Heredity (2010) 104, 482–492

www.nature.com/hdy

ORIGINAL ARTICLE

# Genetic diversity and fitness in small populations of partially asexual, self-incompatible plants

M Navascués[1,2], S Stoeckel[3] and S Mariette[4]

[1]*Unidad de Genética, Centro de Investigación Forestal, INIA, Carretera de La Coruña km 7.5, Madrid, Spain;* [2]*Équipe Éco-évolution Mathématique, CNRS, UMR 7625 Écologie et Évolution, Université Pierre et Marie Curie & École Normale Supérieure, Paris, France;* [3]*UMR Biology of Organisms and Populations applied to Plant Protection, INRA Agrocampus Rennes, Le Rheu, France and* [4]*Unité de Recherche sur les Espèces Fruitières, INRA, Domaine de la Grande Ferrade, Villenave d'Ornon, France*

How self-incompatibility systems are maintained in plant populations is still a debated issue. Theoretical models predict that self-incompatibility systems break down according to the intensity of inbreeding depression and number of *S*-alleles. Other studies have explored the function of asexual reproduction in the maintenance of self-incompatibility. However, the population genetics of partially asexual, self-incompatible populations are poorly understood and previous studies have failed to consider all possible effects of asexual reproduction or could only speculate on those effects. In this study, we investigated how partial asexuality may affect genetic diversity at the *S*-locus and fitness in small self-incompatible populations. A genetic model including an *S*-locus and a viability locus was developed to perform forward simulations of the evolution of populations of various sizes. Drift combined with partial asexuality produced a decrease in the number of alleles at the *S*-locus. In addition, an excess of heterozygotes was present in the population, causing an increase in mutation load. This heterozygote excess was enhanced by the self-incompatibility system in small populations. In addition, in highly asexual populations, individuals produced asexually had some fitness advantages over individuals produced sexually, because sexual reproduction produces homozygotes of the deleterious allele, contrary to asexual reproduction. Our results suggest that future research on the function of asexuality for the maintenance of self-incompatibility will need to (1) account for whole-genome fitness (mutation load generated by asexuality, self-incompatibility and drift) and (2) acknowledge that the maintenance of self-incompatibility may not be independent of the maintenance of sex itself.
*Heredity* (2010) **104,** 482–492; doi:10.1038/hdy.2009.159; published online 18 November 2009

**Keywords:** self-incompatibility; asexual reproduction; number of *S*-alleles; linkage disequilibrium; inbreeding depression; mutation load

## Introduction

Hermaphroditic plant species reproduce with variable rates of selfing, ranging from strict selfing to strict outcrossing (Barrett, 2002). Self-incompatibility (SI) is a reproductive system that prevents self-fertilization. In the case of heteromorphic self-incompatibility, distinct morphologies result in distinct compatibility groups, whereas in the case of homomorphic self-incompatibility, compatible individuals cannot be distinguished by their morphology (de Nettancourt, 1977). Most SI systems depend on physiological mechanisms that prevent pollen germination or pollen tube growth. In sporophytic self-incompatibility (SSI) systems, the compatibility of a pollen grain depends on the diploid genotype of the plant that produced it. In gametophytic self-incompatibility (GSI) systems, the compatibility of a pollen grain depends on its haploid genotype. GSI is more widespread than SSI (Glémin *et al*., 2001).

*Correspondence: Dr S Mariette, Unité de Recherche sur les Espèces Fruitières, INRA, Domaine de la Grande Ferrade, 71 avenue Edouard Bourlaux, BP 81, Villenave d'Ornon 33883, France.*
*E-mail: smariett@bordeaux.inra.fr*
Received 29 April 2009; revised 9 October 2009; accepted 20 October 2009; published online 18 November 2009

Fisher (1941) showed that self-fertilization should have a selective advantage because a selfing genotype will transmit more copies of its genome than a non-selfing genotype (this has been termed the automatic advantage of selfing). However, numerous studies have shown that inbred offspring are less fit than outbred offspring. The relative decrease in the mean fitness of selfed versus outcrossed individuals (inbreeding depression) is generally recognized as the only main factor that counterbalances the selective advantage of selfing (Charlesworth and Charlesworth, 1987).

Consequently, the level of inbreeding depression in populations should have a determining function in the evolution of SI systems. Inbreeding depression decreases as population size becomes smaller due to reduced polymorphism for selection to act on (Bataillon and Kirkpatrick, 2000). Charlesworth and Charlesworth (1979) showed that the number of *S*-alleles also is important in maintaining SI, because a low number of alleles will limit the number of compatible crosses in the population. A decrease in population size can also cause a reduction in the number of *S*-alleles (Brennan *et al*., 2003), and a self-compatible mutant can be positively selected for (Reinartz and Les, 1994). Thus, small populations may be particularly prone to the breakdown

of SI due to weak inbreeding depression and low numbers of *S*-alleles. However, small self-incompatible populations may maintain high levels of inbreeding depression due to a sheltered load of deleterious alleles linked to the *S*-locus (Glémin *et al*., 2001). The existence of a sheltered load has been shown experimentally in *Solanum carolinense* by Stone (2004) and Mena-Alí *et al*. (2009).

Overall, under a wide range of conditions, SI can evolve to self-compatibility. In effect, the loss of SI systems is very frequent in plant evolution (Igic *et al*., 2008). However, the reasons for which some species maintain an SI system whereas other species lose it are not completely understood. It has been suggested that asexual reproduction, 'when an individual produces new individuals that are genetically identical to the ancestor at all loci in the genome, except at those sites that have experienced somatic mutations' (de Meeûs *et al*., 2007), has a function in the maintenance or breakdown of SI. Two studies suggest that asexuality could relieve the main selective pressures that favor the breakdown of SI. First, Chen *et al*. (1997) showed in Australian Droseraceae that all self-incompatible taxa have effective forms of asexual reproduction, whereas the obligatory sexual annual taxon, *Drosera glanduligera*, is self-compatible. Their interpretation is that self-incompatible forms accumulate recessive lethal polymorphisms, especially in association with biparental inbreeding generated by elevated levels of asexual reproduction. The hypothetical high genetic load constitutes the selective pressure that maintains the outbreeding mechanisms. Second, Vallejo-Marín and O'Brien (2007) hypothesized that asexuality could provide reproductive assurance in cross-fertilizing species subject to pollen limitation. They predicted that cross-fertilizing species subject to pollen limitation would often have some means of asexual reproduction, and they found a strong correlation between SI and asexuality in *Solanum* (Solanaceae). Conversely, Young *et al*. (2002) developed a contrasting hypothesis for the case of *Rutidosis leiolepis* in which they found SSI and high rates of asexual reproduction. They suggested that increased asexual reproduction causes mate limitation by reducing genotype diversity at the *S*-locus, favoring a breakdown of SI.

The net effect of asexual reproduction on the maintenance or breakdown of SI systems is therefore still unknown. Moreover, it is unclear to what extent the effects of asexual reproduction discussed by the various authors are actually present in partially asexual, self-incompatible populations. Theoretical studies have described the effects of partial asexuality on the inbreeding depression of self-compatible populations (Muirhead and Lande, 1997) and the number of *S*-alleles (Vallejo-Marín and Uyenoyama, 2008). However, no model has been developed so far to study the joint effect of asexuality, SI and drift on the evolution of diversity and fitness parameters for a hermaphroditic species. Using individual-based simulations, we investigated the effect of (1) partial asexual reproduction and drift on diversity at the *S*-locus, and (2) combined asexuality, SI and drift on the frequency of deleterious alleles. Thus, our main goal was to characterize the dynamics of two key factors (number of *S*-alleles and inbreeding depression) for the maintenance of SI in partially asexual, self-incompatible populations. It was not the aim of this work to estimate the probability of invasion of self-compatible alleles or other modifiers of reproduction.

## Materials and methods

### Genetic model

The model considered in this study was based on the model developed by Glémin *et al*. (2001). This model consists of a population of $N$ (four population sizes evaluated: 25, 50, 100 and 1000) diploid hermaphroditic individuals with a GSI system. In addition, our model also included asexual reproduction at rate $c$ (probability that an individual is generated by asexual reproduction, seven values evaluated: 0, 0.5, 0.8, 0.9, 0.99, 0.999 and 1). We considered an asexual reproduction event as the production of a new independent individual that is an exact copy of the parental individual (or only different by somatic mutation) (de Meeûs *et al*., 2007). As in Glémin *et al*. (2001), each individual genome possessed the *S*-locus, which regulated SI, and a viability locus, whose state determined individual fitness. Two neutral loci were also included for reference. The four loci were considered to be physically unlinked and were inherited independently through sexual reproduction.

