Can females benefit from selfing avoidance? Genetic associations and the evolution of plant gender

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Evolutionary stability of dioecy and nuclear gynodioecy in higher plants requires that females produce over twice as many successful seeds as hermaphrodites. This fitness differential is widely thought to derive primarily from the advantages of outcrossing caused by high selfing rates and inbreeding depression in the hermaphrodite. This study hypothesized that (i) extraordinarily high deleterious mutation rates are necessary to double female seed success due to outcrossing, and (ii) the large difference in outcrossing rates between sex morphs causes differential purging of these mutations, resulting in additional genetic selection on male sterility. Using genetically explicit models, I showed that the phenotypic outcrossing advantage requires at least one new highly recessive deleterious mutation per genome per generation, regardless of selection coefficient. However, under this mutational regime, differential purging created strong genetic selection against recessive male sterility that overwhelmed the phenotypic selection in favour of outcrossing. In very small populations and for dominant male sterility, this genetic selection was weaker or absent. This first genetically explicit study of the outcrossing advantage of unisexual females may shed new light on both the genetic and selective conditions for the evolution of gynodioecy and dioecy.

Keywords: dioecy; gynodioecy; mutation; inbreeding depression; outcrossing; selfing

1. INTRODUCTION

Evolutionary stability of dioecy (a nuclear dimorphism of males and females) and nuclear gynodioecy (a dimorphism of hermaphrodites and females) requires that females produce over double the number of successful seeds as hermaphrodites (Lloyd 1975; B. Charlesworth & D. Charlesworth 1978; Schultz 1994). This appears to be true regardless of the pathway by which dioecy evolves from hermaphroditism (D. Charlesworth & B. Charlesworth 1978; Schultz 1994; Charlesworth 1998, p. 50). This selective advantage has traditionally been attributed to the higher outcrossing rate of females (Mather 1940; Lewis 1941). For example, Lloyd (1982) concluded that 'Among all factors that favor male or female specialization, the only one that currently has convincing supporting evidence is the increased heterosis that accompanies the enforced outbreeding of unisexual females' (p. 578). Charlesworth (1998) found that 'dioecy seems unlikely to evolve unless inbreeding depression is quite large in the initial co sexes' (p. 53). Webb (1998) presented 'strong evidence... for the role of inbreeding depression as the primary factor favouring the evolution of gender dimorphism in plants' (p. 89). If outcrossing is the only advantage of male sterility, then the product of the hermaphrodite selfing rate r and inbreeding depression δ must exceed 0.5 (the 'outcrossing advantage criterion') (e.g. Lloyd 1975). This criterion is approached or exceeded in several gynodioecious angiosperms (Kohn & Biardi

1995; Schultz & Ganders 1996; Sakai *et al.* 1997). This condition not only ensures the spread of females, but also greatly facilitates the spread of males in a gynodioecious population (Charlesworth 1998).

However, the evolutionary consequences of outcrossing are more complex than suggested by this theory. Certainly, enforced outcrossing can select for the female phenotype. However, enforced outcrossing may also select against the male-sterility allele. Previous models (Lloyd 1975; B. Charlesworth & D. Charlesworth 1978; Schultz 1994) have not considered the latter because they did not incorporate the interdependent evolution of alleles causing male sterility and those causing inbreeding depression. Models following the joint evolution at both mating and load loci have shown that selfing can spread to fixation in an outcrossing hermaphroditic population, even under high initial inbreeding depression (e.g. Charlesworth *et al.* 1990; Uyenoyama & Waller 1991a,b; Schultz & Willis 1995). The descendants of a highly selfing genotype can be rapidly purged of deleterious mutations, increasing the genetic fitness of the selfing allele. This differential purging should theoretically also occur in the presence of male- or female-sterility alleles, since both are disproportionately transmitted by outcrossing. In general, such selection is expected to be strongest under high inbreeding depression and a large difference in selfing rates among genotypes at the mating locus (e.g. Schultz & Willis 1995). Ironically, these are precisely the conditions that maximize $r\delta$, the 'outcrossing advantage' of the female phenotype.

My purpose was thus to evaluate the full genetic implications of this outcrossing advantage of females and, in particular, to answer four questions.

- (i) What mutation regime is necessary to satisfy the outcrossing advantage criterion?
- (ii) Does high hermaphrodite selfing and inbreeding depression create genetic selection on female viability?
- (iii) Does this selection depend on the mode of inheritance of male sterility?
- (iv) Does this selection depend on the effective population size?

