Effects of inbred/outbred crosses on progeny sex ratio in *Silene latifolia* (Caryophyllaceae)

Sara Teixeira¹ ² and Giorgina Bernasconi¹

¹Department of Ecology and Evolution, University of Lausanne, 1015 Lausanne, Switzerland; ²Institute of Environmental Sciences, University of Zurich, 8057 Zurich, Switzerland

Author for correspondence: G. Bernasconi, Tel.: +(41) 21 692 47 88, Fax: +(41) 21 692 42 65, Email: giorgina.bernasconi@unil.ch

Summary

- Sex ratio polymorphism has been extensively studied in *Silene latifolia*, but it is neither known whether inbreeding (which is likely to occur under field conditions) affects it, nor which of the proposed mechanisms (Y degeneration, X-linked drive) is more important. Both mechanisms predict reduced pollen performance.
- In this study, females were crossed with pollen from related and unrelated males in single-donor and two-donor crosses, and the sex ratio of offspring (*n* = 866, 60 crosses), sons’ in vitro pollen germination and sex ratios in parental families were scored.
- Flowers receiving only unrelated pollen produced a significant excess of sons. Sex ratios were not significantly correlated between generations. Sons’ in vitro pollen germination was significantly negatively correlated with the ‘sex-ratio phenotype’ of maternal grandfathers, but not of fathers. This generation leap may be consistent with X-linked determinants because Y-linked determinants alone cannot explain it (grandfathers, fathers and sons share the same Y chromosome).
- Further work is required, but inbreeding and limited dispersal may lead to local accumulation of biasing factors, a process potentially countered by conditional shifts to produce more sons in pure outbred crosses.

**Key words:** dioecy, inbreeding, pollen, sex ratio, *Silene alba*.

Introduction

In panmictic populations, selection should favour equal allocation of resources to sons and daughters (i.e. if the costs are equal, an equal number of daughters and sons should be produced) (Fisher, 1930). However, in dioecious flowering plants, adult sex ratios often deviate from equality (Delph, 1999; De Jong & Klinkhamer, 2005). Variation in observed sex ratios may result from mechanisms modifying the secondary sex ratio (i.e. acting on seedlings or later life-stages through differences between the sexes in the timing of reproduction, in survival, or in herbivory) (Harris, 1968; Lloyd, 1974, Lloyd & Webb, 1977, Barrett & Helenurm, 1981; Meagher, 1981; Allen & Antos, 1993; Ågren et al., 1999). Alternatively, the bias may arise at the primary sex ratio, for instance through selfish genetic elements biasing meiosis (Sandler & Novitski, 1957; Werren et al., 1988; Taylor, 1999; Stouthamer et al., 2002) or differential pollen-tube growth of X-bearing and Y-bearing pollen affecting fertilization success (Correns, 1928). As a result of low recombination between the X and Y chromosomes, Y chromosomes are likely to accumulate deleterious mutations, a process aggravated by their reduced effective number in comparison to X chromosomes (Charlesworth & Charlesworth, 2000). Thus, Y-chromosome degeneration could provide a simple mechanism leading to female-biased primary sex ratios. However, it is unclear how this could be maintained, and additional Y-linked polymorphism may play an important role, in particular to explain intrapopulation variability in sex-ratio phenotype and pollen performance (Taylor, 1994; Taylor & Ingvarsson, 2003).
Silene latifolia, a dioecious short-lived perennial plant, has been extensively studied as an example of sex-ratio bias in plants (reviewed by Taylor & Ingvarsson, 2003). Mendel (1870, discussed in Taylor & Ingvarsson, 2003) had previously detected a 3:1 female : male segregation in some seed families. In this species the sex-ratio bias originates before ovule fertilization (Taylor, 1996) (i.e. the involved mechanisms must act on the primary sex ratio). Early pollen competition experiments suggested that sex-ratio bias might arise through competitively inferior Y-bearing pollen (Correns, 1928). However, sex-ratio polymorphism has been shown to be consistent with X linkage and Y linkage (Lawrence, 1963; Taylor, 1994). Consistent with both X-linked meiotic drive and Y-degeneration hypotheses, males with an extreme sex-ratio phenotype (producing 0–20% male progeny) had reduced siring ability in pollen competition (Taylor et al., 1999). These males also showed bimodal distribution of pollen sizes and had nearly 50% reduction in pollen viability (Taylor & Ingvarsson, 2003), suggesting that Y-bearing pollen may be aborted. Interestingly, there was continuous variation in the relationship between pollen viability and progeny sex ratio also in males not producing extremely biased sex ratios (Taylor & Ingvarsson, 2003). These studies provide support for X/Y-linked variation affecting pollen performance and sex ratios, but we still lack direct demonstration for this.

