.* [15] but it addresses different questions (mainly, inbreeding depression for siring success) through the genotyping of one additional generation (offspring resulting from natural pollination in the common garden) and the analysis of novel predictors of siring success; figure 1 summarizes the experimental design and compares the emergent properties between both studies.

(c) Microsatellite genotyping

To infer paternity, we genotyped 29 females, 101 males and 752 seedlings with five nuclear microsatellite markers. We genotyped on average 26 (s.d. 10) offspring per female. DNA was extracted from leaves using the Qiagen Biosprint DNA kit. The microsatellite loci were isolated by Ecogenics GmbH (Zurich, Switzerland; see electronic supplementary material, table S2). DNA was amplified by PCR (see electronic supplementary material, table S2); the products were separated on an ABI PRISM 3100 genetic analyser (Applied Biosystems), and sizes were assigned with the

GENESCAN and GENOTYPER (Applied Biosystems) softwares, using Genescan-350 as the internal size standard.

Genetic diversity indices and deviations from Hardy–Weinberg equilibrium were calculated with GENEPOP 4 [50]. The frequency of null alleles and exclusion probabilities were estimated with CERVUS 3.0.3 [51,52]. The five loci comprised a total of 57 alleles in the 133 genotyped parents (see the electronic supplementary material, table S2), with a mean of 11.4 alleles per locus. All individuals could be characterized by a unique multilocus genotype and the cumulative exclusion probability of the second putative father [53] was 99.6 per cent. All loci showed an excess of homozygotes (see the electronic supplementary material, table S1). Departure from the expected heterozygosity was greatest for the loci *Sillat08* and *Sillat28*, partly because of the possible presence of null alleles, as suggested by the comparison of the genotypes of mothers and offspring.

(d) *Joint estimation of dispersal and male fecundity parameters*

We analysed siring success by performing paternity analyses that apply the spatially explicit mixed mating model (SEMM [21,22]). This approach has the advantage of accounting for the potential biases arising through external pollen flow [54,55], or because some males may sire many seeds because of their proximity to females, rather than their phenotypic advantage. The method stems from the neighbourhood model [56,57], and allows the joint estimation of the pollen dispersal curve, the external pollen flow rate (m) and the impact of several phenotypic or ecological traits on male fecundity using maximum likelihood [21,22,56,58,59]. Here, we adapted the model for dioecious species by removing the possibility of selfing and by considering only the males as potential fathers. As the exponential power kernel [60] performed poorly (results not shown), we assumed a geometric dispersal kernel, whereby the probability of a pollen grain dispersing at positions (x,y) assuming that the father is at position $(0,0)$ is

$$p_g(a,b;x,y) = \frac{(b-2)(b-1)}{2\pi a}\left(1+\frac{r}{a}\right)^{-b}, \qquad (2.1)$$

with $r = \sqrt{x^2 + y^2}$. a represents the scale parameter (the extent to which pollen disperses) and b is the shape parameter of the dispersal curve, which describes the tail of the distribution: low b corresponds to more leptokurtic curves, with much short-distance dispersal but also a substantial proportion of long-distance dispersal [61].

We estimated these dispersal parameters jointly, along with the impact of various factors on the fecundity of males. We treated the level of inbreeding (males arising from inbred crosses versus arising from outbred crosses) as a qualitative factor. In addition, five phenotypic traits were treated as quantitative factors: germination time (mean 7.31 days, s.d. 2.56 days), flowering age (mean 46.31 days, s.d. 4.75 days), pollen *in vitro* germination rate (mean 26.04%, s.d. 7.31%), length of the stems at day 60 (mean 57.27 cm, s.d. 11.90 cm) and total number of flowers (mean 61.86, s.d. 26.81). Finally, we considered relatedness between males and females as a qualitative factor with four levels (unrelated, half-cousins, half-sibs or full-sibs). Relatedness was inferred from the crossing design using the three-generation pedigree information. This factor differs from the others because its value for a given male depends on the female with which he mates; we thus modified the SEMM algorithm [22] accordingly.

