

When gametophytic self-incompatibility meets gynodioecy

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Summary

The occurrence of gynodioecy among angiosperms appears to be associated with self-compatibility. We use individual-based simulations to investigate the conditions for breakdown of a gametophytic self-incompatibility system in gynodioecious populations and make a comparison with hermaphroditic populations where the conditions are well known. We study three types of mutations causing self-compatibility. We track the fate of these mutations in both gynodioecious and hermaphroditic populations, where we vary the number of S-alleles, inbreeding depression and selfing rate. We find that the conditions for breakdown are less stringent if the population is gynodioecious and that the breakdown of self-incompatibility tends to promote stability of gynodioecious populations since it results in a higher frequency of females. We also find that fecundity selection has a large effect on the probability of breakdown of self-incompatibility, in particular if caused by a mutation destroying the female function of the S-locus.

1. Introduction

Plant species show a remarkable flexibility in mating systems and transitions between mating systems occur readily. Two common transitions are the loss of self-incompatibility (hereafter SI), and the emergence of gynodioecy (coexistence of hermaphrodites and females) in hermaphrodite species. Two pioneering papers were published in 1979: (1) Charlesworth & Charlesworth (1979) investigated the conditions for the breakdown of a gametophytic SI system as a function of inbreeding depression, and (2) Charlesworth & Ganders (1979) studied the emergence of cytoplasmic male sterility (with nuclear restoration) in response to female versus hermaphrodite fecundity. Gametophytic SI is most often encoded by a single locus with two tightly linked genes encoding SI specificity in males and females. Incompatibility occurs if a pollen grain meets the corresponding pistil gene in the diploid pistil. Charlesworth & Charlesworth (1979) found that the level of inbreeding depression, at which breakdown of SI by a

disruptive mutation at the S-locus is favoured by selection, is a decreasing function of the selfing rate, and depends on whether the mutation destroys male or female function (or both). The destruction of male function is selected for more readily than the destruction of female function. The conditions for a cytoplasmic male sterility factor to invade require that the total fitness of seeds produced by a female is larger than the total fitness of seeds produced by a hermaphrodite, where fitness depends both on the number of seeds set and their possible inbreeding depression. Thus, if hermaphrodites can self and selfed seeds suffer from inbreeding depression, the male sterility mutation can invade even if females produce fewer seeds than hermaphrodites, and it is therefore predicted that conditions for invasion of a male sterility mutation are less stringent for self-compatible than for self-incompatible species (Charlesworth & Ganders, 1979). This is concordant with the observation that most, but not all, gynodioecious species are self-compatible. In models of gynodioecy, the main factor that prevents invading male sterility mutations from fixing (resulting in all female populations

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being doomed to extinction) is the occurrence of dominant, nuclear mutations leading to restoration of male function (restorer loci), and empirical surveys confirm the existence of such mutations. Several male sterility mutations with corresponding restorer loci are now characterized at the molecular level (e.g. Li *et al.*, 1998; Wise *et al.*, 1999; see also the recent review by Delph *et al.*, 2007), and more than one male sterility mutation has been found to be segregating within gynodioecious populations (e.g. Koelewijn & van Damme, 1995; Manicacci *et al.*, 1996; Charlesworth & Laporte, 1998). If restorer loci are without any detrimental effects, they are expected to go to fixation, begging the question of what can cause stable polymorphism for restorer loci. Several models have examined conditions for maintaining polymorphism (e.g. Charlesworth, 1981; Frank, 1989; Gouyon *et al.*, 1991; Bailey *et al.*, 2003). A necessary condition appears to be the presence of a cost of restoration, i.e. a reduced male fitness for plants carrying a restorer allele which does not match its cytoplasmic male sterility mutation. The equilibrium frequency of females is then a complex function of: (1) the female versus hermaphrodite seed production and quality, (2) the selfing rate, (3) inbreeding depression in self-compatible species, (4) pollen limitation caused by fewer available males and (5) the magnitude of the cost of restoration. Models considering two distinct CMS mutations and two restorer loci predict that stable limit cycles of female frequencies can occur in a homogeneous environment (Gouyon *et al.*, 1991). However, selfing rates and pollen limitation in particular are also expected to vary with changing environmental conditions, with more marginal populations having higher frequencies of females due to founding events where suitable restorer alleles may be temporarily lost (see e.g. Manicacci *et al.*, 1996; Nilsson & Ågren, 2006).