In natural populations of S. latifolia, offspring in one fruit are often sired by several pollen donors (Teixeira & Bernasconi, 2007). However, as a result of limited seed dispersal (McCauley, 1997; Richards et al., 1999; Wright & Meagher, 2004), and high probability of pollen transfer over short distances (Richards et al., 1999), neighbouring individuals may often be closely related, increasing the risk of inbred pollinations. Inbreeding may affect offspring sex ratios in different ways. For instance, if there are heritable components to sex-ratio bias, inbreeding may influence their dynamics in space and time, and also affect progeny sex ratios.

To address whether inbreeding affects variation in offspring sex ratios, and to infer the mechanisms governing it, we conducted inbred and outbred single-donor crosses (with pollen from a male related to the females and from an unrelated male, respectively) and compared them to two-donor crosses with a 1:1 mixture of pollen from the related and the unrelated male (Fig. 1). We monitored offspring sex ratio and pollen performance in sons arising from these crosses, and addressed whether these were explained by sex ratio in the previous generations. As sons, fathers and grandsons share the same Y chromosome, a pattern solely caused by Y-linked variation should consistently result in a positive correlation of sex-ratio phenotypes between the generations, and, assuming that pollen performance reflects the mechanism of sex-ratio bias, also a negative correlation of pollen performance and female bias. On the other hand, under X linkage, genes expressed in males are inherited from the female parent, so expression of X-linked variation in males skips a generation and we then expect, with some probability, a correlation between sons and maternal grandparents, but not with fathers (Fig. 2). Thus, although both mechanisms predict reduced pollen germination rate, they differ for expected correlations with sex-ratio phenotypes when more than one generation is examined.

Materials and Methods

Study species

The white campion (S. latifolia (Miller) Kraus) is a diploid (2n = 24) member of the carnation family, Caryophyllaceae. It is a short-lived perennial, dioecious species with an X/Y chromosomal sex determination system (Westergaard, 1958), with fruits producing c. 200 seeds. It is native to Europe and parts of Asia (Prentice, 1979). It was introduced to North America in the early 19th century, where it has become invasive (Baker, 1948; Wolfe et al., 2004). S. latifolia is a model system in diverse fields, including host–pathogen interactions (Alexander et al., 1996; Kaltz & Shykoff, 2001), sex chromosome evolution (Guttman & Charlesworth, 1998; Ironside & Filatov, 2005), sexual dimorphism (Delph et al., 2004), sex-ratio distortion (Taylor et al., 1999), reproductive ecology (Jolivet & Bernasconi, 2006, 2007a), hybridization (Goulson & Jerrim, 1997) and biological invasions (Wolfe et al., 2004).

Field collection, plant rearing and experimental hand-pollinations

Seeds were collected in September 2003 in Village-Neuf on the banks of the Hunigue canal (France; 47°36'25"N; 7°33'31"E; 245 m above sea level (asl)). The population had an estimated size of approx. 80 flowering individuals, occupying an area of 350 x 25 m. One fruit (seed capsule) was sampled from each of 45 different female plants, which were at least 2 m apart from each other.

In March 2004, 15 out of the 45 field-collected fruits were randomly selected. Twenty seeds of each of the 15 fruits (n = 300 seeds in total) were germinated in 90-mm Petri dishes lined with cotton and filter paper damped with 1 mm giberellic acid in a growth cabinet (21°C, 70% relative humidity (RH), 16 h day/8 h night). After 10 d the seedlings (n = 294) were reotted in 10-cm pots containing a 3:1 soil : sand mixture (BF₄ Tref®, GVZ-Bolltec, Zurich, Switzerland) and kept under glasshouse conditions (23°C, sodium lamps, no pesticides).