Sexual reproduction events were controlled by the *S*-locus: crosses between individuals were only possible when the *S*-allele carried by the pollen grain was different from both *S*-alleles of the pistil (that is, at least three different *S*-alleles were necessary in the population for sexual reproduction to occur). To focus on effects specifically attributable to asexuality on the maintenance and breakdown of SI, we assumed unlimited pollen availability and that sexual crosses were always fruitful. Thus, sexual reproduction was only limited when asexuality and genetic drift reduced the number of *S*-alleles to less than three within a population. In this case, independent of the original rate of asexual reproduction, all individuals reproduced asexually ($c=1$) until mutation introduced a third *S*-allele. Then, $c$ was reset to its original value.

Individual fitness $f$ was determined by the viability locus, which had two alleles $A$ and $a$. Strength of the dominance of allele $A$ over deleterious allele $a$ was regulated by the dominance coefficient $h$, and strength of the selection against $a$ was regulated by the selection coefficient $s$. Thus, relative fitness of genotypes were as follows: $f_{AA}=1$, $f_{Aa}=1-hs$ and $f_{aa}=1-s$ ($0<s<=1$ and $0<h<=1/2$). Three selection regimes at the viability locus were considered: (1) a neutral case, to be able to study the effects of the interaction between SI and asexuality without the interference of selection, where parameters $s$ and $h$ were set to zero; (2) a case of mildly deleterious and partially recessive mutations, for which values assigned to $s$ and $h$ were, respectively, 0.1 and 0.2; and (3) a case of highly recessive lethal mutations, values assigned to $s$ and $h$ were, respectively, 1 and 0.02.

Mutations were allowed in all loci. Neutral loci mutated at rate $\mu_N=10^{-3}$, following a $k$-allele model ($k=100$). Mutation at the *S*-locus followed an infinite-allele model (Kimura and Crow, 1964) with rate $\mu_S$; three values were considered for this rate ($\mu_S=10^{-3}$, $10^{-4}$ and $10^{-5}$ as in Glémin *et al*. (2001)). Mutations at the viability locus occurred at rate $\mu_1=10^{-3}$ from $A$ to $a$ and at rate $\mu_2=10^{-4}$ from $a$ to $A$.

### Individual-based simulations

Forward-time simulations of a population of $N$ diploid individuals with no overlapping generations were

performed. Simulations started with all individuals carrying two unique alleles at the *S*-locus ($2N$ different *S*-alleles in the whole population), alleles *A* or *a* randomly assigned (with equal probability, 0.5) to the viability locus and any of the $k$ alleles ($k=100$) randomly assigned (with equal probability, $1/k$) to the two neutral loci.

At each generation, the number of individuals generated by asexual reproduction $x$ was drawn from a Poisson distribution with mean $Nc$, and the remaining $N-x$ individuals were generated by sexual reproduction (parents contributing to these two types of offspring were not determined in this step). Genotypes of the $N$ individuals were generated one by one. If a given individual originated by asexual reproduction, a genotype $i$ was randomly drawn from all individuals of the previous generation. That genotype was assigned to the individual with a probability equal to the fitness of genotype $i$; this will be called the selection step. If the genotype was not successfully assigned, the genotype draft and the selection step was repeated until a genotype passed the selection step. If the individual originated by sexual reproduction, the simulation followed a different procedure. First, an individual from previous generation was randomly drawn to act as a mother and the ovule haploid genotype was randomly generated. Then, a second individual was drawn to act as a father and the pollen haploid genotype was randomly generated. If the *S*-allele at the pollen grain was not different from both *S*-alleles of the mother (that is, incompatible pollen), the pollen was discarded. New fathers and pollen grain genotypes were generated until a compatible cross was obtained (that is, pollen availability was unlimited). Then, a diploid genotype $i$ was generated from the haploid genotypes of the ovule and the pollen. The selection step was carried out on genotype $i$ as in the case of asexual reproduction; if the genotype was not assigned the process was repeated by drawing two new parents until a genotype was able to pass the selection step. Once all $N$ genotypes were selected, mutations were applied to each individual. The numbers of mutants at the *S*-locus and at the two neutral loci were drawn from Poisson distributions with means $2N\mu_S$ and $2N\mu_N$, respectively. At the viability locus, a mutant was formed with a probability $\mu_1$ if the allele was *A* and a probability $\mu_2$ if the allele was *a*. The rate of asexual reproduction was a fixed parameter of our model and did not change with the selection process.

### Monitored genetic indices

Simulations were run for 50 000 generations before beginning to monitor the genetic indices, this allowed monitored indices to become stable and avoided the influence of initial conditions in their values (Glémin *et al*., 2001). From generation 50 000, indices were calculated every 3000 generations until 107 000 generations (that is, 20 times). For the same combination of parameters, 50 replicates of the simulations were performed providing 1000 (indices calculated 20 times in each of the 50 replicates) evaluations of the monitored variables for each set of parameter values. Indices were calculated from all genotypes of the population (that is, they were not estimates, but the true values of the population).

**Genetic diversity indices:** For each locus we calculated the number of alleles ($n_a$), allele frequencies, effective number of alleles ($n_e = 1/\sum_{i=1}^{n_a} p_i^2$ , where $p_i$ was the frequency of allele $i$), expected heterozygosity ($H_e = 1-1/n_e$), observed heterozygosity ($H_o$, proportion of heterozygous individuals) and the inbreeding coefficient ($F_{IS} = (H_e - H_o)/H_e$). Theoretical predictions for some of these indices were obtained from the work of several authors for some of the scenarios considered:

(a) Vallejo-Marín and Uyenoyama (2008) studied the effects of partial asexuality in the number of alleles of the *S*-locus using a diffusion approximation and found that the number of common alleles ($n_c$, which is approximately equivalent to the effective number of *S*-alleles, $n_e$; Takahata, 1990) is determined by

$$1 = \theta \frac{2Mn_c}{2Mn_c - \theta(n_c-1)(n_c-2)} e^{\frac{2Mn_c}{(n_c-1)(n_c-2)} - \theta} \sqrt{\frac{2\pi(n_c-1)}{Mn_c + \theta(n_c-1)}} \times \left\{ \left[ 1 + \frac{\theta(n_c-1)}{Mn_c} \right] \frac{n_c-2}{n_c} \right\}^{\theta + Mn_c/(n_c-1)} \quad (1)$$

where $M = N(1-c)$ and $\theta = 2N\mu_S$. This equation was solved numerically to obtain the expected effective number of *S*-alleles in the simulations without selection on the viability locus.

(b) Glémin *et al*. (2001) obtained the expected $F_{IS}$ for a neutral locus linked to the *S*-locus as

$$F_{IS} = -\frac{(1+2f_s)(n_c-1)}{1+n_c^2+2f_s(n_c-1)(4Nr'+n_c-1)} \quad (2)$$

where $f_S$ is the scaling factor of the genealogy of the *S*-locus and $r'$ is the net recombination rate between the two loci. For the case of partial asexuality, the number of common alleles was given by Equation (1) and the scaling factor was

$$f_S = \frac{n_c^2(n_c-1)(n_c-2)}{4\theta[2Mn_c - \theta(n_c-1)(n_c-2)]}$$

(Vallejo-Marín and Uyenoyama, 2008). To correct for the reduction of recombination due to asexuality, we used $r' = r(1-c)$, where $r$ was the recombination rate during sexual reproduction (that is, $r = 0.5$ for unlinked loci). This approach ignored mutation on the neutral locus.

(c) Finally, an expression of the expected $F_{IS}$ of a neutral locus in a self-compatible, partially asexual population was derived following Balloux *et al*. (2003):

$$F_{IS} = \frac{\gamma[(1-c)Ns - 1]}{2N + \gamma[1 + cN(s-2) - Ns]} \quad (3)$$

where $\gamma = (1-\mu_N)^2$ and $s$ is the selfing rate. Selfing rate in Balloux *et al*. (2003) model is defined as the rate of self-fertilization of individuals; thus a fertilization event between two individuals from the same genet is considered an outcrossing event. Under random mating, $s = 1/N$ and for negligible mutation rates, $\gamma = 1$.

