

Sex ratio in hybrids between *Silene alba* and *Silene dioica*: evidence for Y-linked restorers

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Sex ratio in *Silene alba* is generally female biased, and the bias is influenced by Y-linked alleles that are polymorphic in natural populations. One interpretation of these alleles is that they enhance the production of males in female-biased populations, i.e. they are restorers. Two *Silene* species, *S. alba* and *S. dioica*, and their reciprocal hybrids were used to investigate the inheritance of sex ratio and the relationship between sex ratio and male fertility. Sex ratio was paternally inherited (i.e. Y-linked), but was also strongly influenced by the maternal parent through an interaction with the Y chromosome. These results corroborate previous work on the inheritance of sex ratio within *S. alba*, and suggest that sex ratio in *S. alba* and *S. dioica* has a similar genetic basis. Examination of the maternal by Y chromosome interaction revealed that the Y chromosome of each species produced a more severe female bias in crosses with females of the opposite species. This is consistent with the hypothesis that the alleles expressed in the maternal parent cause the female bias, while the Y-linked alleles tend to restore sex ratio toward equality.

Keywords: interspecific crosses, restorers, sex ratio, *Silene*, Y-linkage.

Introduction

Fisher (1930) argued that natural selection generally favours a 1:1 sex ratio; in populations with biased sex ratios, selection favours the production of the minority sex, because individuals of that sex have a higher per capita reproductive success. Despite the generality of Fisher's model, many populations exhibit persistently biased sex ratios, and numerous mechanisms of selection favouring a biased sex ratio have been proposed. These include the possibility that one sex is more costly to rear (Fisher, 1930), local mate competition (Hamilton, 1967), group selection (Wilson & Colwell, 1981), inbreeding (Uyenoyama & Bengtsson, 1982; Nunney, 1985), and the spread of selfish genetic elements (e.g. sex-chromosome meiotic drive (Sandler & Novitski, 1957) and cytoplasmic elements (Uyenoyama & Feldman, 1978)). Many of these models of selection for sex ratio bias have received empirical support from studies of animal populations (Hickey & Craig, 1966; Fredga *et al.*, 1976, 1977; Trivers & Hare, 1976; Werren, 1980, 1983; Curt-singer, 1991; Antolin, 1992; Aviles, 1993; Rigaud & Juchault, 1993). In dioecious plants, many species have been shown to have biased sex ratios in the field (Godley, 1964; Lloyd, 1973, 1974), but few studies

have investigated whether the bias is influenced by natural selection.

The most widely documented examples of sex ratio bias in dioecious plants are in the genus *Silene*. Female-biased sex ratios have been reported by numerous authors in *Silene alba* (Correns, 1928; Lawrence, 1963; van Nigtevecht, 1966; Mulcahy, 1967; Lovett Doust *et al.*, 1987; Lyons *et al.*, 1994; Taylor, 1994), and in other dioecious *Silene* species (*S. dioica*: Correns, 1928; Lawrence, 1963; van Nigtevecht, 1966; *S. diclinis*: Prentice, 1984). *S. alba* and *S. dioica* have X/Y sex determination mechanisms, with male heterogamety. Correns (1928) suggested that the sex ratio bias in these species is caused by differential success of X- and Y-bearing gametes during pollen competition. Though Correns' results have been challenged experimentally in *S. alba* (Carroll & Mulcahy, 1993), competition between X- and Y-bearing pollen has been interpreted as the cause of female-biased sex ratios in several other plant genera (Lloyd, 1974).