After all plants had flowered, 20 female plants and 36 male plants were selected for crosses. These plants were derived from 12 different field-collected seed families. For each female plant, one brother was selected from the same field-collected fruit (i.e. a full-brother or a half-brother) as well as one unrelated male. Each male was used only for crosses with one female (with four exceptions, as a result of variation in flowering schedules).
We experimentally manipulated two fixed factors: pollen load (low, two anthers in total, \( n = 10 \) females; and high, four anthers in total, \( n = 10 \) females) and pollen origin, with three flowers on each female receiving pollen from her brother, an unrelated male, or a 1:1 mixture of the two (Fig. 1). In a separate study (Jolivet & Bernasconi, 2007a) we estimated that in this population each anther carries on average (± SD) 1911 ± 446 pollen grains. Thus, both pollen loads in our experiment represented an excess of pollen. An excess of pollen provides conditions of pollen competition and should give rise to maximum seed set. However, substantial scatter in the seed set/pollen load response has been observed in separate experiments with this species (Taylor et al., 1999; A. Burkhardt & G. Bernasconi, unpublished data). We applied pollen from anthers that had dehisced in the previous 36 h, directly from the anthers to the stigma (24 h after opening of the flower), by holding all anthers together and rubbing them over the entire surface of the stigma, starting from the base to the tip.

Fig. 1 Experimental design of inbred, outbred and mixed-pollination crosses in *Silene latifolia*. One replicate shown (\( n = 20 \) replicates in total).
Sex ratio bias as a result of X-linked variation

<table>
<thead>
<tr>
<th>P</th>
<th>X1X2</th>
<th>Xd/Y*</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>X1Xd</td>
<td>X1Y*</td>
</tr>
<tr>
<td></td>
<td>X2Xd</td>
<td>X2Y*</td>
</tr>
</tbody>
</table>

Sex ratio bias as a result of Y degeneration

<table>
<thead>
<tr>
<th>P</th>
<th>X1X2</th>
<th>X3/Y*</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>X1Y*</td>
<td>X2X2</td>
</tr>
<tr>
<td></td>
<td>X2Y*</td>
<td></td>
</tr>
</tbody>
</table>

F2 | X1X1 | X3/Y* | X1Y* | X2X2 | X2Y* |

Fig. 2 Sex chromosome genotypes and expected sex-ratio phenotypes from inbred crosses between siblings originating from a sex ratio-biasing male in the field (P generation) under the hypotheses that sex-ratio bias arises through (top) X-linked variation (for instance, a driving X chromosome, Xd, possibly associated with a Y chromosome lacking the specific restorer, Y*) or (bottom) Y degeneration. Under X linkage, a generation lag in the expression of sex-ratio bias is expected (because the same Xd or Xd/Y* combination found in P reappears with probability of 0.5 in F2 males (i.e. in grandsons), but is not given in F1 males). No generation lag is expected under Y degeneration because the same Y chromosome is passed on from father to son to grandson. Bold text represents X or Y chromosome that carry specific genetic determinant of sex ratio variation.

Immediately after pollination, the flowers were bagged to ensure that no other pollinations could take place. All crosses resulted in successful fruit maturation. To determine progeny sex ratio and pollen performance, we sowed 20 seeds per fruit obtained in each cross (i.e. a total of 1200 seeds) in Jiffy peat pellets (Jiffy7, 703; GVZ-Bolteec) and cultivated them until flowering under glasshouse conditions (21°C; 70% RH).

Microsatellite DNA genotyping in two-donor crosses

To reconstruct the transmission of paternal X chromosomes and Y chromosomes by each of the competing males to the offspring in the mixed pollination, genomic DNA was extracted from leaf tissue of the parents (n = 56, using the cetyltrimethyl ammonium bromide (CTAB) method; Doyle & Doyle, 1990) and of 400 offspring (i.e. 20 offspring in each of the 20 crosses involving pollen competition) using the Nucleospin Plant Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany). We inferred paternal genotypes using three microsatellite DNA loci (S46, S66 and S88, see Teixeira & Bernasconi, 2007 for details of the protocol), which were found to be highly polymorphic in six natural populations of European S. latifolia, including the population used in the present study (Jolivet & Bernasconi, 2007b). Fragments were separated on an ABI 3100 genetic analyser (Applied Biosystems, Foster City, CA, USA) with the internal size standard Genescan 350 and then analysed using GENEMAPPER (Applied Biosystems). Complete analysis of paternity success in mixed-donor pollinations will be examined elsewhere (S. Teixeira, K. Forster and G. Bernasconi, unpublished results).