This new algorithm estimates jointly the difference in fecundity connected both with quantitative and qualitative factors. For qualitative factors, we set the fecundity of one class to 1 and estimated the relative fecundity of the other classes. For quantitative factors, we estimated their impact on fecundity by assuming a linear selection gradient [58,62,63], where the fecundity $f_i(z_{ki})$ for a male m with phenotypic value z_{mi} at trait i is:

$$\ln(f_i(z_{mi})) = \beta_i z_{mi}, \qquad (2.2)$$

where β_i is the regression coefficient; a positive β_i indicates that the trait under consideration is under directional selection for higher values. As in previous work [22,56,63], we assumed that the fecundities for qualitative and quantitative traits were multiplicative; i.e. that the fecundity of a male individual m with phenotypic value z_{mi} at trait i and z_{mj} at trait j was $f_i(z_{mi}) f_j(z_{mj})$. The significance of each estimated parameter was tested with a likelihood-ratio test, comparing the likelihood for the model excluding each parameter one at a time with the likelihood of the full model [22]. We estimated 95% confidence limits as the parameter values below and above the maximum-likelihood estimate associated with a log-likelihood 2 units smaller than the maximum likelihood [64].

3. RESULTS

(a) *Inbreeding and male–female genetic similarity as determinants of siring success*

The experimentally controlled inbreeding level of males significantly affected their siring success: outbred males sired significantly more offspring than inbred males (table 1 and electronic supplementary material, figure S1). The outbred males had, on average, a relative fecundity of 1.61 (95% CI: 1.31, 2.00) compared with inbred males (fecundity set to 1). The genetic relatedness between mating partners also significantly influenced siring success (table 1 and electronic supplementary material, figure S2). Male plants sired fewer offspring of their full sisters than males less closely related to the females. Half-cousins, on the other hand, appeared to have slightly more siring success than unrelated males; however, this tendency only approached significance, as the 95% CI of their siring success included 1.0 (equal relative fertility compared with unrelated males).

(b) *Male phenotypic traits as determinants of siring success*

Males with higher siring success were taller, germinated earlier, flowered later, produced more flowers (table 1 and figure 2) and produced pollen with higher rates of *in vitro* pollen germination. Because of the statistical model used, the estimated effects of phenotype on paternal fitness were independent of the effects of inbreeding on the phenotypic traits, i.e. they indicate effects of phenotypic differences among males that act in addition to those mediated by inbreeding depression on the traits under consideration. Most notably, these results suggest that positive selection may be acting on increased flower production. Because we estimated flower production over six weeks of pollinator exposure, we assume that it approximates the number of open flowers in males at the time of pollination.
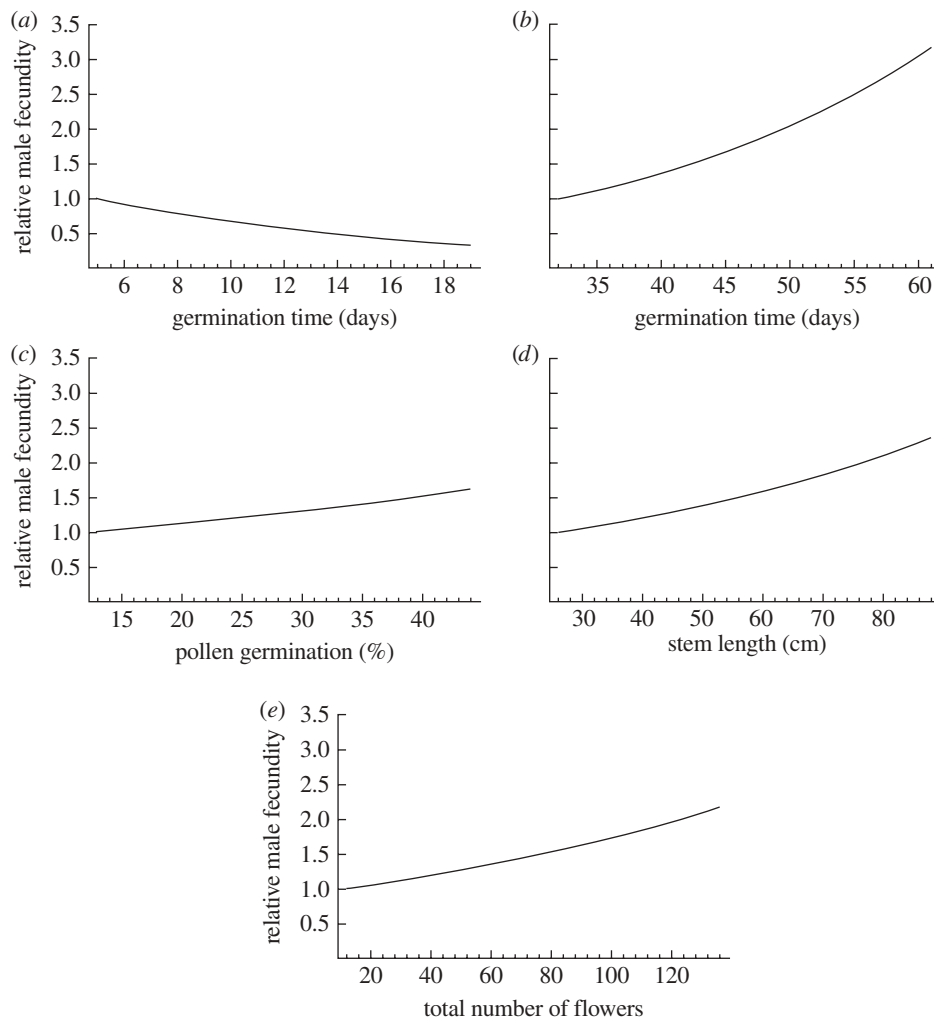
Figure 2. Estimated effects of the five considered traits on relative male fecundity. The curves are drawn using equation (2.2) and the β coefficients given in table 1.