In *S. alba*, the female-biased sex ratio is influenced by a Y-linked sex ratio modifier that is polymorphic in natural populations (Taylor, 1994). Analysis of the ontogeny of the sex ratio bias using molecular markers of the Y chromosome has shown that the variation in sex ratio between *S. alba* crosses most likely originates among the male gametes, or less likely, within two days

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after fertilization (Taylor, 1992). The early development of the sex ratio bias suggests that the Y-linked locus influences either (i) the proportion of X- vs. Y-bearing gametes produced during gametogenesis, (ii) the relative performance of X- vs. Y-bearing gametes during pollen competition (as Correns suggested) or (iii) the viability of male zygotes. There are two general interpretations of this pattern of inheritance. First, the Y-linked alleles may be deleterious, and cause a female bias by producing inviable male zygotes or by decreasing the survival or performance of Y-bearing gametes. Second, the Y-linked alleles may enhance the viability of males or the performance of Y-bearing gametes, increasing the production of males in populations with a pre-existing female bias, i.e. they are restorers.

Restorers can be selected for when sex ratio is biased by the spread of selfish genetic elements (e.g. sex-chromosome meiotic drive or maternally inherited cytoplasmic elements: Thompson & Feldman, 1975; Uyenoyama & Feldman, 1978; Wu, 1983; Frank, 1989; Taylor, 1990). Most documented cases of these so-called non-Mendelian sex ratio distorters are accompanied by specific restorers which counteract their effects (see Novitski, 1947; Stalker, 1961; Hickey & Craig, 1966; Smith, 1975; Wood, 1976; Gileva, 1987; Rigaud & Juchault, 1993). There is circumstantial evidence supporting the presence of a Y-linked restorer in *S. alba*. Restorers are predicted to modify the expression of specific alleles that cause the bias, leading to complex patterns of inheritance (Frank, 1991a), and there is evidence for complex interactions between different sex ratio loci in *S. alba* (Taylor, 1994). Moreover, Y-linked sex ratio modifiers appear to be common in *S. alba* populations, which would not be expected if they were highly deleterious (Taylor, 1994).

One way to test for sex ratio restorers is to use crosses between different populations or species to break up the association between restorer genes and genes that bias sex ratio. The expectation is that if restorers are present, the sex ratio bias will be more severe in the hybrid crosses because the genes that cause the bias are no longer repressed. This approach has been especially productive in detecting autosomal restorers of cytoplasmic male sterility in plants (Frank, 1989; Couvet *et al.*, 1991), but may also be useful for detecting restorers of any type of sex ratio distorter. Frank (1991a) and Hurst and Pomiankowski (1991) suggested that restored meiotic drive systems may be very common, and proposed that the derepression of meiotic drive in interspecific crosses may explain Haldane's Rule, i.e. that it is the heterogametic sex which is generally absent, rare or sterile in interspecific crosses (Haldane, 1922). The plausibility of this

mechanism as a general explanation for Haldane's Rule is the subject of considerable debate (Coyne *et al.*, 1991; Frank, 1991b; Charlesworth *et al.*, 1993; Pomiankowski & Hurst, 1993). Nevertheless, when the underlying genetic mechanisms of sex ratio bias do involve intragenomic conflict, interspecific crosses may be useful for uncovering them. The objective of this study was to look for evidence of sex ratio restorers in *Silene alba* by examining the inheritance of sex ratio and patterns of hybrid fertility in crosses between *S. alba* and a closely related species, *S. dioica*.

Methods

Silene alba (Miller) E.H.L. Krause (= *S. latifolia* Poiret) and *Silene dioica* (L.) Clairv. are dioecious, short-lived perennial herbs native to Europe. *S. alba* and *S. dioica* are closely related species in the section *Elisanthe* (Fenzl) Fenzl (Prentice, 1984), and both have an X/Y sex determination system with male heterogamety (Westergaard, 1940). Once recognized as a single species, *S. alba*, *S. dioica* and their hybrids have been used in numerous studies of sex-linkage and sex ratio bias in the genus (Correns, 1928; Warmke, 1946; Westergaard, 1940, 1958; Lawrence, 1963; van Nigtevecht, 1966).