Estimates of sex ratios

The F1 sex ratio is the percentage of females in the field-collected seed families based on 20 offspring/fruit. It expresses the effect of sex-determining factors in their parents (P generation). The F1 plants were reared in the glasshouse and crossed. The F2 sex ratio is the percentage of daughters produced by these hand pollinations, and expresses the effect of sex-determining factors of the F1 parents. To increase precision, F2 sex ratios were based on a larger sample of 27 ± 7 (mean ± SD) offspring/fruit.

In vitro pollen germination rates

We assessed in vitro pollen germination rates of sons from inbred and outbred crosses using a medium prepared from a stock 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, stored at 4°C). One day before pollen sampling, we mixed 10 g of sucrose, 10 ml of stock solution and deionised water in a total volume of 100 ml, then added 0.5 g of agar and heated gently until the agar dissolved and the solution was completely clear. The medium was poured into 0.35 mm Petri dishes and allowed to cool. We rubbed three desiccant anthers from each male against the solidified medium. Pollen was allowed to germinate and grow for 3 h at 27°C and was then stored at −20°C before counts. For each anther, we examined 100 pollen grains under × 50 magnification and counted those that had germinated (identified by the presence of a visible pollen tube). Thus, for each male, the in vitro pollen germination rate was assessed for a total of 300 pollen grains.

Results

Progeny sex ratios

In total, we scored the gender at flowering of 866 offspring in 60 crosses. This revealed that experimental pollination of flowers on the same female plant, with pollen from a related male, an unrelated male or a mixture of the two, significantly affected progeny sex ratios. Offspring sex ratios of inbred (mean ± SD: 49 ± 13%, n = 20; over all crosses, 255 daughters and 255 sons), mixed-pollination (49 ± 10%, n = 20; over all 255 daughters and 272 sons) and outbred (42 ± 11%, n = 20; overall 244 daughters and 339 sons) crosses differed significantly (Friedman test: X = 10.11, degrees of freedom (df) = 2, P = 0.006). Specifically, sex ratio in outbred crosses
Table 1  Number of female (F) and male (M) offspring, as well as sex ratios (proportion of females out of all offspring, n) resulting from pollinating three replicate flowers per maternal plant with pollen from a brother (inbred cross), an unrelated male (outbred cross) and a 1:1 mixture of pollen from both males (mixed-donor cross) in Silene latifolia

<table>
<thead>
<tr>
<th>Pollen load</th>
<th>Outbred cross</th>
<th>Mixed donor cross</th>
<th>Inbred cross</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>M</td>
<td>F/n</td>
</tr>
<tr>
<td>High</td>
<td>16</td>
<td>17</td>
<td>0.485</td>
</tr>
<tr>
<td>High</td>
<td>8</td>
<td>19</td>
<td>0.296</td>
</tr>
<tr>
<td>High</td>
<td>12</td>
<td>14</td>
<td>0.461</td>
</tr>
<tr>
<td>High</td>
<td>9</td>
<td>21</td>
<td>0.300</td>
</tr>
<tr>
<td>High</td>
<td>16</td>
<td>17</td>
<td>0.485</td>
</tr>
<tr>
<td>High</td>
<td>11</td>
<td>20</td>
<td>0.355</td>
</tr>
<tr>
<td>High</td>
<td>15</td>
<td>15</td>
<td>0.500</td>
</tr>
<tr>
<td>High</td>
<td>15</td>
<td>17</td>
<td>0.469</td>
</tr>
<tr>
<td>High</td>
<td>8</td>
<td>22</td>
<td>0.267</td>
</tr>
<tr>
<td>High</td>
<td>17</td>
<td>15</td>
<td>0.531</td>
</tr>
</tbody>
</table>

Average                              0.415          0.506          0.501
SD                                    0.102          0.110          0.107

Italic values represent the one-tailed error probabilities (P-value) under the null hypothesis for the binomial test when sons and daughters are produced in equal proportions (i.e. F/n = M/n = 0.5; Siegel & Castellan, 1988, Table D).