Table 1. Estimated parameters of pollen dispersal and male fecundity in the experimental *S. latifolia* population exposed to natural pollinators (with 95% CI). The algorithm jointly estimates the pollen immigration rate (m), the scale (a) and shape (b) parameters of the pollen dispersal curve, and the impact of inbreeding and male–female relatedness and male traits on fecundity. For qualitative traits, the fecundity of one of the classes was fixed to be 1 (the fixed classes were 'inbred' for the level of inbreeding, and 'unrelated' for male–female relatedness), thus estimates represent relative fecundities. For quantitative traits, the estimated effects are regression slopes (β). The significance of each factor was tested by removing the factors one by one from the full model and by comparing the reduced models with the full model by a likelihood ratio test.

parameter/effect	estimate	log likelihood	p-value
m (pollen immigration rate)	0.153 (0.124, 0.185)		
a	0.004 (0, 0.701)		
b	0.715 (0.605, 0.823)		
level of inbreeding (outbred versus inbred male)	1.620 (1.31, 2.00)	5615	less than 10^{-4}
male–female relatedness		5612	0.006
half-cousins versus unrelated	1.55 (0.994, 2.28)		
half-sibs versus unrelated	1.46 (0.798, 2.40)		
full-sibs versus unrelated	0.256 (0.043, 0.73)		
male phenotypic traits			
germination time (days)	−0.079 (−0.125, −0.039)	5611	0.002
age at first flowering (days)	0.040 (0.020, 0.059)	5611	0.003
pollen germination *in vitro* (%)	0.015 (0.002, 0.029)	5608	0.033
stem length (cm)	0.014 (0.005, 0.022)	5611	0.002
total number of flowers	0.006 (0.0025, 0.010)	5611	0.002
full model		5606	

Among the five male traits that we considered, only germination time and age at first flowering correlated significantly (Spearman rank test, $r_s = 0.40$, $p < 0.001$; electronic supplementary material, table S3) and none of these five traits differed significantly between inbred and outbred individuals (Wilcoxon test, all p-values > 0.16; electronic
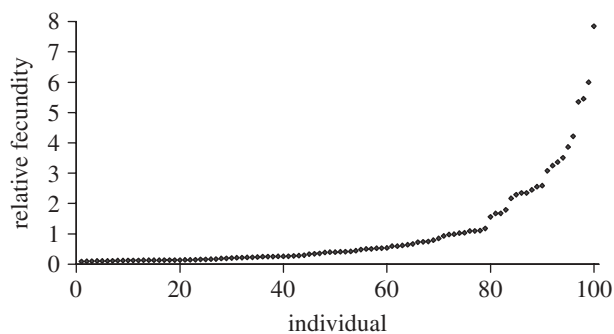
Figure 3. Estimated individual male fecundities of the 101 genotyped males, normalized so that the average male fecundity equals 1.0 and ranked by increasing fecundities.

Figure 4. Graphical representation of the inferred dispersal kernel (geometric distribution with parameters $a = 0.004$ and $b = 0.715$).

supplementary material, table S4). Because two traits ('Germination time (days)' and 'Age at first flowering (days)') diverged significantly from normality (Shapiro–Wilk test $p = 0.00571$ and $p = 1.14 \times 10^{-13}$, respectively), we could perform only non-parametric tests. Nevertheless, even if two variables are correlated, their effects on siring success are independent of each other, as each predictor was removed stepwise from the full model and all the likelihood-ratio tests performed were significant (see §2). Thus, the significance of these phenotypic effects (as well as all other factors considered simultaneously in the model) cannot be attributed to correlations among them, and each of the phenotypic traits with significant effects contributed independently to the variation in siring success. The summed effects of all measured traits yielded strong heterogeneity in the estimated fecundities of individual males (figure 3).