These questions were resolved by theoretical models that followed the joint evolution of male sterility and inbreeding depression due to recurrent mutation throughout the genome.

2. METHODS

(a) *Mutation parameters*

What deleterious mutation rate is necessary to double the female fitness at introduction? To calculate the minimum rate, I assumed inbreeding depression was caused by partially recessive, deleterious mutations at the mutation–selection equilibrium. The minimum rate is that which satisfies the inequality $r\delta > (1 - k)/2$, where r is the selfing rate of hermaphrodites, δ is the population inbreeding depression and k combines all remaining selective advantages of male sterility not attributable to outcrossing (B. Charlesworth & D. Charlesworth 1978). Because these remaining advantages are widely seen to result from resource allocation trade-offs (e.g. Charnov 1982; Ashman 1994; Seger & Eckhart 1996), I labelled the quantity k the ‘allocation advantage’. If this inequality is satisfied, then the combined benefits more than double female fitness. I arbitrarily considered $k < 0.3$ and, thus, $r\delta > 0.35$ as defining the parameter space within which the selective advantage of male sterility can be said to derive mainly from outcrossing.

The minimum mutation rate was found by iteration of single-locus, population-genetic, recursion equations which calculate the frequency of mutated alleles each generation (presented in the appendix of Schultz & Willis (1995)). These equations take the starting allele frequency, the per-locus mutation rate, the selection (s) and dominance (h) coefficients of mutations and the selfing rate of the population as their parameters (1, $1 - hs$ and $1 - s$ are the fitnesses of the non-mutant, heterozygous and homozygous genotypes, respectively; fitnesses combine multiplicatively across loci). The mutation parameters used were those estimated in mutation–accumulation experiments in *Drosophila* (Crow & Simmons 1983) and in inbreeding depression studies in angiosperms (Klekowski & Godfrey 1989; Charlesworth *et al.* 1994; Johnston & Schoen 1995). Two major classes of mutations were assumed: highly recessive lethals with $s = 1.0$ and $h = 0.02$ and nearly additive milds with $s = 0.01$ and $h = 0.3$. In addition, I explored the behaviour of three intermediate classes (see the descriptions in figures 1 and 2). This model was tested by a comparison with Schultz & Willis (1995) and gave identical results.

(b) *Dynamic inbreeding depression models: deterministic*

The evolutionary dynamics of male sterility were then analysed using a mutation regime that was able to satisfy

Figure 1. The female outcrossing advantage $r\delta$ resulting from the indicated mutation parameters and selfing rate in hermaphrodites and the additional allocation advantage necessary for the spread of females, according to fixed inbreeding depression models. The results show that empirically supported deleterious mutation rates fail to maintain a high inbreeding depression for even moderate rates of selfing. Thus, the spread of females requires an additional, very large, allocation advantage, in the order of 0.6–1.0. The mutation parameters were those measured for lethals and milds in *Drosophila* ($U = 0.02$ and $U = 1.0$, respectively) (see Crow & Simmons 1983; Drake *et al.* 1998) and estimated in red mangrove from chlorophyll deficiency mutations ($U = 0.2$ to lethals) (Klekowski & Godfrey 1989; Lande *et al.* 1994). The mutation rate and dominance coefficient for milds in *Drosophila* are similar to those estimated for milds in highly selfing plants (Charlesworth *et al.* 1994; Johnston & Schoen 1995).

$r\delta > 0.5$ while maintaining the high genetic fitness of outcrossed individuals. This regime was not dependent on the selection coefficient s of individual mutations, but rather on the genomic mutation rate U and dominance h . If mutations were partially dominant, then $r\delta > 0.5$ could not be satisfied unless the mean outcross viability was in the order of $\exp(-U) = \exp(-4) = 0.018$ (Haldane 1927), which is unrealistically low. For highly recessive mutations, the outcrossing advantage criterion could be satisfied with moderate mutation while maintaining reasonably high outcross viability (for $U = 1$ and $h = 0.02$, for example, the mean outcross viability was 0.45). Because the selection coefficient had virtually no effect on the equilibrium inbreeding depression, it was set to 0.95 in most runs to correspond to that estimated for highly recessive mutations in *Drosophila* (Crow & Simmons 1983). Test runs (not shown) for selection coefficients as low as 0.5 (at which computing limitations were reached) gave results virtually identical to those for nearly lethal mutations. Previous work has shown that low selection coefficients do not prevent differential purging of outcrossing alleles in infinite populations (see Schultz & Willis 1995, p. 1216 and table 3c).