*, P ≥ 0.10.

was significantly more male-biased than either the sex ratio produced in inbred crosses (Wilcoxon signed-rank test: z = −2.05, P = 0.04) or in mixed-pollination crosses (z = −2.50, P = 0.013, Fig. 3). Moreover, the sex ratio in the outbred crosses was significantly male biased (one-sample t-test for angularly transformed sex ratios against the expectation of equal sex ratios: t = −3.52, df = 19, P = 0.002). The same pattern of male bias in outbred crosses arose for both high pollen loads (over all crosses, daughters/sons: 121/118, 122/126 and 127/177 for inbred, mixed and outbred crosses, respectively) and low pollen loads (134/137, 133/146, 117/162 for inbred, mixed and outbred crosses, respectively; Table 1, Fig. 3).

When comparing the transmission of paternal X chromosomes and Y chromosomes through each of the competing males with the offspring of known paternity in the mixed pollination, there was no significant evidence that Y-bearing pollen vs X-bearing pollen originating from either the related or the unrelated males were preferentially transmitted (Wilcoxon signed rank tests, all P > 0.40; overall, a total of 58 X from the brother and 67 X from the unrelated male; and a

Fig. 3 Proportion of daughters among the offspring of inbred, outbred and mixed-pollination crosses (F2 sex ratio) of Silene latifolia. Irrespective of pollen load (low represents two anthers per flower; high represents four anthers per flower), outbreeding leads to significantly male-biased progeny.
Table 2  Expected correlations between F1 and F2 sex ratios and F2 pollen germination rates under the hypotheses of either X-linked variation or Y degeneration. The two hypotheses make different predictions for the F1 sex ratio/F2 sex ratio correlation and for the F2 sex ratio/F2 pollen germination correlation.

<table>
<thead>
<tr>
<th>Correlation examined</th>
<th>Expected under X linkage</th>
<th>Expected under Y degeneration</th>
<th>Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$r$</td>
</tr>
<tr>
<td>Inbred crosses</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1 sex ratio/F2 sex ratio</td>
<td>Uncorrelated</td>
<td>Positive</td>
<td>-0.05</td>
</tr>
<tr>
<td>F1 sex ratio/F2 pollen germination</td>
<td>Negative</td>
<td>Negative</td>
<td>-0.51</td>
</tr>
<tr>
<td>F2 sex ratio/F2 pollen germination</td>
<td>Uncorrelated</td>
<td>Negative</td>
<td>0.38</td>
</tr>
<tr>
<td>Outbred crosses</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1 sex ratio/F2 sex ratio</td>
<td>Uncorrelated</td>
<td>Positive</td>
<td>-0.24</td>
</tr>
<tr>
<td>F1 sex ratio/F2 pollen germination</td>
<td>Negative</td>
<td>Negative</td>
<td>-0.53</td>
</tr>
<tr>
<td>F2 sex ratio/F2 pollen germination</td>
<td>Uncorrelated</td>
<td>Negative</td>
<td>0.36</td>
</tr>
</tbody>
</table>

total of 63 Y from the brother and 67 Y from the unrelated male, offspring of known gender at flowering and known paternity). Therefore, there was no evidence that the unrelated male would produce a male-biased sex ratio among the offspring it sired in mixed crosses.

Pollen germination of F2 offspring

In vitro pollen germination of F2 males was not significantly affected by pollen load (mean ± SD: high: 0.259 ± 0.06, $n = 29$; low: 0.256 ± 0.06, $n = 30$; univariate ANOVA: $F_{1,53} = 0.09$, $P = 0.77$), pollen origin (brother: 0.263 ± 0.07, $n = 20$; unrelated male: 0.249 ± 0.04, $n = 20$; mixed pollination: 0.260 ± 0.07, $n = 20$; $F_{2,53} = 0.28$, $P = 0.76$), or by their interaction ($F_{2,53} = 2.08$, $P = 0.14$). In the following, we examined the correlation of pollen germination with F1 and F2 sex ratios, and thereby restricted the analysis to the single-donor crosses to examine in more detail the pattern of inheritance. Variation in in vitro pollen germination of F2 males was significantly explained by the F1 sex ratio (Table 2), which expresses the sex ratio-determining genotype of the maternal grandfather (Fig. 2). The correlation was negative for both inbred and outbred males (Table 2). By contrast, pollen germination rates of F2 males were not significantly explained by the F2 sex ratio, which expresses the sex ratio genotype of the parents (F1) in the experimental crosses (Table 2). Sex ratios in the F1 and in the F2 were not significantly correlated (Table 2).