Both species were thought to have been introduced to the eastern U.S.A. in the late 1800s, though *S. dioica* never became established in North America (McNeill, 1977). *S. alba* is a weed of cultivation, inhabiting open fields and wastelands throughout the northern regions of the U.S.A., Canada and in upland areas farther south (McNeill, 1977). *S. dioica* in Europe is restricted to forest edges and less disturbed sites (Baker, 1947). The species are interfertile, and occasionally form hybrid swarms in nature (Baker, 1948). Both species are easily grown and crossed, producing between 50 and 400 seeds per capsule. The seeds have a germination percentage of approximately 90 per cent. Grown under long days in the greenhouse, *S. alba* and *S. dioica* start to flower in approximately 6 and 8 weeks, respectively.

Two generations of crosses were used to examine the inheritance of sex ratio. For the parental generation, five male and five female *S. dioica* plants were randomly selected from among a large sample of plants collected as seed from a population near Polperro, Cornwall, U.K. Five male and five female *S. alba* individuals were randomly selected from plants grown from seed collected from several different populations in Giles County, Virginia, U.S.A. One plant of each sex from both species was assigned to one of five diallel crosses. Each plant was crossed with one individual of its own species and one individual of the other species producing both pure species and the reciprocal hybrids

in the F_1 . All crosses were made using as much pollen as possible from a single male flower. The pollen delivered by this method was far in excess of that necessary to maximize seed set (Taylor, 1992). To minimize the effects of among flower variation in pollen load, stigma receptivity and pollen viability on sex ratio, all crosses used male and female flowers that had opened in the previous 24 h.

Several seeds were sown in each of 60 2.5 cm diameter \times 10 cm deep pots (ConetainersTM), and when more than one plant germinated per pot, all plants except the one nearest to the centre were removed. The sex of each plant was recorded when it flowered. The sex ratio data were analysed for maternal and paternal species effects using categorical data analysis (SAS Proc CATMOD).

The paternal parent has been shown to be of overwhelming importance in determining sex ratio in *S. alba* (Taylor, 1994). Hence, for the F_1 generation, I decided to focus primarily on the sex ratios produced by males with different genetic backgrounds. For each diallel, four F_1 males were selected; one of each pure species and one of each reciprocal hybrid. Each male was crossed with two females, the pure *S. alba* F_1 female and the pure *S. dioica* F_1 female from the same diallel. This crossing scheme made it possible to identify whether paternal effects on sex ratio were inherited from the paternal grandmother (indicating X-linkage), the paternal grandfather (indicating Y-linkage), or equally from both paternal grandparents (indicating autosomal inheritance). The sex ratios produced by the F_1 parents were assayed from a sample of 50 F_2 progeny as in the parental generation. The sex ratio data were analysed for maternal species effects and paternal ancestry effects (i.e. paternal grandmother and paternal grandfather effects) using categorical data analysis (SAS Proc CATMOD).

Sex ratio distorters often have detectable effects on fertility. For example, meiotic drive can operate by inactivating male gametes that do not carry the drive allele, reducing male fertility (Policansky, 1979). Maternally inherited cytoplasmic incompatibility alleles are often son killers (Frank, 1991a), that reduce female fertility. To test for such effects, the fertility of each F_1 male was estimated as the product of total pollen production per flower and the proportion of pollen that were viable. Pollen production and viability were estimated as in Taylor (1994). Pollen production data were analysed for maternal and paternal parent effects using an analysis of variance (SAS GLM procedure). Pollen viability data were analysed for heterogeneity among maternal and paternal parents using *G*-statistics (Sokal & Rohlf, 1981).

To estimate female fertility, each F_1 female was crossed with eight males. The males were the F_1 progeny from two of the five diallel crosses, so there were two males of each species and two of each reciprocal hybrid. Crosses were performed as in the parental generation. Each cross was replicated three times using the same plants, but different male and female flowers. The fertility of each female was estimated as the seed production per capsule. The data were analysed for maternal and paternal grandmother and grandfather species effects using analysis of variance (SAS GLM procedure).