The evolution of male sterility was analysed under the above mutation regime using the combinatorial framework introduced by Kondrashov (1985) (Charlesworth *et al.* 1990; Schultz & Willis 1995). The deterministic ‘dynamic inbreeding depression’

Figure 2. The minimum genomic mutation rate necessary for the spread of females in fixed inbreeding depression models for the indicated allocation advantage, mutation parameters and selfing rates in hermaphrodites. The selfing rates are (a) $r=0.5$ and (b) $r=1.0$. The results show that mutations that are expressed in the heterozygote (higher dominance coefficient h) contribute less to inbreeding depression than highly recessive mutations (lower dominance coefficient). Thus, lower mutation rates for the latter are necessary to satisfy the outcrossing advantage criterion for the spread of females: in the order of one per generation. These results were not dependent on the magnitude of the selection coefficient s .

model assumed an infinite population and an infinite number of mutable loci and followed the frequency distribution of deleterious mutations each generation in each genotype at the sterility locus (for details, see Schultz & Willis 1995).

The initial hermaphrodite population was allowed to reach mutation–selection equilibrium, after which the male-sterility allele was introduced as a heterozygote. The minimum allocation advantage of females (k) necessary for their spread was calculated twice: first, from models that assume fixed inbreeding depression and, second, from repeated trials of different k -values in the dynamic inbreeding depression model until females persisted in the population. Persistence was defined as a constant or increasing frequency for at least 2000 generations after introduction. In the fixed inbreeding depression models, the minimum k is simply $1 - 2r\delta$, where r and δ are the hermaphrodite selfing rate and equilibrium inbreeding depression at the introduction of male sterility, respectively. The ‘inbreeding depression’ of a sterility genotype (not the population mean) was calculated as the mean reduction in fitness of selfs of that genotype relative to random outcrosses involving that genotype. This model was tested by a comparison with

those of Lande *et al.* (1994) and Schultz & Willis (1995) and gave identical results.

(c) *Dynamic inbreeding depression models: stochastic*

I analysed the effects of finite population size in a stochastic, individual-based version of the above dynamic inbreeding depression model (see Schultz & Lynch 1997). In the stochastic version, new mutations were assigned to each zygote as a Poisson random deviate with mean U and heterozygous mutations were segregated as a random binomial deviate. Biparental inbreeding was simulated as an increase in the expected number of homozygous mutations based on the inbreeding coefficient, which is assumed to be $F=1/(2N)$, where N is the adult population size. Although F will generally be somewhat greater than this in natural gynodioecious populations, moderate biparental inbreeding is predicted to affect the outcrossing advantage only slightly (Yahara & Maki 1993). After the initial hermaphrodite population reached mutation–selection equilibrium, I introduced the male-sterility allele as a heterozygote and monitored its frequency, the total number of mutations carried by every individual in the population each generation and the total survivorship of zygotes produced by selfing. This model was tested by a comparison with the deterministic version and the results were found to converge as population size increased. For comparison, control runs were performed in which inbreeding depression was set as a parametric constant.

3. RESULTS AND DISCUSSION

The results, which are summarized below, suggest that the outcrossing advantage of unisexual females relative to hermaphrodites may have conflicting phenotypic and genotypic effects, net influence of which on fitness depends on the genetic basis of male sterility as well as on the population effective size.

(a) *Mutation parameters*

A high genomic mutation rate to deleterious, highly recessive alleles ($U > \sim 1$), regardless of the selection coefficient of individual mutations, is required to cause the spread of females due solely or mainly to their outcrossing advantage (figures 1 and 2). This is over ten times the rates that have been directly measured in mutation–accumulation studies, which, to date, have only been measured in invertebrate animals and *Escherichia coli* (see Crow & Simmons 1983; Drake *et al.* 1998). For these rates, the spread of nuclear male sterility requires an extraordinary additional selective advantage, in the order of 60% or greater (figures 1 and 2). Thus, if the deleterious mutation rate in dioecious and nuclear gynodioecious species is no higher than the current empirically supported values, then either the primary selective advantage of females must be unrelated to outcrossing or inbreeding depression must be caused primarily by genetic factors other than recessive mutation, but still largely unknown. However, some higher plants might experience more frequent mutation each generation than invertebrates due, for example, to a lack of a germ line, large size, vegetative reproduction, greater gene expression in developing embryos and greater activity of transposable genetic elements (Klekowski & Godfrey 1989; Jürgens *et al.* 1991; Krebs & Hancock 1991; Aizen &