In this study we use individual-based simulations to model the interaction of gynodioecy and a gametophytic SI system. Specifically we study how the conditions for the breakdown of SI depend on a gynodioecious condition. Our investigation was motivated by the presence in *Plantago maritima* of polymorphisms for both systems. Nilsson (2005) and Nilsson & Ågren (2006) reported that *P. maritima* populations in the archipelagos at the Swedish east coast express a gradient in both female frequency and presence of a functional SI system. *Plantago maritima* is self-incompatible in populations of the southernmost Grot archipelago closer to the species' central distribution and self-compatible in populations of the northernmost Skepsvik archipelago approaching the margin of the species distribution. Female frequency was scored from more than 100 populations in four archipelagos along a 600 km gradient from North to South. Female frequency was 7.7 times higher in the

northern compared with the southern populations. Populations in between these extremes show both self-incompatible and self-compatible individuals and a mean female frequency intermediate between the northern and southern extremes (Nilsson, 2005; Nilsson & Ågren, 2006; Nilsson pers. comm.). Thus, it is likely that the gynodioecy and SI systems interact in this species.

2. Model description

We compare the conditions for invasion of self-fertility alleles in gynodioecious and hermaphrodite species (Fig. 1). To do so, we combine Gouyon *et al.*'s (1991) model of gynodioecy with Porcher & Lande's (2005) model of maintenance of SI. Table 1 provides an overview of the parameters and abbreviations used in the study. Gouyon *et al.*'s model assumes that each cytoplasm in the population carries one of two male sterility mutations (C_1 , C_2). Two nuclear restorer loci have matching dominant alleles R_1 , R_2 that can restore male fertility in each CMS background (respectively C_1 and C_2). If the cytoplasm is not restored, the individual is female and has a female fecundity advantage relative to a hermaphrodite. Furthermore, a cost of restoration is assumed in cases when an individual carries a restorer allele which does not correspond to the cytoplasmic male sterility factor. Female fecundity advantage (hereafter FF) and cost of restoration (hereafter CR) together can lead to stable polymorphism, often with cyclic fluctuations in frequencies of alleles (Gouyon *et al.*, 1991).

The SI system is modelled as a gametophytic system with co-dominance among n different alleles. All functional alleles are assumed to have equal frequencies (Wright, 1939) as expected in a large population due to the negative frequency-dependent selection and exchangeability of S-alleles. Self-compatibility is assumed to be due to mutations at the S-locus and can affect male function, female function or both (see Fig. 2). Only the last situation was modelled by Porcher & Lande (2005). A self-compatibility allele is associated with a selfing rate s , and selfing is associated with a fixed inbreeding depression δ . This differs from Porcher & Lande (2005), who allowed inbreeding depression to evolve. The selfing rate s denotes the proportion of self pollen received and the selfing model we assume is therefore similar to the mass action model of Holsinger (1991). This implies that hermaphrodites heterozygous for a self-compatibility (SC) allele have a lower realized selfing rate than hermaphrodites homozygous for the self-compatibility allele.

The SI system restricts certain matings. Thus if pollen is limiting, then the seed production of females and hermaphrodites may be affected by their

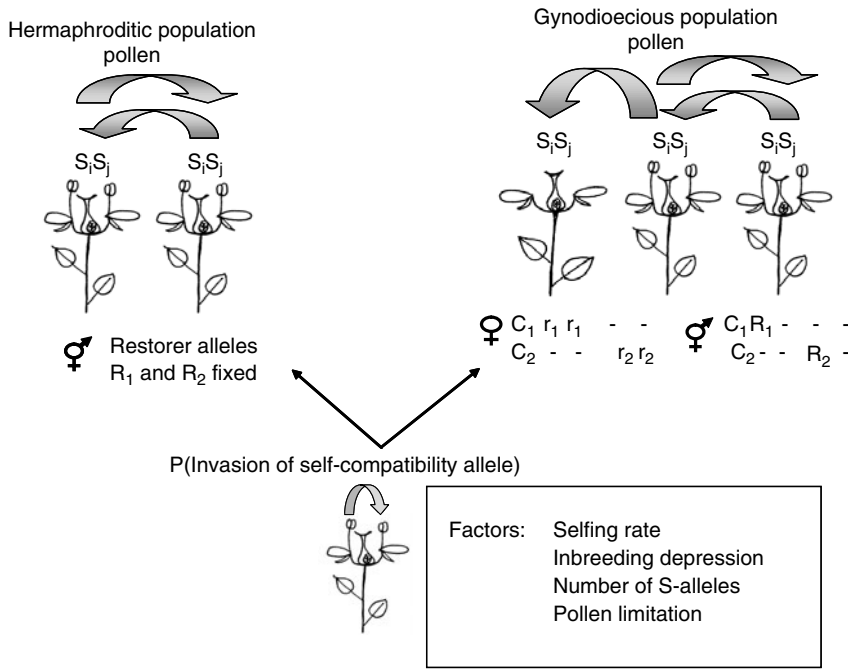


Fig. 1. Schematic illustration of the model used to investigate the invasion of an SC allele into either a hermaphroditic or gynodioecious population, and which factors it depends on. The hermaphroditic population has full restoration of the male sterility mutation and thus no females. In the gynodioecious population, females lack restoration of their cytoplasmic male sterility mutation. Arrows show possible pollen flow. For a definition of symbols, see Table 1.