**Linkage disequilibrium:** The linkage disequilibrium between pairs of loci (*S*-locus-viability locus and the two neutral loci) was studied with the correlation coefficient between two loci, $R_{GGD}$, developed by Garnier-Géré and Dillmann (1992), which is particularly appropriate for partially asexual diploids (de Meeûs and Balloux, 2004).

Fitness: Mean fitness of the population ($\bar{W}$) was calculated from the genotype frequencies as $\bar{W} = f(AA)\times 1 + f(Aa)\times(1-hs) + f(aa)\times(1-s)$, and the average fitness of individuals potentially produced by selfing ($W_s$), outcrossing ($W_o$) and asexuality ($W_a$) were calculated as $W_s = f(AA)\times 1 + f(Aa)\times\left\lfloor \frac{1}{4}\times 1 + \frac{1}{2}\times(1-hs) + \frac{1}{4}\times(1-s)\right\rfloor + f(aa)\times(1-s)$, $W_o = 1\times p_A^2 + (1-hs)\times 2 \times p_A \times p_a + (1-s)\times p_a^2$ (where $p_A$ and $p_a$ are the frequencies of alleles $A$ and $a$) and $W_a = \bar{W}$. Inbreeding depression ($\delta$) and mutation load ($L$) were calculated as $\delta = 1 - (W_s/W_o)$ and $L = 1 - \bar{W}$. In addition, we compared the fitness of individuals produced by asexual reproduction ($W_a$) to the fitness of individuals produced sexually, using the ratio $W_o/W_a$. $W_s$, $W_o$ and $W_a$ were calculated as the fitness of potential offspring if all individuals were produced by selfing, by outcrossing and asexually, respectively. However, these modes of reproduction might not be actually present in the different scenarios considered (for example, in self-incompatible populations, no selfing occurs, but potential $W_s$ and inbreeding depression can be calculated).

Fixation probabilities: To interpret the results, we calculated the probabilities of four events: fixation of alleles $A$ and $a$ in the viability locus, 'fixation' of viability locus heterozygote ($Aa$) and fixation of a genotype in the $S$-locus (reduction of number of $S$-alleles to two, this was only monitored in self-incompatible populations). These fixation probabilities were estimated on 1000 evaluations of the monitored variables as the proportion of times in which $p_a = 0$, $p_a = 1$ and $H_o = 1$ for the viability locus, or $n = 2$ for the $S$-locus.

## Results

### Effect of asexuality and drift on linkage disequilibrium between loci

Probably the most obvious effect of partial asexual reproduction was the reduction of recombination. Asexuality generated identical genotypes in offspring, so allele associations were transmitted in the same way that they would be transmitted if they were physically linked throughout the whole genome. In fully asexual populations, maximum linkage disequilibrium was expected (equivalent to the linkage disequilibrium between fully linked loci) and in partially asexual populations, recombination was reduced proportionally to the rate of asexual reproduction. Figure 1 shows how nonrandom associations of alleles increased with asexual reproduction rates. Linkage disequilibrium levels also depended on population size because drift produced a departure from the expected values of frequencies of allele combinations (Figure 1). Strong drift might have even caused some combinations of alleles to be absent in the population.

In small populations, some generations with complete allelic association started to appear at asexual reproduction rates of 0.8 and were predominant at rates of 0.99 and higher. In larger populations, complete allelic association was only found in the fully asexual scenario. These associations of alleles were observed between loci subject to selection and between neutral loci, which showed that linkage disequilibrium was not a product of selective processes (Figures 1a and b).

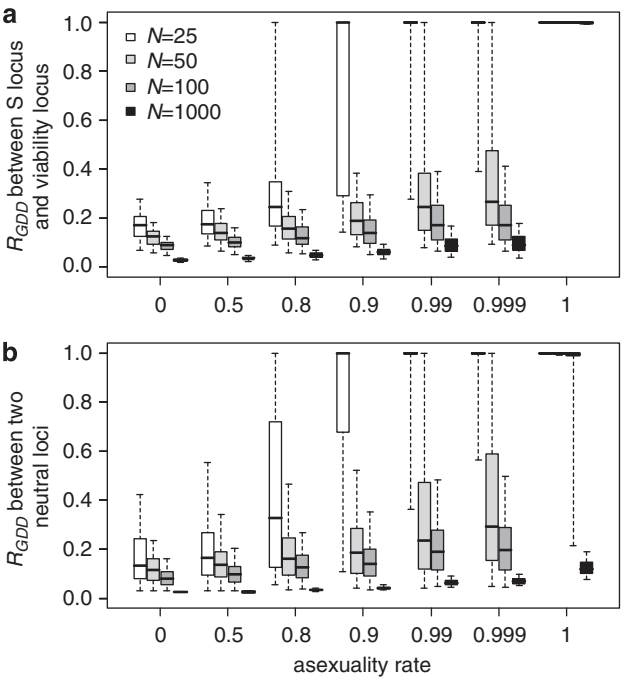


**Figure 1** Linkage disequilibrium ($R_{GDD}$ index, Garnier-Géré and Dillmann, 1992) at increasing rates of asexual reproduction. Box plots represent median (black line), first and third quartiles (box) and 5 and 95% percentiles (whiskers) of 1000 observations from simulations performed at seven rates of asexual reproduction ($c = 0$, 0.5, 0.8, 0.9, 0.99, 0.999 and 1) and four population sizes ($N = 25$, 50, 100 and 1000).(**a**) $R_{GDD}$ index between $S$-locus and viability locus ($s = 0.1$, $h = 0.2$); (**b**) $R_{GDD}$ index between two neutral loci.

Physical linkage between the $S$-locus and loci with deleterious alleles can decrease the number of $S$-alleles (Uyenoyama, 2003). It can also dramatically increase inbreeding depression in small populations (Glémin *et al*., 2001). Therefore, for the present model, it was important to discern whether the linkage disequilibrium generated by asexual reproduction is sufficient for the selective forces of the $S$-locus to interact with the viability locus. The results are presented below taking this perspective into account.

### Effect of asexuality and drift on diversity parameters: number of S-alleles and heterozygote excess

The effective number of $S$-alleles decreased with asexuality (Figure 2) as shown analytically by Vallejo-Marín and Uyenoyama (2008). Balancing selection on the $S$-locus only occurred during sexual reproduction events. Because these events were scarce at high rates of asexual reproduction, the influence of balancing selection (which promotes high allelic diversity) on the population diminished. This can temporarily stop sexual reproduction by reducing the number of $S$-alleles to two (that is, fixation of an $S$-locus genotype, Table 1).

No significant differences in the number of $S$-alleles were found among the three different selection regimes for the viability locus (frequency distributions of the number of $S$-alleles among different selection regimes were undistinguishable in a Kolmogorov–Smirnov test,

*P*-value > 0.98). It must be noted that small populations (with high linkage disequilibrium) were fixed for the wild-type allele *A* most of the time (Table 2b) and this may explain the weak influence of deleterious alleles on the *S*-locus.

Another general effect of partial asexual reproduction in diploids was the reduction of allele segregation. This can increase heterozygosity by the independent accumulation of mutations on the two alleles of an asexual lineage (Pamilo, 1987; Birky, 1996). To quantify this effect, we measured the inbreeding coefficient $F_{IS}$, which compares observed and expected heterozygosities.