Results

The sex ratios from the crosses in both generations, pooled over the five replicates, are shown in Fig. 1. The sex ratios produced by the parental generation crosses were determined primarily by the species identity of the paternal parent (Table 1a). Though the sex ratios produced by both paternal parents were significantly female biased, *S. dioica* males produced a more severe bias (28.6 per cent male, $G_1 = 98.20$, $P < 0.0001$) than *S. alba* males (43.5 per cent male, $G_1 = 9.25$, $P = 0.0024$). Note that in the categorical data analysis (Table 1), some independent variables were specified, but not included in the model. The effects of these variables, and their degrees of freedom, are lumped into the likelihood ratio term. The significance of this term, therefore, indicates the extent to which these unspecified variables could have improved the fit of the model to the data. In Table 1, the likelihood ratio term estimates the cumulative effects of variation among replicate diallel crosses, plus any interactions. Hence, there is evidence for variation in sex ratio among replicates that could only be accounted for by intraspecific variation in sex ratio. I did not have sufficient replication in this experiment, however, to address this in detail. Inheritance of sex ratio within *S. alba* has been studied elsewhere (Taylor, 1994).

When the F_1 progeny were used as parents, the paternal effect on sex ratio in the F_2 was influenced strongly by the species identity of the paternal grandfather, indicating Y-linkage (Table 1b, Fig. 1). There were also interaction effects involving the paternal X chromosome (paternal grandmother effects, Table 1b), but these were only marginally significant. Again, the highly significant likelihood ratio term indicates that there was substantial variation in sex ratio in one or both of the species.

The paternal inheritance was also strongly influenced by an interaction with the species identity of the maternal parent (Table 1b), the maternal parent having a particularly strong effect on males that had *S. dioica*

Table 1 Categorical data analysis of (a) maternal parent effects, paternal parent effects and their interaction on the sex ratios of progeny from the parental generation crosses between *S. alba* and *S. dioica* and (b) maternal parent effects, paternal ancestry effects and their interactions on the sex ratios of the progeny from F_1 *S. alba* and *S. dioica* females crossed with F_1 *S. alba*, *S. dioica* and hybrid males

Source	d.f.	χ^2	<i>P</i>
(a)			
Intercept	1	81.79	<0.0001
Maternal parent	1	0.01	0.7877
Paternal parent	1	25.48	<0.0001
Maternal parent \times paternal parent	1	0.44	0.5087
Likelihood ratio	16	68.05	<0.0001
(b)			
Intercept	1	35.46	<0.0001
Maternal parent	1	5.91	0.0150
Paternal grandmother	1	2.59	0.1074
Paternal grandfather	1	26.16	<0.0001
Maternal parent \times paternal grandmother	1	4.12	0.0424
Maternal parent \times paternal grandfather	1	23.94	<0.0001
Paternal grandmother \times paternal grandfather	1	4.60	0.0321
Maternal parent \times paternal grandmother \times paternal grandfather	1	0.44	0.5095
Likelihood ratio	28	92.99	<0.0001

Parameters were estimated using maximum likelihood.

The response variable was the generalized logits ($\ln(\text{no. males}/\text{no. females})$).