Table 1. Notation used in the model

Symbols	Description
δ	Inbreeding depression (ID) in selfed offspring (assuming no biparental inbreeding)
CR	Cost of restoration
N	Number of S-alleles
s	Selfing rate associated with self-compatibility allele
FF	Female fecundity advantage
PL	Pollen limitation
C_1, C_2	Cytoplasmic male sterility types
$R_1/r_1, R_2/r_2$	Restorer loci with dominant restorer alleles (R_1, R_2)
SI	Self-incompatibility allele
SC- <i>a</i> , SC- <i>b</i> , SC- <i>c</i>	Self-compatibility allele expressed in pollen (<i>a</i>), pistil (<i>b</i>) or both (<i>c</i>)

genotype at the S-locus. Such selection can be termed *fecundity selection* (Vekemans *et al.*, 1998). We model the two extreme situations of no fecundity selection (seed set independent of the S-locus) and full fecundity selection (each putative incompatible mating results in one fewer zygote produced).

Our model tracks four loci, with two haploid CMS types (C_1 and C_2) at locus 1, three genotypes at loci 2 and 3 (restorer loci) and three at the S-locus (0, 1 or 2 copies of the self-compatibility allele). This results in a total of 54 possible genotypes of which 18 are female and 36 hermaphrodite.

Breakdown of the self-incompatibility system

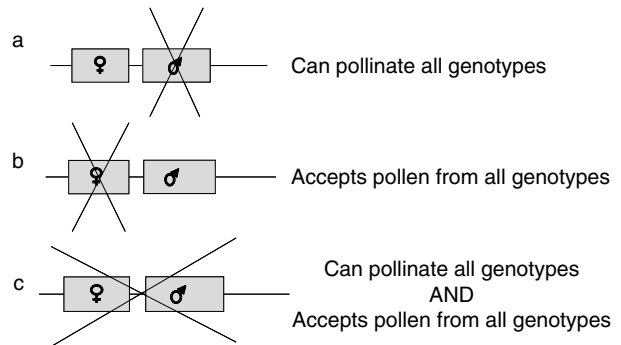


Fig. 2. Schematic illustration of the type of mutational breakdown of the self-incompatibility system for mutations affecting the two components of the S-locus: (a) a mutation in the pollen gene causing the S-allele to lose its pollen specificity, (b) a mutation in the pistil gene causing the S-allele to lose its pistil specificity and (c) a mutation (deletion) in both pollen and pistil gene causing the S-allele to lose both pollen and pistil specificity.

3. Simulations

It would be possible, but fairly tedious, to derive deterministic recursion equations for these 54 genotypes. Therefore, we choose instead to write a stochastic simulation program that samples a new generation of individuals from the previous generation by sampling parental gametes according to the reproductive fitnesses of each genotype and modelling

inbreeding depression by discarding fractions of zygotes.

In the simulations, each individual of the next generation was created as follows: First an ovule parent was randomly chosen from the 54 genotypes, weighted by their fitnesses (taking into account pollen limitation, female fecundity advantage and cost of restoration effects). If this individual could self, it was chosen to self with a probability determined by the selfing rate, and the resulting zygote was discarded with probability determined by the amount of inbreeding depression. If the individual was not selfing, a random pollen parent was chosen. Random gametes were then created from each parent. With a probability of $1/n$ the ovule and pollen gamete was assumed to have the same S-allele specificity, and the mating was incompatible. In that case either a new pollen parent was chosen while keeping the ovule parent (no fecundity selection) or a new pollen and a new ovule parent was chosen (fecundity selection).

Individuals were thus generated until the same number of individuals as in the previous generation was sampled (typically 1000). The use of stochastic simulations has the advantage that the strength of selection can also be evaluated against genetic drift, but has the disadvantage that very weak selection can only be investigated with very large population sizes. The main focus here is to contrast probabilities of invasion of self-compatibility alleles in gynodioecious and hermaphrodite populations. Thus, we focused our simulations on populations with 1000 individuals, where the deterministic effects of selection generally dominate. However, if parameter values are chosen very close to the deterministic threshold for invasion of an SC allele, selection can be weak compared with genetic drift. In the cases studied here, self-compatibility alleles introduced will always be either fixed or lost in the deterministic case. However, in our simulations, stochastic effects close to the deterministic equilibria result in certain parameter values where a self-compatibility allele can both be fixed and lost in a certain proportion of cases. We show results where this is the case.