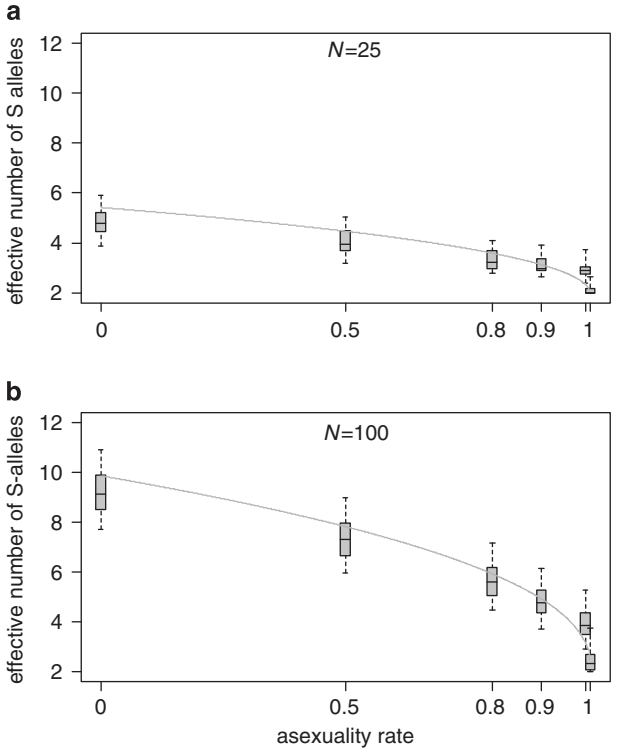


**Figure 2** Effective number of *S*-alleles at increasing rates of asexual reproduction. Box plots represent median (black line), first and third quartiles (gray box) and 5 and 95% percentiles (whiskers) of 1000 observations from the simulations performed at six rates of asexuality ($c = 0$, 0.5, 0.8, 0.9, 0.99 and 1), population size $N = 25$ (**a**) and $N = 100$ (**b**), mutation rate $\mu_S = 10^{-5}$, $s = 0$ and $h = 0$ for the viability locus. Gray line represents the theoretical expected number of common *S*-alleles obtained numerically from Equation (1) (Equation 3.7 in Vallejo-Marín and Uyenoyama (2008)).

Theoretical equilibrium values for $F_{IS}$ at a neutral locus (unlinked to the *S*-locus) were very similar in self-compatible and self-incompatible populations (Figure 3), and, for large population sizes, the simulated populations followed the same pattern (Figure 3b). However, for small population sizes, drift generated strong linkage disequilibrium between the *S*-locus and other loci. Thus, balancing selection on the *S*-locus had a hitchhiking effect on other loci, increasing their heterozygosity. This explained the contrasting $F_{IS}$ values on simulated self-compatible and self-incompatible populations (Figure 3a). Analytical predictions do not reflect this dramatic difference because they do not account for linkage disequilibrium. $F_{IS}$ decreased with asexuality and this decrease was enhanced by SI in small populations, particularly noticeable at asexual reproduction rates higher than 0.8. This decrease in $F_{IS}$ observed in neutral loci also occurred at the viability locus (both for the mildly deleterious and lethal recessive cases), which implied an increase in the frequency of the deleterious allele (Figure 4).

### Effect of asexuality and drift on fitness

For both self-compatible and self-incompatible populations, mutation load globally increased with asexuality and population size, but a greater variance among generations and populations was observed for very small populations (Figure 5). As mentioned above, the frequency of the deleterious allele increased with asexuality in small self-incompatible populations, due to a hitchhiking effect of the *S*-locus over the viability locus. In the SI system, when the population size was small enough, the mutation load increased with asexual reproduction rates; this load resulted from the increase of the deleterious allele frequency (Figures 5a and c). Under similar conditions in the self-compatible system, mutation loads were lower than the mutation load observed in the self-incompatible system (Figures 5b and d). In contrast, inbreeding depression had similar values in self-incompatible and self-compatible populations (Figure 6), showing some increases with asexual reproduction rate for lethal recessive mutations that was more apparent in large populations.

The average fitness was higher in sexual populations than in asexual populations due to Mendelian segregation (for example, Figure 5; Chasnov, 2000; Kirkpatrick and Jenkins, 1989). The relative fitness between individuals produced by sexual and asexual reproduction was influenced very little by partial asexuality in a self-

**Table 1** Genotype fixation probability at the *S*-locus under two different selection regimes at the viability locus

| *Population size* (N) | *Selection regime* | *Rate of asexual reproduction* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | c = *0* | c = *0.5* | c = *0.8* | c = *0.9* | c = *0.99* | c = *0.999* | c = *1* |
| 25 | Mildly deleterious[a] | 0.000 | 0.000 | 0.036 | 0.325 | 0.784 | 0.807 | 0.997 |
| 25 | Lethal recessive[b] | 0.000 | 0.000 | 0.018 | 0.315 | 0.790 | 0.805 | 0.994 |
| 50 | Mildly deleterious | 0.000 | 0.000 | 0.000 | 0.002 | 0.072 | 0.117 | 0.990 |
| 50 | Lethal recessive | 0.000 | 0.000 | 0.000 | 0.001 | 0.075 | 0.090 | 0.989 |
| 100 | Mildly deleterious | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.983 |

The probability of genotype fixation at the *S*-locus (only two alleles in the population) was estimated as the proportion of observations in which this event was recorded. Results in this table are for populations with a mutation rate of $\mu_S = 10^{-5}$ at the *S*-locus.
[a]Viability locus selection and dominance coefficients: $s = 0.1$ and $h = 0.2$.
[b]Viability locus selection and dominance coefficients: $s = 1$ and $h = 0.02$.

**Table 2** Fixation probabilities at the viability locus in a self-incompatible population under two different selection regimes

| *Population size* (N) | Selection regime | *Rate of asexual reproduction* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | c = *0* | c = *0.5* | c = *0.8* | c = *0.9* | c = *0.99* | c = *0.999* | c = *1* |
| *(a) Fixation of deleterious allele a* | | | | | | | | |
| 25 | Mildly deleterious[a] | 0.066 | 0.049 | 0.056 | 0.050 | 0.085 | 0.081 | 0.067 |
| 50 | Mildly deleterious | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.001 | 0.001 |
| 100 | Mildly deleterious | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| *(b) Fixation of allele A* | | | | | | | | |
| 25 | Mildly deleterious | 0.670 | 0.714 | 0.663 | 0.519 | 0.264 | 0.279 | 0.082 |
| 25 | Lethal recessive[b] | 0.831 | 0.783 | 0.767 | 0.581 | 0.293 | 0.317 | 0.085 |
| 50 | Mildly deleterious | 0.583 | 0.541 | 0.574 | 0.507 | 0.474 | 0.466 | 0.139 |
| 50 | Lethal recessive | 0.661 | 0.635 | 0.596 | 0.564 | 0.518 | 0.493 | 0.150 |
| 100 | Mildly deleterious | 0.314 | 0.306 | 0.320 | 0.294 | 0.274 | 0.290 | 0.187 |
| 100 | Lethal recessive | 0.437 | 0.361 | 0.318 | 0.313 | 0.269 | 0.296 | 0.189 |
| *(c) Fixation of genotype Aa* | | | | | | | | |
| 25 | Mildly deleterious | 0.000 | 0.000 | 0.023 | 0.184 | 0.472 | 0.467 | 0.723 |
| 25 | Lethal recessive | 0.000 | 0.000 | 0.013 | 0.212 | 0.551 | 0.569 | 0.879 |
| 50 | Mildly deleterious | 0.000 | 0.000 | 0.000 | 0.000 | 0.026 | 0.050 | 0.585 |
| 50 | Lethal recessive | 0.000 | 0.000 | 0.000 | 0.000 | 0.032 | 0.041 | 0.721 |
| 100 | Mildly deleterious | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.230 |
| 100 | Lethal recessive | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.317 |

The probability of fixation at the viability locus (fixation of genotypes, *AA*, *aa* or *Aa*) was estimated as the proportion of observations in which fixation was recorded.
[a]Viability locus selection and dominance coefficients: $s = 0.1$ and $h = 0.2$.
[b]Viability locus selection and dominance coefficients: $s = 1$ and $h = 0.02$.