Figure 3. Minimum allocation advantage for the spread of females in the infinite population dynamic inbreeding depression model. The results show that a high outcrossing advantage $r\delta$ creates net selection against recessive male sterility (thick solid line), as indicated by the enormous allocation advantage necessary for the spread of females. In contrast, dominant male sterility (thin solid line) evolves exactly as predicted by fixed inbreeding depression models. Mutation parameters: $U = (a) 1$ or $(b) 2$, $s = 0.95$ and $h = 0.02$. Partially dominant mutations were not analysed, because these yielded near zero viabilities of outcrossed zygotes for mutation rates above the minimum. Also shown is the equilibrium inbreeding depression at the introduction of females.

Rovere 1995; Clegg *et al.* 1997). More research is needed on mutation rates and effects in plant species.

(b) *Recessive male sterility*

Even if both the genomic mutation rate and the selfing rate in hermaphrodites were high enough to double the seed success of females, the male-sterility allele still would not necessarily spread. On the contrary, if male sterility was recessive, recessive deleterious mutations would not promote but prevent the spread of females in a population of highly selfing hermaphrodites (figures 3 and 5). This occurs for two reasons. First, because heterozygous hermaphrodites (Mm) are on average produced by outcrossing events more often than homozygous hermaphrodites (MM), the former carry more heterozygous mutations and the latter more homozygous mutations as adults (figure 5). This pattern, in itself, does not result in net selection on m .

Second, however, hermaphrodites are capable of self-fertilization, while females are not. Selection against deleterious recessive mutations as homozygotes, then, occurs only in the progeny of hermaphrodites. This selection against mutations kills more progeny of the heterozygous hermaphrodite Mm than of the homozygote MM , because these heterozygotes contain more heterozygous

Figure 4. Trajectories of female frequency and number of surviving selfed zygotes for small ((a) population effective size = 100) and large ((b) population effective size = 10 000) populations in the finite population dynamic inbreeding depression model. The results show that, in a large population, sufficient selfs survived to create strong viability selection against the male-sterility allele, causing female frequency to fall to zero. This occurred even though the allele was introduced at high frequency and the female seed success was double that of hermaphrodites due to their outcrossing advantage. In contrast, zero survival of selfed zygotes in a small population over hundreds of generations allows the spread of females to the equilibrium predicted by fixed inbreeding depression models. In both populations $U = 2$, $s = 0.95$, $h = 0.02$, $k = 0$ and the selfing rate $r = 0.6$. Similar results were obtained for a range of selection coefficients above 0.5 and mutation rates down to 1.0. Because near lethal mutations cannot accumulate due to random drift, these finite populations are not at risk of genetic extinction.

mutations (figure 5). This results in a lower mean viability of female zygotes mm (figure 5). Stated in the simplest terms, most purging of deleterious mutations also purges the male-sterility allele, since hermaphrodites carrying the allele also carry the most heterozygous mutations.

Thus, for a high selfing rate, the higher the mutation rate to recessive deleterious mutations, the lower the viability of female zygotes. Because the viability selection occurs within the selfed component of the population, this selection outweighs the phenotypic selection only above a threshold of the hermaphrodite selfing rate (figure 3). Selection against females is strongest at the highest selfing rate for which inbreeding depression is still not purged, precisely the conditions maximizing the phenotypic outcrossing advantage. Females could overcome this selection only with a compensatory fitness increase several times that of hermaphrodites. Whereas classical models imply that a doubling of female fertility is sufficient for the spread of females, dynamic inbreeding

depression models show that female fertility must increase by a factor as high as 20 to outweigh the viability selection against a recessive male-sterility allele (figure 3). A high outcrossing advantage ($r\delta > 0.5$) may more than double the number of successful seed produced by a female, but it can nullify the viability of female zygotes.

(c) Dominant male sterility

If male sterility was dominant, however, the purging of deleterious alleles would not differentially eliminate the male-sterility allele. As a result, male sterility spread to the equilibrium frequency predicted by previous fixed inbreeding depression models (figure 3). For dominant male sterility, females are heterozygous at the sterility locus (Mm) and homozygotes for the sterility allele (MM) are not possible. Selfing in the hermaphrodite (mm) does not select against the M allele because it does not involve the M allele. Thus, in both infinite and finite populations and under all mutation parameters, selfing rates and inbreeding depression, dominant male sterility evolved to the exact equilibrium frequency predicted by fixed inbreeding depression models. The fate of such an allele due to viability selection clearly depends on its dominance.