Replicate simulation runs for a given set of parameters were performed as follows: We first simulated a number of generations with a fully functional SI system to ensure an approximate equilibrium of the gynodioecious system before introducing self-compatibility alleles. For a hermaphroditic population, equilibrium is reached very quickly. For a gynodioecious population we chose parameters, following Gouyon *et al.* (1991), leading to a stable gynodioecious population with little cycling in allele frequencies at restorer loci (mostly $FF=1.7$ and $CR=0.4$). SC alleles were introduced at a frequency of 5% and we recorded how often (out of 100

replicates) the SC allele was either lost or swept to fixation (defined as reaching a frequency above 90%). Typically, fewer than 1000 generations were necessary for either loss or fixation to occur.

We checked the simulation program using previous predictions for gynodioecy and SI individually. When restorer alleles are fixed and hence the population is always hermaphroditic, the outcomes of the model were in agreement with the results of Porcher & Lande (2005). We also checked that the results of Gouyon *et al.* (1991) are obtained in our model under similar parameters of female fertility advantage and cost of restoration.

The simulation program was written in Python and is available from www.daimi.au.dk/~mheide

4. Results

(i) Invasion of an SC allele into gynodioecious and hermaphroditic populations

In general, SC alleles invade under a broader range of conditions in a gynodioecious population than a hermaphroditic population. The conditions for the invasion of SC in hermaphrodites closely match the predictions by Charlesworth & Charlesworth (1979). The presence of females in a population allows for the evolution of selfing in hermaphrodites under higher levels of inbreeding depression, selfing rate and number of S-alleles compared with a situation where females are absent (Figs. 3, 4; light grey bars always higher than or equal to dark grey bars).

The type of mutational breakdown of the SI system (Fig. 2) has a large impact on the invasion probability of an SC allele. This is true both for hermaphroditic and gynodioecious populations (compare *a*, *b* and *c* in Figs. 3 and 4). This difference is more pronounced when there is no fecundity selection. When the mutation occurs in the pollen gene (SC type *a*; Figs. 3*a*, 4*a*), invasion occurs under higher levels of inbreeding depression than when the mutation happens in the pistil gene (SC type *b*; Figs. 3*b*, 4*b*). Invasion probability generally increases as the number of S-alleles decreases (Fig. 4). An exception is SC type *b*, where invasion is independent of the number of S-alleles for levels of inbreeding depression less than 0.65 (data not shown).

Another difference in invasion conditions between SC types *a* and *b* is the effect of selfing rate when there is no fecundity selection. Assuming similar levels of inbreeding depression and number of S-alleles, the invasion probability of an SC allele of type *a* decreases with increasing selfing rate, whereas the reverse is true for an SC of type *b* (Fig. 3, no fecundity selection; compare *a* and *b*).

Fecundity selection generally favours the invasion of an SC allele because there is no selection against