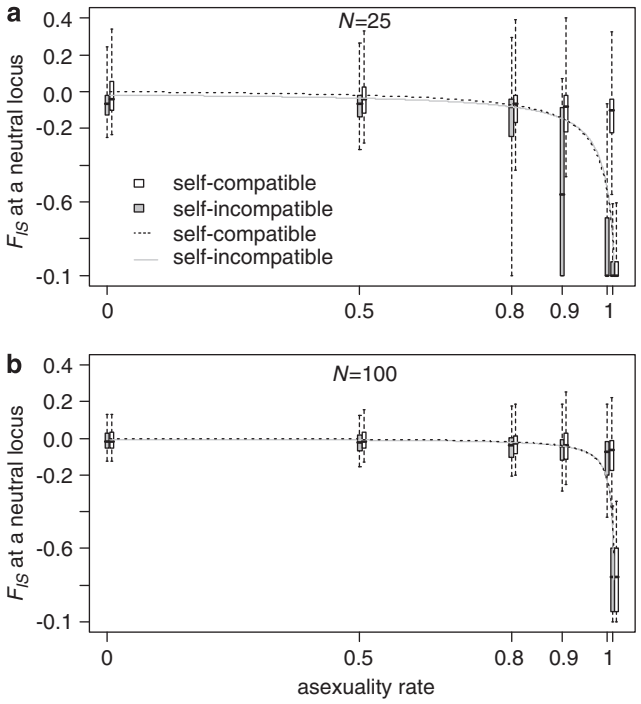


**Figure 3** Inbreeding coefficient $F_{IS}$ at a neutral locus at increasing rates of asexual reproduction. Box plots represent median (black line), first and third quartiles (white and gray boxes, for self-compatible and self-incompatible populations, respectively) and 5 and 95% percentiles (whiskers) of 1000 observations from the simulations performed at six rates of asexuality ($c = 0$, 0.5, 0.8, 0.9, 0.99 and 1), mutation rates $\mu_S = 10^{-5}$ and $\mu_N = 10^{-3}$, and population size (**a**) $N = 25$ and (**b**) $N = 100$. Theoretical equilibrium values for $F_{IS}$ at a neutral locus in a self-compatible, random-mating population (dotted black line, from Equation (3) neglecting mutation) and in a self-incompatible population (continuous gray line, from Equation (2)) are also represented.

compatible population (Figures 7b and d). However, in small self-incompatible populations, the asexual reproduction rate increased the advantage of asexual compared with sexual reproduction (Figures 7a and c). These populations consisted mainly of heterozygous individuals (Figure 3). Therefore, asexual reproduction produced mainly *Aa* individuals, with only slightly lower fitness than the fittest *AA* haplotype. However, sexual reproduction produced a high proportion of *aa* individuals, reducing the average fitness of offspring, a reduction that was not compensated by the production of the fittest *AA* individuals. This could potentially lead to a positive feedback effect on the asexual reproduction rate, but this was not studied in our model where the asexual reproduction rate was a fixed parameter.

## Discussion

The number of *S*-alleles and inbreeding depression are considered to be the main factors that influence the maintenance or breakdown of SI systems (Charlesworth and Charlesworth, 1979). A decrease in the number of *S*-alleles is expected to favor the breakdown of SI because self-compatible mutants can invade the population, whereas the maintenance of inbreeding depression within populations prevents the breakdown of SI. In this study, we examined how drift and partial asexuality may modify the dynamics of these two key parameters in a self-incompatible population.

### Drift, combined with partial asexuality, dramatically reduced the number of S-alleles

As already predicted by Yokoyama and Hetherington (1982), we showed that the number of *S*-alleles decreases with the effective size of the population. Thus, in small

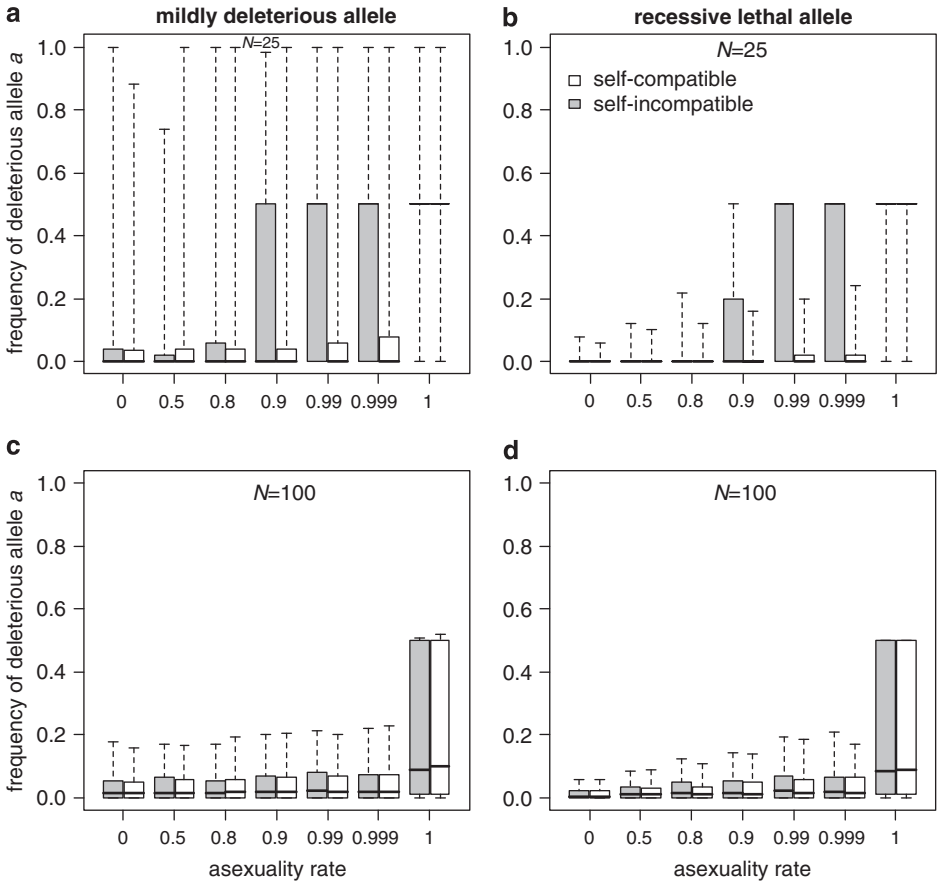


**Figure 4** Frequency of deleterious alleles at increasing rates of asexual reproduction under two selection regimes. Box plots represent median (black line), first and third quartiles (white and gray boxes, for self-compatible and self-incompatible populations, respectively) and 5 and 95% percentiles (whiskers) of 1000 observations from the simulations performed at seven rates of asexuality ($c = 0$, 0.5, 0.8, 0.9, 0.99, 0.999 and 1), mutation rates $\mu_S = 10^{-5}$ and $\mu_N = 10^{-3}$. Population size and viability locus coefficients: (**a**) $N = 25$, $s = 0.1$, $h = 0.2$; (**b**) $N = 25$, $s = 1$, $h = 0.02$; (**c**) $N = 100$, $s = 0.1$, $h = 0.2$; and (**d**) $N = 100$, $s = 1$, $h = 0.02$.

populations, repeated bottlenecks or founder events may lead to the breakdown of the SI system. However, Karron (1987) found no differences in mating systems between rare and widespread congeners across several families (but see also a study on some Brassicaceae species, Kunin and Shmida (1997)). As for the impact of asexuality, our simulation study confirmed a recent result shown analytically by Vallejo-Marín and Uyenoyama (2008). The number of *S*-alleles decreased with asexuality due to the weakened influence of balancing selection in a partially asexual population (Figure 2). In contrast with other effects of asexuality that were only observed for very high rates of asexuality, intermediate asexual reproduction rates were sufficient to significantly reduce the number of *S*-alleles. High rates of asexual reproduction combined with drift produced extremely low numbers of *S*-alleles. This effect was strong enough to even reduce the number of *S*-alleles to two (that is, fixation of an *S*-locus genotype, Table 1), which stopped all possibility of sexual reproduction until a new *S*-allele arose from mutation or migration. Therefore, in such extreme cases, this drastic effect of asexuality on the number of *S*-alleles should favor the breakdown of SI. However, paradoxically, the number of *S*-alleles may not be very relevant in a system where asexuality serves to provide reproductive assurance.

### Drift, combined with partial asexuality and SI, led to an increase in mutation load

Drift influenced the level of mutation load. As population size decreased, the frequency of the deleterious allele decreased and mutation load decreased due to more effective purging. We also observed a much greater variance in mutation load in very small populations (Table 2 and Figure 5, $N = 25$). In this case, selection was overwhelmed by drift and this caused a higher probability of fixation of the deleterious allele and an increase in mutation load. Similar results have been described by Bataillon and Kirkpatrick (2000), Glémin (2003) and Haag and Roze (2007).