Discussion

As a result of limited seed dispersal (McCauley, 1997), S. latifolia plants may often grow next to related individuals. Particularly in small isolated patches, they may often incur the risk of receiving pollen from these related neighbours, as population size and the distance between populations influence pollination opportunities (Richards et al., 1999). In S. latifolia, plant patches can display sex-ratio bias. Sex-ratio bias has been demonstrated to affect primary sex ratios (Taylor, 1996), consistent with a genetic mechanism. However, it is not known how inbreeding affects the observed sex ratios. Inbreeding may affect the expression of progeny sex ratios and the dynamics of sex-ratio bias factors. We found that experimental inbreeding/outbreeding significantly affected progeny sex ratios: progeny sex ratios were significantly male biased when the flower received pollen only from the unrelated male. By contrast, inbred and mixed-donor crosses produced even sex ratios. All three cross types were replicated within individual females. Thus, male bias under single-donor outbreeding cannot be ascribed to variation among females, not to the time sequence of the three treatments within females, which was randomized. Male-biased progeny of single-donor outbred crosses were consistently observed for both high and low pollen loads. Male-biased sex ratios in the single-donor outbred cross were further confirmed by rearing several hundreds of additional offspring from these seed families (A.-M. Labouche et al., unpublished data). Interestingly, the unrelated male did not transmit more Y-bearing than X-bearing pollen in mixed-donor pollinations. This suggests that the observed male bias is a shift that depends on pollination conditions. However, the mechanism producing this shift is not known.

A significant effect of inbreeding/outbreeding on progeny sex ratios was also observed in an experimental study of the gynodioecious Silene vulgaris. In S. vulgaris, self-pollinated hermaphrodites produced more daughters than outcrossed hermaphrodites (Glaettli & Goudet, 2006). It would be interesting to examine the mechanisms (including potential commonalities with other congeneric species), and also the consequences, of male-biased sex ratios under single-donor outcrossing for reproductive success in natural stands of S. latifolia. In natural large populations, multiple-donor pollination is highly prevalent (Teixeira & Bernasconi, 2007) and
thus single-donor outcrossing may be more likely to occur for females that are either isolated (in space, or in time, Elzinga et al., 2007) or present in female-biased patches.

When examining the correlation across generations between sex-ratio phenotypes and pollen performance, we found that the sex ratio produced by F1 males (i.e. the ratio of female and male F2 offspring) was not significantly correlated to the pollen performance of their sons (in vitro pollen germination of F2 males). Such a correlation would be expected, however, if both pollen performance and sex-ratio phenotype were strongly dependent on Y-linked variation, because fathers and sons share the same Y chromosome. In fact, although it may be argued that statistical power was low (0.37–0.41, as calculated using GPOWER version 3, Erdfelder et al., 1996), the estimated correlation coefficient was positive, and as such not even in the direction expected under the hypothesis of Y degeneration. Under the hypothesis that males which produce an excess of daughters do so because of the low performance of their Y-bearing pollen (as a result of Y degeneration), we would expect both fathers producing an excess of daughters and their sons to have low pollen germination.

By contrast, pollen germination of F2 males was significantly negatively correlated with F1 sex ratios (i.e. the sex-ratio phenotype of their maternal grandfathers with whom they are likely to share the X chromosome) in 50% of cases. Thus, the observed negative correlation between pollen performance and F1 sex ratio supports the idea that both pollen performance and sex ratio phenotype are at least partly affected by X-linked variation. In this study we did not determine the sex ratio phenotype of sons (i.e. in the next generation); instead we measured sons’ pollen performance as a proxy. Thus, the above reasoning rests on the assumption that sex-ratio determining factors also affect pollen traits. In future studies, it would be important to establish that males producing a biased sex ratio also have lowered pollen performance or produce fewer viable pollen grains. Consistent with this, as outlined in the Introduction, a previous study with different populations found that sex-ratio biasing males produced pollen with c. 50% reduced viability compared with males producing progenies of even sex ratios (Taylor & Ingvarsson, 2003). An effect of the maternal parent on variation in the primary sex ratio was also found in an experimental study involving controlled crosses in Urtica dioica (Glawe & De Jong, 2007). In agreement with previous work (Taylor & Ingvarsson, 2003), the reduced pollen germination rate of males derived from grandparents producing a female-biased sex ratio is consistent with a mechanism that acts on pollen formation and may reside in the elimination, incapacitation or lower performance of Y-bearing pollen. One possible mechanism might be selfish genetic elements that act as a ‘pollen killer’, which have been described in other species (wheat, Loegering & Sears, 1963; tobacco, Cameron & Moav, 1957; rice, Sano, 1990). Continuous variation in this relationship also confirms that the underlying determinants are likely to be polymorphic or to involve multiple loci, as suggested for pollen viability (Taylor & Ingvarsson, 2003).