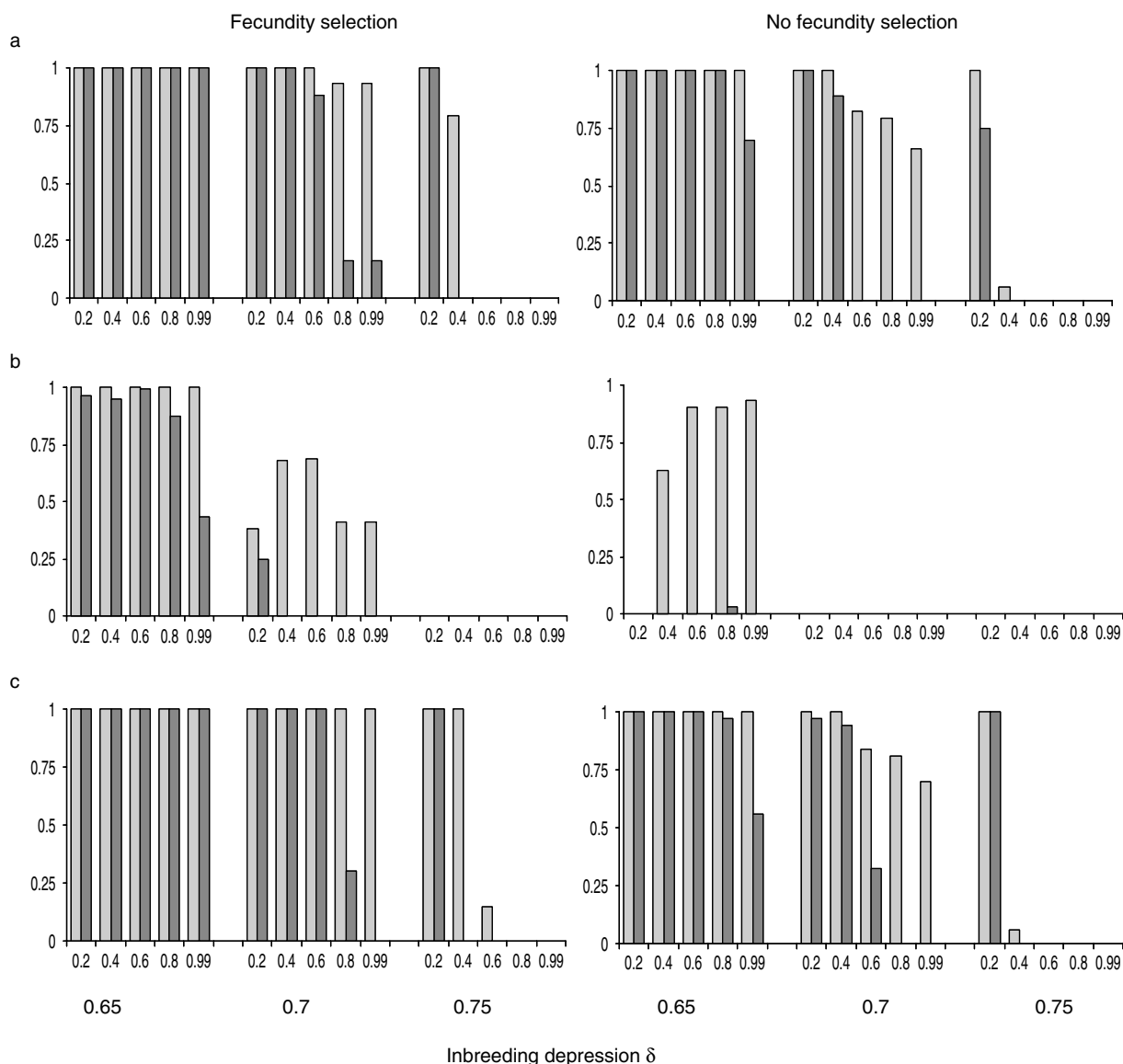


Fig. 3. Probability of invasion of an SC allele into either a gynodioecious (light grey bars) or a hermaphroditic (dark grey bars) population as a function of inbreeding depression, δ , and selfing rate (ranging from 0.2 to 0.99), with a fixed number of S-alleles (30). Scenarios with and without fecundity selection are in figures to the left and right, respectively. (a) Invasion of an SC allele with no pollen specificity (type a); (b) invasion of an SC allele with no pistil specificity (type b); and (c) invasion of an SC allele with neither pollen nor pistil specificity (type c).

S-alleles through female function. Thus, the difference is most striking for a small number of S-alleles. The fate of different types of SC alleles becomes more similar because there is less asymmetry in selection through male and female function.

In order to better understand why invasion of an SC allele was facilitated in a gynodioecious population, we tracked, for every generation, the proportion of SC alleles passed on in the offspring of females and in hermaphrodites (Fig. 5). Under conditions where invasion of an SC allele is possible in a gynodioecious but not in a hermaphroditic population, females pass on a significantly higher

proportion of SC alleles than do hermaphrodites (Fig. 5, sign test; null hypothesis: females = hermaphrodites, $G = 42.9$, $P < 0.001$).

(ii) Effect of invasion of the SC allele on the female frequency

The frequency of females in gynodioecious populations is determined by their fitness relative to the other sex morph – hermaphrodites. Females are relatively more fit in a population of self-compatible hermaphrodites practising some selfing and suffering some inbreeding depression compared with a