Drift is also a key component of mutation load in asexual populations (Haag and Roze, 2007). Indeed, large asexual and sexual populations showed comparable frequencies of the deleterious allele and comparable mutation loads (Figure 5; see also Haag and Roze (2007)). However, mutation load was greater in small asexual populations than in small sexual ones. This could be explained by the absence of segregation in asexual populations, in which heterozygous individuals are present in high frequencies (Table 2 and Figure 5; see also Haag and Roze (2007)).

In this study, we additionally showed that SI had a function in increasing mutation load in partially

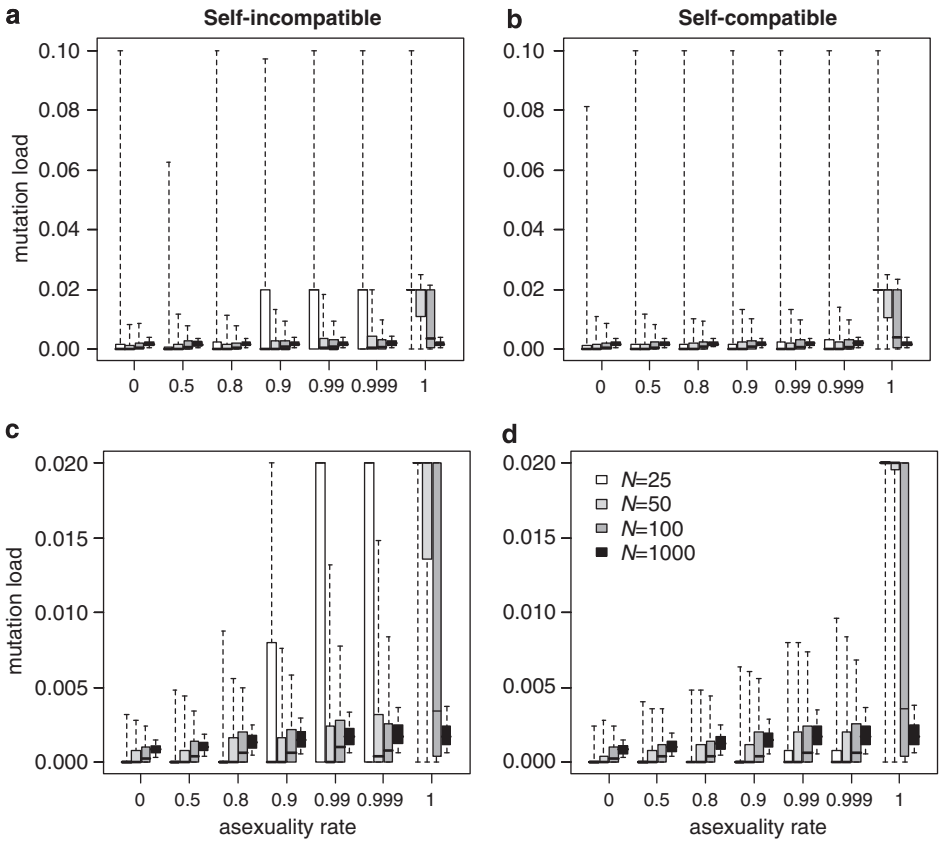


**Figure 5** Mutation load at increasing rates of asexual reproduction under two different selection regimes according to reproductive system. Box plots represent median (black line), first and third quartiles (box) and 5 and 95% percentiles (whiskers) of 1000 observations from simulations performed at seven rates of asexual reproduction ($c = 0$, 0.5, 0.8, 0.9, 0.99, 0.999 and 1) and four population sizes ($N = 25$, 50, 100 and 1000). (**a**) Self-incompatible population with a viability locus with mildly deleterious allele ($s = 0.1$, $h = 0.2$), (**b**) self-compatible population with a viability locus with mildly deleterious allele ($s = 0.1$, $h = 0.2$), (**c**) self-incompatible population with a viability locus with highly recessive lethal allele ($s = 1$, $h = 0.02$) and (**d**) self-compatible population with a viability locus with highly recessive lethal allele ($s = 1$, $h = 0.02$).

asexual, self-incompatible populations. Partial asexual reproduction and small population sizes produced strong associations (that is, linkage disequilibrium) between deleterious alleles (on any locus of the genome) and *S*-alleles. Glémin *et al*. (2001) have already shown that deleterious mutations on loci linked to the *S*-locus may be sheltered by balancing selection acting on rare *S*-alleles. Asexuality and SI both favored the increase of the heterozygote genotype *Aa* within the population, leading to an increase of mutation load in small self-incompatible, partially asexual populations.

### Inbreeding depression remained low in small, partially asexual, self-incompatible populations

Inbreeding depression followed different patterns than the ones observed for mutation load. For very small populations, inbreeding depression is expected to decrease (Bataillon and Kirkpatrick, 2000), due to the absence of polymorphism at the viability locus. This was observed in small self-compatible and self-incompatible populations (Glémin *et al*. (2001), and our own simulations, Figure 6). As for the impact of asexuality, our results showed no large differences between the self-compatible and the self-incompatible cases for small populations. The effect of strong drift, in the presence of strong asexuality, was to reduce genotype diversity. In most cases, populations were fixed at one allele, but sometimes the population was fixed at the heterozygote genotype *Aa*. This mainly occurred in self-incompatible populations (see Table 2). With no or very low genotype diversity, inbreeding depression had very low values, even if deleterious alleles were present at high frequencies in the population.

Chen *et al*. (1997) proposed that asexuality would favor the maintenance of SI. Their reasoning was that asexual reproduction would increase a population's genetic load due to lethal recessive mutations, which was confirmed by our simulation results. However, mutation load and inbreeding depression did not show parallel trends, and the high level of mutation load due to the combination of drift, asexuality and SI was accompanied by low levels of inbreeding depression. Nevertheless, inbreeding depression generally tended to increase with asexuality due to the accumulation of deleterious alleles and this should favor the maintenance of SI.

### Evolution of partially asexual, self-incompatible populations: a third possible outcome

Until now, the evolutionary interest of partially asexual, self-incompatible populations has been focused on two

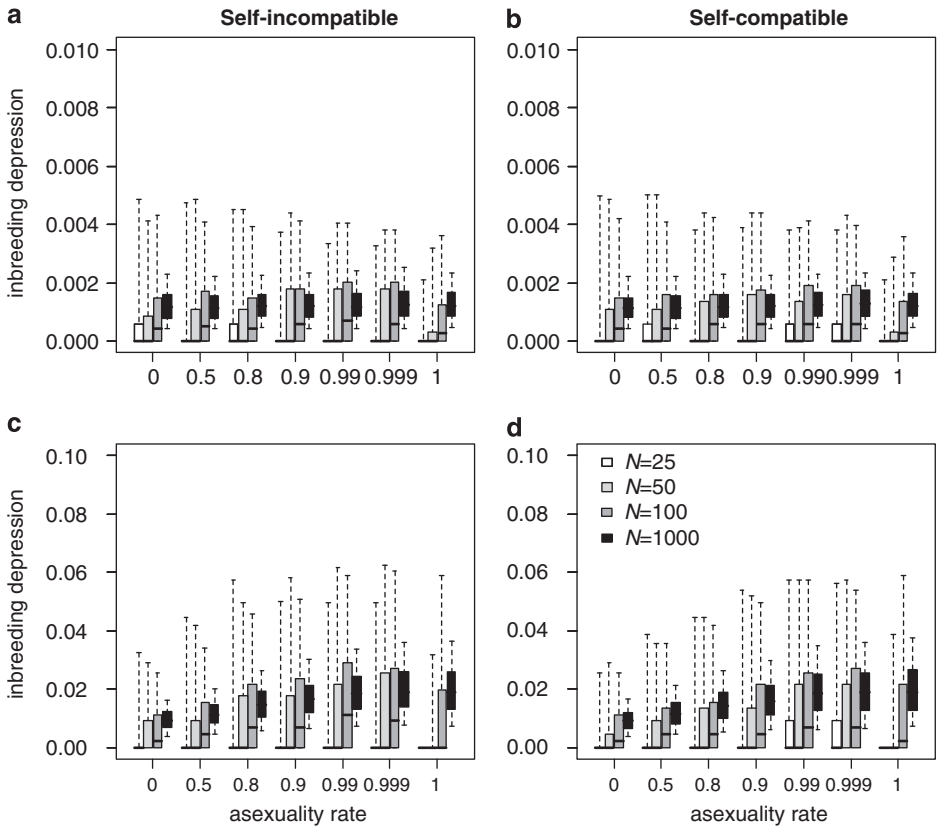


**Figure 6** Inbreeding depression at increasing rates of asexual reproduction and under two different selection regimes according to reproductive system. Box plots represent median (black line), first and third quartiles (box) and 5 and 95% percentiles (whiskers) of 1000 observations from simulations performed at seven rates of asexual reproduction ($c = 0$, 0.5, 0.8, 0.9, 0.99, 0.999 and 1) and four population sizes ($N = 25$, 50, 100 and 1000). (**a**) Self-incompatible population with a viability locus with mildly deleterious allele ($s = 0.1$, $h = 0.2$), (**b**) self-compatible population with a viability locus with mildly deleterious allele ($s = 0.1$, $h = 0.2$), (**c**) self-incompatible population with a viability locus with highly recessive lethal allele ($s = 1$, $h = 0.02$) and (**d**) self-compatible population with a viability locus with highly recessive lethal allele ($s = 1$, $h = 0.02$).