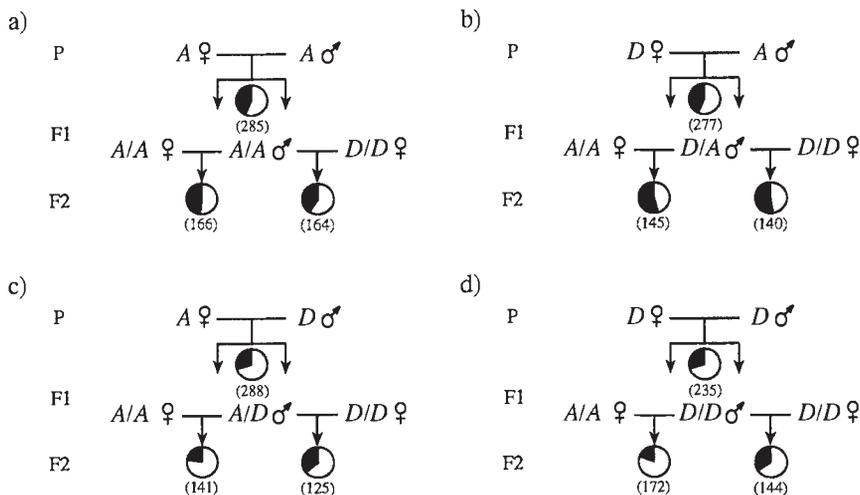


Fig. 1 Pedigrees showing the crossing scheme with the sex ratios and sample sizes. Sex ratios are represented by the pie diagrams, with the black portion representing the proportion of males. The entire crossing design was replicated five times, and the sex ratios are pooled across replicates. The species designations are as follows: A = *S. alba*, D = *S. dioica*, the F_1 s are identified by their maternal and paternal parents' species designations separated by a slash.

Y chromosomes (Fig. 1). On average, *S. dioica* Y chromosomes crossed with *S. alba* maternal parents expressed a more severe female bias than when they were crossed with *S. dioica* maternal parents, though this effect was not apparent in the previous generation (Fig. 1).

To examine this interaction in detail, the overall sex ratio produced by each paternal parent was calculated,

and the sex ratio of each cross was expressed as the deviation from that paternal mean. The deviations from the paternal means were analysed for maternal effects and paternal grandfather effects (and their interactions) using analysis of variance (SAS Proc GLM). The transformation removed the Y chromosome main effect and all the effects of genetic variation within species, but the maternal parent Y chromosome.

interaction effect persisted ($F_{1,33} = 23.55$, $P < 0.0001$). Examination of the mean deviations showed that the sex ratios produced by the Y chromosome of each species were significantly more female biased when crossed with a maternal parent of the opposite species (Table 2). This maternal effect was more pronounced for males with *S. dioica* Y chromosomes, causing a reduction in sex ratio of 0.15 when crossed with *S. alba* females, as opposed to a sex ratio reduction of 0.06 when males with *S. alba* Y chromosomes were crossed with *S. dioica* females.

There was no evidence that the hybrid progeny suffered a significant or consistent reduction in male or female fertility relative to the two parent species. Pollen production was greater in *S. alba* than in *S. dioica*, and was paternally inherited in the hybrids (Table 3). Pollen viability was determined by strong maternal by paternal interaction effects, being reduced relative to both pure species in hybrids with *S. alba* paternal parents, but enhanced in the reciprocal hybrids (Table 3). The fertility of the *S. alba*, *S. dioica* and hybrid female progeny was highly significantly influenced by the species ancestry of the female (maternal effects, Table 4), but also by the Y chromosome constitution of the pollen donor (paternal grandfather effects, Table 4).

There was a significant relationship between the fertility of F_1 males and the sex ratio they produced (Fig. 2). *S. alba* and *S. dioica* F_1 males differ in sex ratio and male fertility, creating a significant correlation between the traits across species. The two species,

however, are expected to differ in numerous traits that have no effect on sex ratio, but are correlated with sex ratio due to linkage disequilibrium. If variation in male fertility in these species caused the variation in sex ratio, or vice versa, then the correlation should persist in the interspecific crosses. In the F_1 hybrids, however, sex ratio did not segregate with male fertility, and the correlation disappeared (Fig. 2).