(d) Finite populations

Differential purging of the recessive male-sterility allele does not occur in small populations (N in the order of 100 or less) over many hundreds of generations. This allows recessive females to increase to the equilibrium frequency predicted by fixed inbreeding depression models (figure 4). The reason is straightforward: under very high inbreeding depression, many generations can pass without the survival of a single selfed zygote. Thus, the population is effectively completely outcrossing, except that females produce over double the surviving seed of hermaphrodites ($r\delta > 0.5$).

However, if populations are large (N in the order of several thousand), then large numbers of selfed zygotes are produced each generation and, each generation, some of these by chance segregate out most or all the deleterious mutations. Since these also overwhelmingly lack the recessive male-sterility allele, selfing again strongly selects against male sterility. Females are eliminated even though their outcrossing advantage more than doubles their successful seed ($r\delta > 0.5$) and even though they are introduced repeatedly at artificially high frequencies (figure 4). In comparison, when inbreeding depression is set to a parametric constant, females spread if $r\delta > 0.5$ regardless of the magnitude of N . The viability selection against a recessive male-sterility allele clearly depends on the population effective size.

These results could conceivably explain some formerly puzzling and controversial observations on male sterility in nature. First, females established at high frequencies in natural gynodioecious populations are rarely the result of a simple nuclear recessive allele (Jain 1959; Edmondson 1970; Gouyon & Couvet 1987), even though recessive male sterility is a large component of the segregational load in seed plants (Aslam *et al.* 1990; Krebs & Hancock 1990; Willis 1993; Carr & Dudash 1997). Pollen is easily made inviable and recessive mutations at many loci cause pollen

Figure 5. Inbreeding depression, viability and deleterious mutations carried by hermaphrodites and females in the infinite population dynamic inbreeding depression model. The results show that female zygotes had a viability of zero due to differential purging of the male-sterility allele along with deleterious mutations in the progeny of the heterozygous hermaphrodite. In this population $U = 1$, $s = 0.95$, $h = 0.02$, $k = 0$ and $r = 0.6$.

sterility in flowering plants. In some hermaphroditic species, up to 40% of individuals in populations carry recessive male-sterility alleles in the heterozygous state, probably at many loci. Nevertheless, male-sterile homozygotes remain at very low frequencies even under substantial selfing and inbreeding depression (see Willis 1993). Recessive nuclear male sterility is ubiquitous but may be selected against by differential purging of the allele from the selfed progeny of hermaphrodites, making the evolution of gynodioecy and dioecy in large populations dependent on mutational events that are less frequent than previously assumed.

Second, these results predict that females spread more easily during a population bottleneck, when genetic associations break down and no longer interfere with the female outcrossing advantage. Bottlenecks are predicted to occur in two situations that are correlated with dioecy: oceanic island endemism and the production of fleshy, animal-dispersed fruit (Renner & Ricklefs 1995; Sakai & Weller 1998). In both cases, occasional, long-distance dispersal of small numbers of seed to novel habitats is more frequent than in taxa outside these categories (see Janzen 1975; Loiselle *et al.* 1995; Sun *et al.* 1997). If these colonists find poor pollinating conditions in their new environs, they might undergo an increase in self-pollination. In combination with a high inbreeding depression from a past history of outcrossing, the outcrossing advantage criterion may be satisfied. Unhindered by genetic associations, a recessive male-sterility allele can then spread rapidly from the standing recessive genetic load present in the small group of colonists (figure 4; see also Ganders 1978; Delph 1990; Weller *et al.* 1998).

4. CONCLUSIONS

Very high mutation rates to highly recessive alleles are necessary to satisfy the outcrossing advantage criterion for the spread of females in a hermaphroditic population. Nevertheless, even if mutation is frequent enough, the purging of these alleles in selfed progeny is predicted to purge a recessive male-sterility allele simultaneously from a large population. If selfing and inbreeding depression are high, then this selection against the sterility genotype overwhelms the outcrossing advantage of the female phenotype. However, the low fitness of selfs may make small populations effectively outcrossing for many generations, thus weakening or eliminating this dynamic. These findings represent in part a reversal of our traditional understanding of the outcrossing advantage of females, assumed to exist since Darwin (1877; Mather 1940; B. Charlesworth & D. Charlesworth 1978; Schultz 1994). Further research is necessary to evaluate the frequency and power of these genetic processes in the evolution of female sterility and in natural populations generally (see Mutikainen & Delph 1998).

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