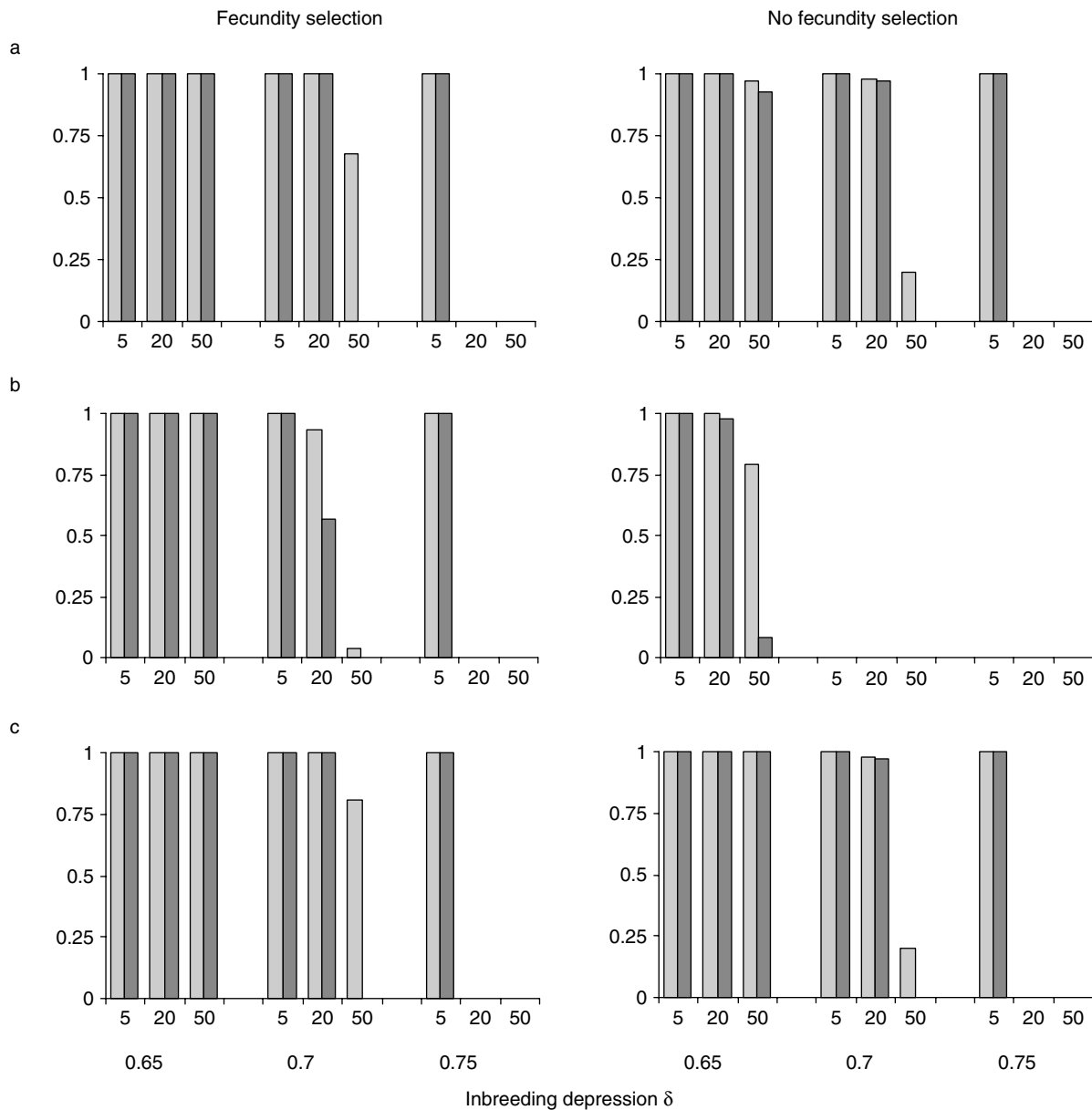


Fig. 4. Probability of invasion of an SC allele into either a gynodioecious (light grey bars) or a hermaphroditic (dark grey bars) population as a function of inbreeding depression, δ , and number of S-alleles (5, 20 or 50), with a fixed selfing rate of 0.6. Scenarios with and without fecundity selection are in figures to the left and right, respectively. (a) Invasion of an SC allele with no pollen specificity (type a); (b) invasion of an SC allele with no pistil specificity (type b); and (c) invasion of an SC allele with neither pollen nor pistil specificity (type c).

population where self-incompatible hermaphrodites never self and hence never suffer any inbreeding depression: This is illustrated in Fig. 6b, where female frequency is high when SC is fixed, and drops significantly at levels of inbreeding depression, δ , where the SC allele cannot invade and the population remains self-incompatible (Fig. 6b: $\delta > 0.7$). Hence female frequency increases in SC populations when δ increases (Fig. 6c), and the effect should be highest in populations where hermaphrodites have high selfing rates and negligible when hermaphrodites self only at very low rates (Fig. 6a).