Discussion

Sex ratio in crosses between *S. alba* and *S. dioica* showed a distinct pattern of paternal inheritance. There was a strong paternal effect on progeny sex ratio that was, in turn, shown to be inherited from the paternal grandfather. I have interpreted this paternal inheritance as Y-linkage, though I cannot reject the possibility of paternally-inherited cytoplasmic elements. It is interesting to note that, in addition to sex ratio, male fertility and the pollen donor effect on female fertility were paternally inherited, suggesting that the Y chromosomes in these species carry some important functional loci. The sex chromosomes in *S. alba* and *S. dioica* are also unusual in that the Y chromosome is much larger than the X chromosome. The large size of the Y chromosome, and the fact that it is not inert, may reflect an intermediate level of sex-chromosome differentiation in these species (Charlesworth, 1991).

The maternal parent also strongly affected progeny sex ratio, but only through an interaction with the Y chromosome. This pattern of Y-linkage and mater-

Table 2 Mean deviations from the paternal mean sex ratios produced by males with *S. alba* or *S. dioica* Y chromosomes when crossed with *S. alba* or *S. dioica* maternal parents

Maternal parent	Paternal grandfather (Y chromosome origin)	Deviation from paternal mean
<i>S. alba</i>	<i>S. alba</i>	0.030*
<i>S. alba</i>	<i>S. dioica</i>	-0.063†
<i>S. dioica</i>	<i>S. alba</i>	-0.029†
<i>S. dioica</i>	<i>S. dioica</i>	0.084*

Means marked with the same symbol were not significantly different using a comparison of the Least Square Means (SAS Institute, 1988).

The tests for differences in the mean deviations between (i) *S. alba* Y chromosomes crossed with the two maternal parents, and (ii) *S. dioica* Y chromosomes crossed with the two maternal parents were of *a priori* interest, and withstood a Bonferroni correction for making two statistical tests (Rice, 1989). All the remaining significant *a posteriori* tests withstood the Bonferroni correction for making all six pairwise tests.

Table 3 Mean pollen production per flower and per cent pollen viability for *S. alba*, *S. dioica* and reciprocal hybrid F_1 males

Maternal parent	Paternal parent	Pollen production per flower (thousands)	Percent viable pollen
<i>S. alba</i>	<i>S. alba</i>	62.12*†	80.51*
<i>S. alba</i>	<i>S. dioica</i>	53.80*	95.72†
<i>S. dioica</i>	<i>S. alba</i>	67.50†	58.95‡
<i>S. dioica</i>	<i>S. dioica</i>	50.66*	82.85§

Means marked with the same symbol were not significantly different.

Pollen production means were compared using Least Square Means (SAS Institute, 1988) and though there is a significant paternal effect (see text) the individual comparisons did not withstand a Bonferroni correction for multiple comparisons (Rice, 1989).

Mean pollen viabilities were tested for heterogeneity in a pairwise fashion using *G*-statistics (Sokal & Rohlf, 1981) and all significant differences withstood the Bonferroni correction.

nal by Y chromosome interaction effects on sex ratio between species is identical to the pattern of inheritance of sex ratio modifiers that are polymorphic within *S. alba* populations (Taylor, 1994). These results, therefore, corroborate previous work on the inheritance of sex ratio in *S. alba*, and suggest that sex ratio in *S. alba* and *S. dioica* has a similar genetic basis.

In evaluating the hypothesis that the Y-linked sex ratio modifiers in *S. alba* are restorers, it is important to consider the specific action of the genes involved. For example, Frank's (1991a) and Hurst & Pomiankowski's (1991) models assumed that all sex ratio loci

are expressed in the heterogametic sex, altering the segregation of the sex chromosomes. They suggested that restored systems of sex-chromosome meiotic drive may be very common, and predicted that when such systems in different populations or species have diverged, the F_1 hybrid males may be sterile. This type of severe disruption of sex ratio or fertility was not observed in male *S. alba*/*S. dioica* hybrids. In *Silene*, however, sex ratio is influenced by interactions between at least one Y-linked locus expressed in the paternal parent and at least one other locus expressed in the maternal parent. Here, a Y-linked restorer may fail to influence the sex ratio bias produced by the maternal parent of a different species, and the bias would be more severe in interspecific crosses. This is one interpretation of the result in this study where the female bias produced by Y-chromosomes of each species was more severe when the crosses involved females of the opposite species.