(c) Assortative mating for flower number
We found evidence of assortative mating only for flower number (see the electronic supplementary material, table S5) such that males with more flowers tended to mate with females with more flowers. No significant patterns of assortative mating were found for germination time, age at first flowering or stem length (see the electronic supplementary material, table S5).

(d) Pollen dispersal curve
The SEMM model also allowed us to estimate the parameters of the dispersal kernel (table 1 and figure 4). The shape parameter (b) of the geometric dispersal kernel was very low ($b = 0.715$), indicating frequent short-distance dispersal and rare long-distance dispersal events. The rate of external pollen flow (m) was estimated at around 15 per cent.

4. DISCUSSION
Pollen fate within plant populations depends on several factors, including spatial structure, plant phenotype, pollinator behaviour and genetic interactions among mating pairs. A key question that has been understudied, owing to limited access to genetic markers in the past, is whether the high levels of inbreeding and relatedness that often characterize plant populations also impact pollen fate. Higher fitness of outbred individuals is a central prediction of evolutionary biology [32], and inbreeding and relatedness are expected to have major effects on the
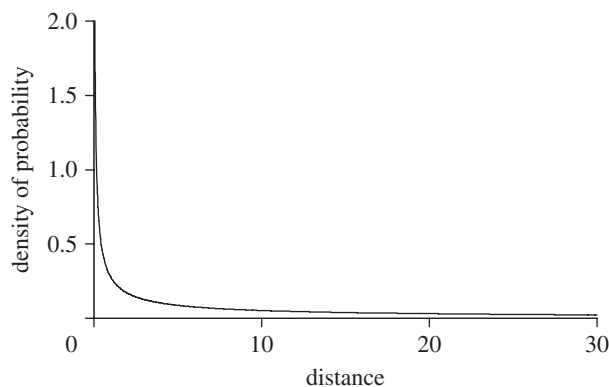
number, quality and genetic variability of offspring [4]. Here, we examined whether inbreeding, relatedness, plant phenotype and pollen dispersal jointly affected male fitness in an experimental population in which the level of inbreeding was experimentally controlled and spatial structure was randomized and accounted for statistically.

(a) Inbreeding depression in siring success and avoidance of inbreeding
While the impact of inbreeding on male reproductive success was shown for some animal species [18,30,32], this study provides the first direct experimental evidence in plants that inbreeding directly and strongly decreased male fitness (siring success) under natural conditions. Previous studies reported inbreeding depression for *in vitro* pollen germination [26,28] and pollen performance in controlled pollinations [29], including *S. latifolia* [15]. However, these experimental approaches do not necessarily reflect pollination patterns under natural conditions, especially for entomophilous species. Here we show that under conditions of natural pollination and competition among multiple donors, the siring success of outbred males is more than 50 per cent higher than that of inbred males. Because all traits were tested independently and inbred and outbred plants did not differ for the measured phenotypic traits, the difference in mating success must stem from inbreeding depression on traits that were not assessed in our study, e.g. flower attractiveness (nectar production or quality, UV reflectance, fragrance, floral morphology, etc.) or pollen production.

The avoidance of mating between full-sibs also has strong evolutionary implications. Inbreeding can be avoided by female choice or early-acting inbreeding depression, yielding within-fruit selective abortion of seeds or a lower competitive ability of inbred seeds within fruits. Mating events between close relatives are potentially frequent in this species, given the low level of pollen and seed dispersal shown by fine-scale studies [33] and the leptokurtic pollen dispersal curve estimated here (figure 4). Thus, even if some rare events of long-distance dispersal occur, the large majority of pollen dispersal will occur at much shorter distances, between related plants. Inbreeding avoidance will decrease the frequency of full-sib mating events, and therefore, reduce the opportunities for purging deleterious mutations. A substantial genetic load can thus be maintained.