5. Discussion

We have used stochastic simulations to investigate the conditions for the breakdown of SI in a gynodioecious species and contrasted them with the conditions in a hermaphrodite species, where our results closely matched previous deterministic models (e.g. Porcher & Lande, 2005; Charlesworth & Charlesworth, 1979). The main new result is that we confirm our intuitive belief that gynodioecy facilitates the breakdown of SI and that a breakdown of SI stabilizes the gynodioecious system. Below we discuss

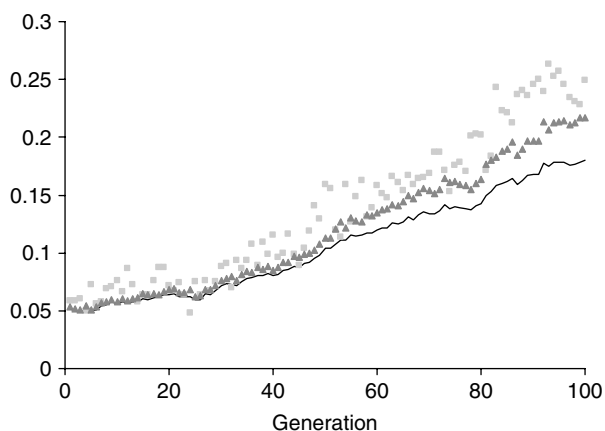


Fig. 5. The proportion of SC alleles passed on per generation in hermaphrodites (dark grey triangles) and females (light grey squares). The unbroken line represents the frequency of the SC allele in the population. The trajectory is from a typical run of a population with 10 000 individuals where the stochastic fluctuations are small.

the evolutionary forces behind these conclusions in detail.

(i) *Do females facilitate the invasion of a self-fertility allele?*

One main factor preventing the spread of a self-fertility (SC) allele is the level of inbreeding depression expressed when an individual carrying an SC allele produces selfed offspring. A main difference between hermaphrodites and females is that females can never self. The female fitness associated to the SC allele is thus not affected by inbreeding depression. An SC allele which has lost its pollen specificity (type *a*) will always have a selective advantage in the outcrossed pollen pool as it will be able to germinate on all pistils while the pollen of a functional S allele will be rejected with probability $1/n$.

When a female's seed set does not depend on the S-locus (no fecundity selection), she will reject any pollen carrying an active S-allele similar to her own, but will never reject an SC type *a* pollen, increasing the chance of her ovules being pollinated by an SC allele (especially when the number of S-alleles is reduced). As this type of SC allele only has its effect in the pollen, fertilization is possible even for females which already carry the allele. Females, regardless of their genotype, will thus never discard zygotes containing the SC allele. This process will automatically allow females to pass on a relatively higher proportion of SC alleles than hermaphrodites which, when selfing, will discard the zygotes carrying the SC alleles proportional to the level of inbreeding depression.

In contrast to the loss of pollen specificity, invasion of an SC allele which has lost its pistil specificity (type

b) will not be transmitted differently in females relative to hermaphrodites when there is no fecundity selection acting. Females will reject any SC pollen of type *b* (where specificity is still active in pollen) at the same frequency as a non-mutated S-allele, and the self-fertility allele will not have any transmission advantage in females over that of hermaphrodites.

The condition for invasion of the different types of SC alleles changes, however, under fecundity selection. If an ovule receives incompatible pollen, it does not get a 'second chance' of meeting a compatible pollen. Therefore an SC allele with a mutation affecting the pistil function will automatically be selected for in females. Females carrying this allele will transmit it at higher frequency than hermaphrodites as females will never discard a zygote with an SC ovule, whereas hermaphrodites will discard a fraction δ of selfed zygotes due to inbreeding depression.

(ii) *Positive feedback of female frequency and SC invasion*

We observe a reinforcing effect between invasion of an SC allele and female frequency when hermaphrodites show high levels of inbreeding depression (and selfing rates). The invasion of SC alleles results in an increase in female frequency and, as females accelerate the invasion of an SC allele by transmitting it at a proportionally higher rate, this in turn may increase the invasion of SC alleles.

(iii) *Invasion of the three SC types*

We found very little difference in the conditions for invasion of an SC allele of type *a* (no pollen specificity) versus *c* (no pollen and pistil specificity), whereas invasion of type *b* (no pistil specificity) occurs under stricter conditions. The effects of number of S-alleles and inbreeding depression for the invasion of SC alleles in a hermaphroditic species are in concordance with those reported by Charlesworth & Charlesworth (1979) and Uyenoyama *et al.* (2001). Type *a* (and *c*) can invade when inbreeding depression is below a certain threshold. This threshold increases when the number of functional S-alleles decreases. The reason for this is that a reduced number of active S-alleles decreases the number of available mates and thus increases pollen limitation due to the S-locus, favouring pollen carrying an SC allele that can pollinate any pistil. In contrast to this, a type *b* mutation does not have any effect in the pollen (and hence does not have the advantage of being favoured in the out-cross pollen pool under a low number of S-alleles). However, an individual carrying a type *b* self-fertility allele will accept all pollen irrespective of its specificity, and the invasion of the SC allele is therefore