If this interpretation is correct, then the genes that cause sex ratio bias in *Silene* may be expressed in the maternal parent. Most documented cases of sex ratio modifiers that are expressed in the maternal parent are maternally-inherited cytoplasmic elements. Often these elements are actually microorganisms that reside in the cytoplasm and enhance their transmission by producing a female bias (Williamson & Poulson, 1979; Werren *et al.*, 1986; Rigaud & Juchault, 1993). In addition to maternally-inherited genes, the maternal parent effect may be due to deleterious recessive X-linked alleles that reduce the viability of the hemizygous male progeny. Alternatively, if a female bias is favoured by natural selection in the form of local mate competition (Hamilton, 1967) or interdemic selection (Wilson & Colwell, 1981), the maternal effects may be caused by autosomal or X-linked alleles that alter the ratio of maternal investment in the two sexes or the relative performance of X- vs. Y-bearing pollen tubes in the style. Unfortunately, I have no genetic data on the

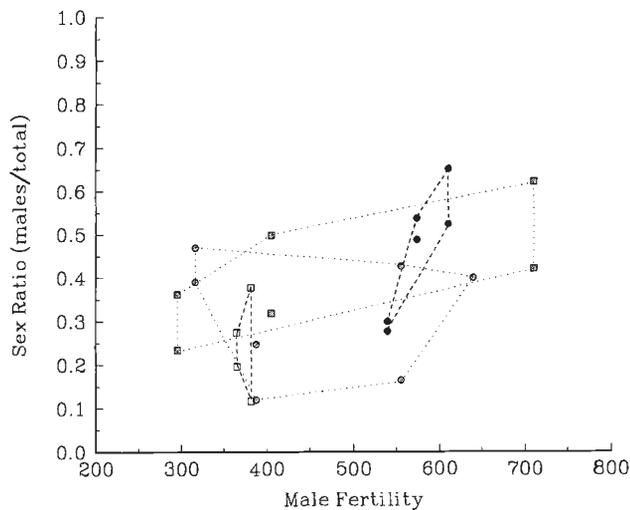


Fig. 2 Sex ratio vs. male fertility (number of pollen grains produced \times the proportion of pollen that was viable) for the F_1 males. Solid circles = *S. alba*, open squares = *S. dioica*, shaded circles = *S. alba*♀/*S. dioica*♂ hybrids, shaded squares = *S. dioica*♀/*S. alba*♂ hybrids. Polygons connect the outermost points. The linear regression of sex ratio vs. male fertility using the pure species males (solid circles and open squares) was highly significant ($r^2 = 0.611$, $P = 0.008$), but the same regression using the hybrid males (shaded symbols) was not significant ($r^2 = 0.138$, $P = 0.211$).

Table 4 Analysis of variance table for the effects of maternal and paternal species ancestry on the speed production from crosses between *S. alba*, *S. dioica*, and hybrid males and females

Source	d.f.	MS	F	P
Maternal grandmother	1	1383205	140.55	<0.0001
Maternal grandfather	1	603367	61.31	<0.0001
Paternal grandmother	1	4384	0.45	0.5048
Paternal grandfather	1	76361	7.76	0.0055
Maternal grandmother \times maternal grandfather	1	45827	4.66	0.0314
Paternal grandmother \times paternal grandfather	1	18038	1.83	0.1764
Error	502	9841		

SAS GLM procedure, Type III sums of squares; SAS Institute (1988).

maternal effects on sex ratio in *Silene*, and the importance of a subdivided population structure in creating selection for a female bias is unknown. There is no relationship, however, between the mass of a seed and the sex of its zygote in *S. alba*, suggesting that selection has not altered the ratio of maternal investment in males and females (Taylor, 1992). How selection influences sex ratio in natural populations of *S. alba*, and the genetic basis of the maternal effects on sex ratio are currently under investigation.