possible outcomes: (1) the system is stable and SI is maintained or (2) the system is unstable and SI disappears (Chen *et al*., 1997; Young *et al*., 2002; Vallejo-Marín, 2007; Vallejo-Marín and O'Brien, 2007). However, a third possible outcome should be considered: being the unstable system, sexual reproduction completely disappears. This type of event was observed in our simulations due to a reduction in the number of *S*-alleles to two (see above). However, we also studied how selection on the viability locus could contribute to the potential loss of sexuality. Our results showed that the fitness of individuals produced by asexual reproduction ($W_a$) was greater than that of individuals produced by random sexual mating ($W_o$) in small populations (Figure 7). Fitness of individuals (potentially) produced by selfing ($W_s$) was also lower than $W_a$ in small self-incompatible populations, suggesting that asexuality may have an immediate advantage over selfing under such circumstances.

Therefore, we speculate that asexual reproduction could take over sexual reproduction more easily than self-compatibility over SI when low numbers of *S*-alleles reduce mate availability. Despite the effect of asexuality on inbreeding depression and *S*-locus diversity, asexual reproduction may offer an alternative means of reproduction under these adverse conditions and SI may be temporarily dormant instead of breaking down. Asexual populations could subsequently recover a sexual self-incompatible reproductive system when new *S*-alleles are introduced in the population (for instance, by migration). However, prolonged periods of asexual reproduction may facilitate the accumulation of mutations affecting sexual traits, including SI, because selective pressures are ineffective for these traits (Eckert, 2001). Thus, alternative periods of sexual and asexual reproduction may be necessary for such dynamics, which is probably the case for plants combining asexual and sexual reproduction (Bengtsson and Ceplitis, 2000). Nevertheless, the scenarios described here need to be confirmed by further and more complex simulations and other factors may favor self-compatible sexual reproduction, such as the more effective purging of deleterious mutations.

## Perspectives

In this study, we used a model with physically unlinked loci and a single viability locus. However, because the linkage disequilibrium produced by asexuality affected the whole-genome, population genomic models may be necessary to fully study the effect of deleterious alleles on the *S*-locus for small, partially asexual populations. In

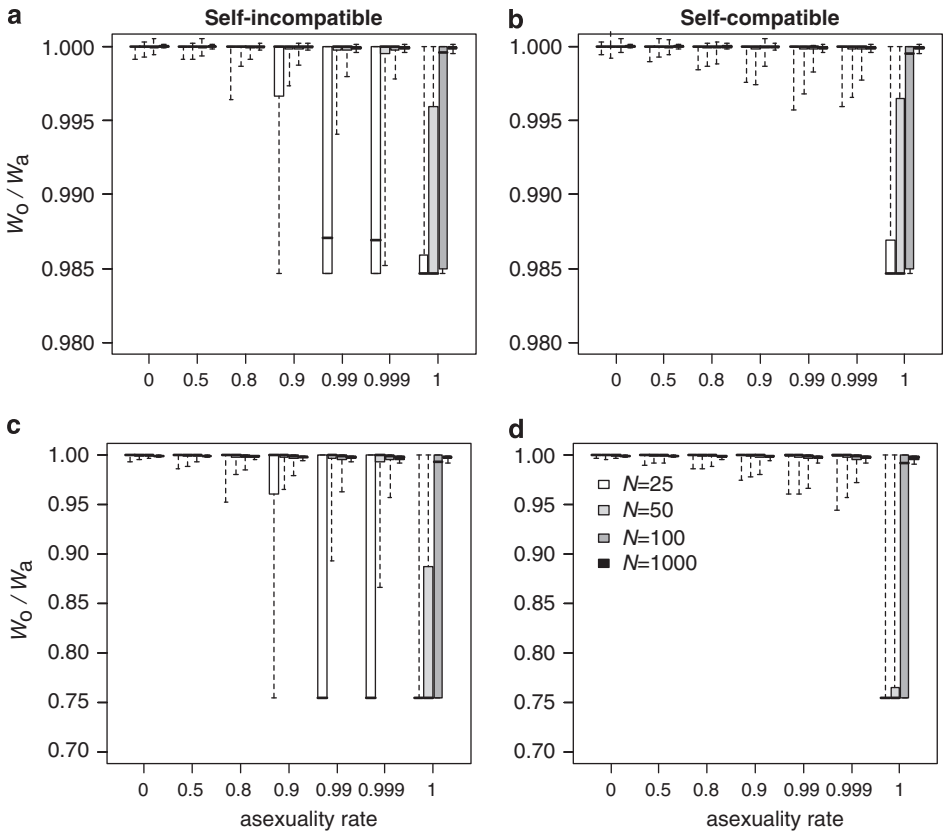


**Figure 7** Fitness ratio between sexually and asexually produced individuals at increasing rates of asexual reproduction under two different selection regimes according to reproductive system. Box plots represent median (black line), first and third quartiles (box) and 5 and 95% percentiles (whiskers) of 1000 observations from simulations performed at seven rates of asexual reproduction ($c = 0$, 0.5, 0.8, 0.9, 0.99, 0.999 and 1) and four population sizes ($N = 25$, 50, 100 and 1000). (**a**) Self-incompatible population with a viability locus with mildly deleterious allele ($s = 0.1$, $h = 0.2$), (**b**) self-compatible population with a viability locus with mildly deleterious allele ($s = 0.1$, $h = 0.2$), (**c**) self-incompatible population with a viability locus with highly recessive lethal allele ($s = 1$, $h = 0.02$) and (**d**) self-compatible population with a viability locus with highly recessive lethal allele ($s = 1$, $h = 0.02$).

addition, physically linked loci with deleterious alleles (which were not studied here) may reduce the number of *S*-alleles (Uyenoyama, 2003) and the strength of that effect may be influenced by the rate of asexuality as discussed by Vallejo-Marín and Uyenoyama (2008). Moreover, in this study, it was not possible to investigate the evolutionary dynamics of mating system and asexuality modifiers because these were assumed to be fixed parameters. It would be then interesting to develop a model to test the invasion propensity of self-compatible or asexual mutants. Finally, models incorporating migration or population structure are necessary to study more realistic dynamics of partially asexual, self-incompatible populations. Population structure is expected to affect the genetic diversity of viability and the *S*-locus; for instance, by introducing a third allele by migration within subpopulations fixed at two *S*-alleles. These models should also take into account the specificities of the different types of asexual reproduction where, for instance, the asexual propagules may have a lower dispersal capacity (for example, vegetative reproduction) and may create spatial clusters of individuals with the same genotype.

Recent theoretical work (Vallejo-Marín and Uyenoyama, 2008) and our own model lead to several predictions about *S*-locus diversity, mutation load and inbreeding depression within partially asexual self-incompatible populations. However, we need more experimental observations to test and validate these theoretical advances. First, as outlined by Vallejo-Marín and O'Brien (2007), if asexuality relieves selective pressures favoring the breakdown of SI, the co-occurrence of asexuality and SI should be frequent. Association of SI and asexuality has been studied in only a few monospecific studies (*R. leiolepis*, Young *et al*., 2002; *Eucalyptus morrisbyi*, Jones *et al*., 2005; *Prunus avium*, Stoeckel *et al*., 2006). The association was found at a multispecific level by Chen *et al*. (1997) in Australian Droseraceae and by Vallejo-Marín and O'Brien (2007) in Solanaceae. More reports on species combining SI and asexuality would enhance our understanding of pressures explaining their maintenance. Second, experimental measurements of asexual reproduction rates and number of *S*-alleles in species showing various levels of asexual reproduction would be useful to test predictions from our simulations. Although experimental measurements of inbreeding depression in self-incompatible species are available (Mena-Alí *et al*., 2008), studies comparing inbreeding depression from different populations with contrasting sizes and rates of asexual reproduction are lacking.