Like previous studies (Lyons et al., 1994; Taylor, 1994, 1999), we found substantial variation in sex ratios among seed families. However, there were important differences. First, these previously published studies revealed female-biased, rather than male-biased, plant sex ratios in natural populations. Second, controlled crosses specifically performed to address the genetic basis of sex ratio in S. latifolia previously found a strong male influence on sex ratios, with sex ratios produced by males generally resembling the sex ratios produced by their male parents (Taylor, 1994). This clearly differs from our results because we did not find any significant correlation between the sex ratio in the parental families and among offspring of the experimental crosses. A probable explanation is that the factors affecting sex-ratio determination in this species vary across populations (Taylor, 1999). In agreement with the idea that evolution of the determining factors may occur at a relatively small spatial scale, we recently also found that the effect of pollen donor number on progeny sex ratios was dependent on the population and family of origin of the female parent (Jolivet & Bernasconi, 2007a). Importantly, among-population variation implies that results found in a single-population study (like the present study) cannot be generalized to the species level. However, the present study suggests that in addition to these sources of variation (population and family of origin, pollination conditions), expression of the ‘sex-ratio phenotype’ is influenced by relatedness among the male and the female parents. It will thus be interesting to investigate the level of inbreeding and patterns of gene flow in natural pollinations.

Together, these results suggest that to explain sex-ratio variation, and its relationship to pollen germination rates, requires X-linked elements, and that sex ratio variation and its relationship to pollen germination are not (or at least not very strongly) consistent with solely effects of Y degeneration on pollen performance. However, neither the actual genetic basis of sex-ratio bias, nor its link to pollen performance, is known. Moreover, the X-linked and Y-linked hypotheses for sex-ratio variation are not mutually exclusive, and both maternally inherited and paternally inherited elements may concur to explain the observed variation. Our results generate the hypothesis that the benefits to S. latifolia females conditionally producing male-biased progeny under outcrossing may indeed reside in how inbreeding/outbreeding modifies the risk of transmission and expression of the putative sex-biasing elements.

In conclusion, pollen/recipient relatedness significantly affected progeny sex ratios in S. latifolia, with females producing significantly male-biased sex ratios in single-donor crosses with unrelated males. Sex-ratio bias was associated with reduced pollen performance, but this correlation showed a generation leap (i.e. there was a significant, negative correlation only between maternal grandparents and sons). As a result of the
different patterns of inheritance of the X chromosomes vs the Y chromosomes, this is consistent with the idea that maternally inherited elements contribute to sex-ratio variation in *S. latifolia*. Although the results are consistent with previous studies suggesting X-linked drive, direct evidence of distortion of segregation at the meiosis level, separate estimates of the performance of Y-bearing and X-bearing pollen, identification of the actual mechanistic basis involved, and quantification of fitness consequences of sex-ratio bias still require further investigation.

Acknowledgements

We thank Anne Atlan, Spencer Barrett, Deborah Charlesworth, Dmitry Filatov, Jérôme Goudet, Greg Hurst, Laurent Keller, Rolf Küümmerli, Bernhard Schmid, Douglas R. Taylor and the reviewers for valuable comments, Rui Candeias, Céline Jolivet and Ana Ribeiro for practical help and Elena Conti for discussion. We acknowledge financial support by Swiss NSF (grants no. 3100A0-10331/1 and PPOOA-102944/1 to GB) and the Young Investigator Research Award of Zurich University (Forschungskredit no. 560065).

References