In this study, support for the hypothesis that the Y-linked genes in *S. alba* are restorers of a pre-existing female bias comes from the increased severity of the female bias when males with Y chromosomes of one species were crossed with females of another species. The Y-linked restorer hypothesis must, however, be viewed as tentative for two reasons. First, the maternal parent by Y chromosome interaction was not observed in the parental generation crosses. Regardless of whether the Y-linked genes are restorers or simply deleterious alleles, there is no obvious explanation for the sudden appearance of the maternal effect in the F_1 generation crosses.

A second precautionary note in interpreting these results as evidence for restorers is that the severity of sex ratio bias is not as great as is often seen in cases of intragenomic conflict. Many documented cases of sex-linked meiotic drive or cytoplasmic sex ratio distorters, for example, cause extreme sex ratio biases within broods, with one sex often exceeding 95 per cent of the progeny (e.g. Hickey & Craig, 1966; Curtsinger & Feldman, 1980; Werren *et al.*, 1986). For males with *S. dioica* Y chromosomes, the average effect on being crossed with an *S. alba* female translated to a 40 per cent reduction in the proportion of males in the progeny, a significant, but less striking, effect.

The expectation that a severe sex ratio bias would emerge from the interspecific crosses, however, may have been overly optimistic. Crosses between *S. alba* families from different populations do not show the extreme sex ratio biases that characterize many meiotic drive systems. In addition, *S. alba* and *S. dioica* have been shown to be interfertile (Baker, 1948) and were not expected to exhibit the hybrid breakdown that characterizes many interspecific crosses. In retrospect, the similarity of the genetic basis of sex ratio in *S. alba* and *S. dioica* suggests that dramatic effects of interspecific crosses on sex ratio should be sought elsewhere. There are *Silene* species that show more severe patterns of sex ratio distortion in hybrids with *S. alba*. *S. diclinis*, for example, produced no males at all in reciprocal crosses with *S. alba* (Prentice, 1978). This pattern of sex ratio bias in *S. alba*/*S. diclinis* hybrids is similar in direction to the results from *S. alba* and *S.*

dioica, and the two results could well have the same genetic basis. If so, then the rarity or absence of males in crosses between some *Silene* species would be an example of Haldane's Rule being caused by interactions between loci influencing sex ratio, perhaps involving intragenomic conflict. This pattern would bear some resemblance to the models involving restorers of meiotic drive (Frank, 1991a; Hurst & Pomiankowski, 1991), though it is important to emphasize that with no relationship between sex ratio and male fertility in this system (Taylor, 1994; this study), there is no clear evidence of meiotic drive.

It is also likely that the factors causing a paucity of males in some *Silene* hybrids are quite different from those involved in the most widely studied examples of Haldane's Rule, e.g. *Drosophila*. Hybrid sterility in *Drosophila* is widespread among closely related species (Coyne & Orr, 1989a), but inviability is only common in hybrids between more distantly related species (Wu & Davis, 1993). This general pattern is incorporated into models which predict that increasing divergence between sex chromosomes has increasingly deleterious effects on hybrid fitness (Charlesworth *et al.*, 1987; Coyne & Orr, 1989b). In contrast, the present results indicate that factors causing sex ratio bias in *Silene* hybrids have evolved before fertility barriers.

It is interesting that detailed studies of the sex ratio genetics in a plant genus have revealed some unique features. *Silene* hybrids express an unusual form of Haldane's Rule which is difficult to reconcile with proposed explanations of the more general pattern of hybrid sterility. This difference is probably the result of sex ratio in *Silene* having a unique genetic basis. Moreover, sex ratio in *Silene* may involve restorers and selection among gametes, but probably not meiotic drive. Instead, sex ratio may be modified by genes which influence the competitive performance of male gametes (Correns, 1928; Taylor, 1994), a pattern which may be unique to plants and other taxa with an elaborate haploid phase.

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