Inbreeding avoidance mechanisms should also play an important role in the context of the metapopulation dynamics of this species. In patches with inbred and outbred individuals, outbred individuals will often result from recent external pollen flow. As they should enjoy greater reproductive success, these outbred individuals may contribute to genetic rescue, favouring the persistence of local demes [34,37,38]. Our results indicate that such a genetic rescue effect can even be reinforced in the generation that follows the immigration event, as evidenced by the deficiency of successful mating between close relatives. This was suggested indirectly in a previous study [34], where greater rates of external pollen flow were found in experimental *S. latifolia* patches consisting of full-sibs than in patches consisting of unrelated individuals.

(b) *Phenotypic selection on male fecundity*
We found substantial variation in male reproductive success under natural pollination, with increased success correlated with several phenotypic traits. In particular, flower production increased male reproductive success, which may be a consequence of either greater pollen production [41] or pollinator attraction. Indeed, males with larger floral displays receive more pollinator visits [40]. The fitness increase associated with the higher production of flowers in males has important implications for the evolution of sexual dimorphism [41,65,66], which is thought to be driven by selection for increased flower number in males [41,42], the most extremely dimorphic trait [67]. For selection to occur, genetic variation should translate into differences in fitness. The differences in flower number among males in our study should reflect genetic variation because we controlled for environmental variation by growing plants under uniform controlled conditions. Our study, therefore, shows that a genetically determined increase in male flower number increases fitness, providing direct evidence that this trait is under selection in *S. latifolia*. In addition, there was assortative mating for flower number, which is also consistent with the fact that pollinators are attracted to larger displays (see the electronic supplementary material, table S5).

Increased siring success was also associated with other male sporophytic traits: larger plant height, later flowering and earlier germination. Taller plants may attract more pollinators, as was shown, for instance, in *Sorbus torminalis* [22], whereas the effect of flowering time may be a consequence of increased phenological overlap with females. The effect of germination time is best explained by correlations with other traits (K. Foerster & G. Bernasconi 2009, unpublished data), which may include traits that influence pollinator behaviour. The corollary of this result is that genetic variation in early life-history traits can translate into differences in the siring success (fitness) of individuals.

Pollen germination rate is expected to play an important role in siring success. Because it is heritable in *S. latifolia* [43,49], it is expected to be under selection. However, most paternity studies performed in natural pollination have focused only on sporophytic traits [21,22,56,63]. Our study also considered the gametophytic phase and found that males producing pollen with faster *in vitro* germination had greater male fecundity. This positive relationship may result from gametophytically expressed genes, or genes expressed in the sporophytic stage that influence pollen efficiency (e.g. resources stored in the pollen grain [26]). In *S. latifolia*, fruits are often sired by many fathers, suggesting that pollen competition is very likely [3], increasing selection pressures on pollen germination rate. Our result that rapid pollen germination increases siring success is consistent with the finding that pollination timing strongly affects the proportion of seeds sired [68].

Altogether, our results indicate that selection can act simultaneously on several phenotypic traits for the male function. The persistence of variability in these traits despite strong selection may be due to non-additive effects or interaction with environmental factors [69]. Moreover, because many traits are simultaneously under selection, it becomes less efficient and more genetic variability can be retained [70]. The efficiency of selection should decrease further in the presence of pleiotropy [71,72], as some alleles can be favourable for one trait and unfavourable for another. Because *S. latifolia* is dioecious, variability in dimorphic traits such as flower number, stem length or age at first flowering [73] may also be retained because of antagonistic selection between the sexes. Such antagonistic selection has been shown to be common and relatively strong in many species, in particular for reproductive traits [74]. As many quantitative trait loci involved in dimorphic traits are autosomal [75], the alleles will be found in males and in females, facing opposite selective pressures. This will contribute to maintaining a fair level of diversity for these traits.

5. CONCLUSION
This study demonstrates that inbreeding and genetic dissimilarity among mates directly affect male fitness under natural pollination, and unveils the influence of multiple quantitative traits on male mating success. Outbred males enjoy greater reproductive success, which may contribute to genetic rescue as outbred males will often result from recent immigration. Inbreeding avoidance mechanisms counterbalance the high incidence of matings between relatives that are expected to occur owing to restricted dispersal. While selection is usually studied for single genes or traits independently, it is shown here for several traits simultaneously, indicating that the outcome of selection will be much more unpredictable. These joint effects may explain the maintenance of many deleterious alleles and large phenotypic variability that are observed in *S. latifolia*. It would be of great interest to perform similar studies on the female function, considering in particular sexually dimorphic traits. This would provide insights on the maintenance of variability through antagonistic selection. In addition, investigating seed dispersal would allow us to better understand the interplay between inbreeding and dispersal in the metapopulation context of this species.

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