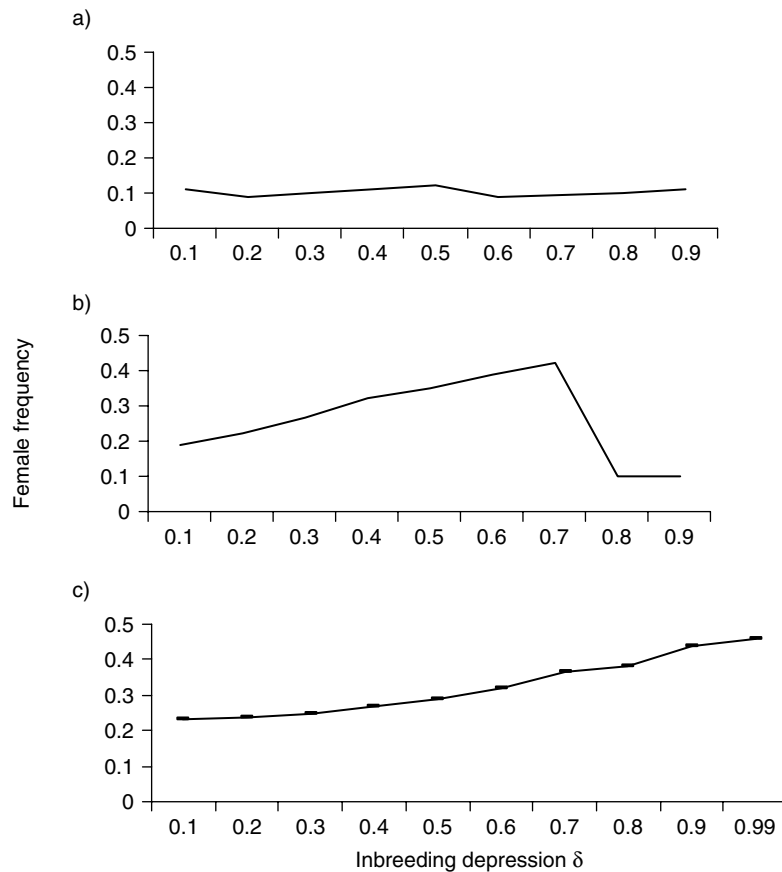


Fig. 6. Frequency of females in a gynodioecious population as a function of the level of inbreeding depression δ . (a) Scenario with 20 S-alleles and a selfing rate s of 0.1, (b) scenario with 20 S-alleles and a selfing rate of 0.8, (c) scenario where the SC alleles are fixed (5 S-alleles and a selfing rate of 0.6).

independent of the number of active S-alleles present in the population.

Another difference between type *a* and *b* is the effect of selfing rate. As inbreeding depression expressed in hermaphrodites is an increasing function of the selfing rate, one would expect high selfing rates to be associated with a higher degree of inbreeding depression – a factor which in general should select against the SC allele. The probabilities of invasion of SC alleles of type *a* and *c* are indeed higher at low than high selfing rates in the instances of high levels of inbreeding depression. Note, however, that this conclusion could be different in a more sophisticated model where inbreeding depression was allowed to evolve (e.g. Porcher & Lande, 2005).

In contrast, for type *b* mutations, we observe under certain conditions a higher probability of invasion with increasing selfing rate. However, invasion does not occur when inbreeding depression is above some threshold between 0.65 and 0.7. Below this threshold, the selective force acting on invasion may be dominated by the automatic selfing advantage and invasion therefore increases with selfing rate, whereas above the threshold, the main selective force acting on

the SC allele is inbreeding depression which here prevents the invasion of a SC type *b*.

The effect of fecundity selection is largest for the fate of type *b* mutations. Thus, pollen limitation should be an important parameter for determining whether SI breaks down most often due to loss of male or female function. Current knowledge of the prevalence of each of these types of mutations and of mutations at modifier loci (not modelled in this study) is still limited (Stone, 2002).

(iv) Implications for interpretation of results from *Plantago maritima*

Our main finding is that gynodioecy greatly facilitates invasion of a self-fertility allele and that the frequency of females in a population increases as selfing in hermaphrodites increases. This presents a plausible scenario for what might have occurred in *Plantago maritima* (Nilsson, 2005; Nilsson & Ågren, 2006), where female frequencies are larger in SC populations and SI is lost in more marginal populations. High female frequencies are predicted in newly established populations where balance between CMS and nuclear

restorer alleles has not yet been obtained or where proper restorer alleles are lacking all together (e.g. Manicacci *et al.*, 1996). If populations of the northern distribution range have a high turnover rate this would mean that newly established populations could contain fewer S-alleles and have a higher female frequency – both factors facilitating invasion of a self-compatible allele. Once self-compatibility has established this may be favoured even further by high turnover rate as the possibility for selfing may facilitate the founding of new populations.

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