## Conclusions

This study investigated for the first time the effect of partial asexual reproduction on the fitness of self-incompatible populations. For loci unlinked to the *S*-locus and large populations, fitness values were similar

to those of a self-compatible population. However, in small populations, the combination of drift, asexuality and SI increased mutation load due to the accumulation of deleterious alleles. This increase in the mutation load was accompanied by a selective advantage of asexually produced offspring compared to sexually produced offspring. Therefore, future studies addressing the maintenance of SI in partially asexual populations (by studying the invasibility of self-compatible genotypes) will need to (1) account for whole-genome fitness and (2) acknowledge that the process may not be independent of the maintenance of sex itself (that is, will need to establish whether modifiers of asexual reproduction rates invade more easily than self-compatible alleles).

## Acknowledgements

Collaboration between MN and SM was promoted through the implementation of the REPROFOR project, financed by the Spanish Ministry for Education and Science. We thank Santiago C González-Martínez and Ricardo Alía for helping to develop this collaboration. E Porcher is acknowledged for helpful discussions on self-incompatibility evolution. We are also very thankful to P Garnier-Géré for a detailed discussion on linkage disequilibrium estimators.

## References

Balloux F, Lehmann L, de Meeûs T (2003). The population genetics of clonal and partially clonal diploids. *Genetics* **164**: 1635–1644.

Barrett SCH (2002). The evolution of plant sexual diversity. *Nat Rev Genet* **3**: 274–284.

Bataillon T, Kirkpatrick M (2000). Inbreeding depression due to mildly deleterious mutations in finite populations: size does matter. *Genet Res* **75**: 75–81.

Bengtsson BO, Ceplitis A (2000). The balance between sexual and asexual reproduction in plants living in variable environments. *J Evolution Biol* **13**: 415–422.

Birky CW (1996). Heterozygosity, heteromorphy, and phylogenetic trees in asexual eukaryotes. *Genetics* **144**: 427–437.

Brennan AC, Harris SA, Hiscock SJ (2003). The population genetics of sporophytic self-incompatibility in *Senecio squalidus* L (Asteraceae): avoidance of mating constraints imposed by low S-allele number. *Philos Trans R Soc B* **358**: 1047–1050.

Charlesworth D, Charlesworth B (1987). Inbreeding depression and its evolutionary consequences. *Annu Rev Ecol Syst* **18**: 237–268.

Charlesworth D, Charlesworth B (1979). The evolution and breakdown of S-allele systems. *Heredity* **43**: 41–55.

Chasnov JR (2000). Mutation-selection balance, dominance and the maintenance of sex. *Genetics* **156**: 1419–1425.

Chen L, Stace HM, James SH (1997). Self-incompatibility, seed abortion and clonality in the breeding systems of several Western Australian *Drosera* species (Droseraceae). *Aust J Bot* **45**: 191–201.

Eckert CG (2001). The loss of sex in clonal plants. *Evol Ecol* **15**: 501–520.

Fisher RA (1941). Average excess and average effect of a gene substitution. *Ann Eugen* **11**: 53–63.

Garnier-Géré P, Dillmann C (1992). A computer program for testing pairwise linkage disequilibria in subdivided populations. *J Hered* **83**: 239.

Glémin S (2003). How are deleterious mutations purged? Drift versus nonrandom mating. *Evolution* **57**: 2678–2687.

Glémin S, Bataillon T, Ronfort J, Mignot A, Olivieri I (2001). Inbreeding depression in small populations of self-incompatible plants. *Genetics* **159**: 1217–1229.

Haag CR, Roze D (2007). Genetic load in sexual and asexual diploids: segregation, dominance and genetic drift. *Genetics* **176**: 1663–1678.

Igic B, Lande R, Kohn JR (2008). Loss of self-incompatibility and its evolutionary consequences. *Int J Plant Sci* **169**: 93–104.

Jones RC, McKinnon GE, Potts BM, Vaillancourt RE (2005). Genetic diversity and mating system of an endangered tree *Eucalyptus morrisbyi*. *Aust J Bot* **53**: 367–377.

Karron JD (1987). A comparison of levels of genetic polymorphism and self-compatibility in geographically restricted and widespread plant congeners. *Evol Ecol* **1**: 47–58.

Kimura M, Crow JF (1964). The number of alleles that can be maintained in a finite population. *Genetics* **49**: 725–738.

Kirkpatrick M, Jenkins CD (1989). Genetic segregation and the maintenance of sexual reproduction. *Nature* **339**: 300–301.

Kunin WE, Shmida A (1997). Plant reproductive traits as a function of local, regional, and global abundance. *Conserv Biol* **11**: 183–192.

de Meeûs T, Balloux F (2004). Clonal reproduction and linkage disequilibrium in diploids: a simulation study. *Infect Genet Evol* **4**: 345–351.

de Meeûs T, Prugnolle F, Agnew P (2007). Asexual reproduction: genetics and evolutionary aspects. *Cell Mol Life Sci* **64**: 1355–1372.

Mena-Alí J, Keser L, Stephenson A (2008). Inbreeding depression in *Solanum carolinense* (Solanaceae), a species with a plastic self-incompatibility response. *BMC Evol Biol* **8**: 10.

Mena-Alí J, Keser L, Stephenson A (2009). The effect of sheltered load on reproduction in *Solanum carolinense*, a species with variable self-incompatibility. *Sex Plant Reprod* **22**: 63–71.

Muirhead CA, Lande R (1997). Inbreeding depression under joint selfing, outcrossing, and asexuality. *Evolution* **51**: 1409–1415.

de Nettancourt D (1977). *Incompatibility in Angiosperms*. Springer Berlin Heidelberg: New York.

Pamilo P (1987). Heterozygosity in apomictic organisms. *Hereditas* **107**: 95–101.

Reinartz JA, Les DH (1994). Bottleneck-induced dissolution of self-incompatibility and breeding system consequences in *Aster furcatus* (Asteraceae). *Am J Bot* **81**: 446–455.

Stoeckel S, Grange J, Fernandez-Manjarres JF, Bilger I, Frascaria-Lacoste N, Mariette S (2006). Heterozygote excess in a self-incompatible and partially clonal forest tree species—*Prunus avium* L. *Mol Ecol* **15**: 2109–2118.

Stone JL (2004). Sheltered load associated with S-alleles in *Solanum carolinense*. *Heredity* **92**: 335–342.

Takahata N (1990). A simple genealogical structure of strongly balanced allelic lines and trans-species evolution of polymorphism. *Proc Natl Acad Sci USA* **87**: 2419–2423.

Uyenoyama MK (2003). Genealogy-dependent variation in viability among self-incompatibility genotypes. *Theor Popul Biol* **63**: 281–293.

Vallejo-Marín M (2007). The paradox of clonality and the evolution of self-incompatibility. *Plant Signal Behav* **2**: 265–266.

Vallejo-Marín M, O'Brien HE (2007). Correlated evolution of self-incompatibility and clonal reproduction in *Solanum* (Solanaceae). *New Phytol* **173**: 415–421.

Vallejo-Marín M, Uyenoyama MK (2008). On the evolutionary modification of self-incompatibility: implications of partial clonality for allelic diversity and genealogical structure. In: Franklin-Tong VE (ed). *Self-Incompatibility in Flowering Plants. Evolution, Diversity, and Mechanisms*. Springer: Berlin, pp 53–71.

Yokoyama S, Hetherington LE (1982). The expected number of self-incompatibility alleles in finite plant populations. *Heredity* **48**: 299–303.

Young AG, Hill JH, Murray BG, Peakall R (2002). Breeding system, genetic diversity and clonal structure in the subalpine forb *Rutidosis leiolepis* F Muell. (Asteraceae). *Biol Conserv* **106**: 71–78.