

The mutation load under tetrasomic inheritance and its consequences for the evolution of the selfing rate in autotetraploid species

J. RONFORT*

Laboratoire de Génétique et d'Amélioration des Plantes, Institut National de la Recherche Agronomique, Domaine de Melgueil, 34130 Mauguio, France

(Received 8 July 1998 and in revised form 5 November 1998 and 15 January 1999)

Summary

Single-locus equilibrium frequencies of a partially recessive deleterious mutation under the mutation–selection balance model are derived for partially selfing autotetraploid populations. Assuming multiplicative fitness interactions among loci, approximate solutions for the mean fitness and inbreeding depression values are also derived for the multiple locus case and compared with expectations for the diploid model. As in diploids, purging of deleterious mutations through consanguineous matings occurs in autotetraploid populations, i.e. the equilibrium mutation load is a decreasing function of the selfing rate. However, the variation of inbreeding depression with the selfing rate depends strongly on the dominance coefficients associated with the three heterozygous genotypes. Inbreeding depression can either increase or decrease with the selfing rate, and does not always vary monotonically. Expected issues for the evolution of the selfing rate consequently differ depending on the dominance coefficients. In some cases, expectations for the evolution of the selfing rate resemble expectations in diploids; but particular sets of dominance coefficients can be found that lead to either complete selfing or intermediate selfing rates as unique evolutionary stable state.

1. Introduction

Inbreeding depression, the reduced fitness of inbred relative to outbred offspring, is of major importance for population biology. For example, it plays a primary role in the evolution of mating systems (Thornhill, 1993) and more notably in that of the selfing rate (Jarne & Charlesworth, 1993; Uyenoyama *et al.*, 1993; Charlesworth & Charlesworth, 1998). Two genetic models can account for the genetic basis of inbreeding depression, namely partial dominance and overdominance (Charlesworth & Charlesworth, 1987). I consider here the former only, as its importance has generally been supported by empirical studies. Under this model, an interesting result is the decrease of inbreeding depression with increasing selfing rates. This has been obtained by analysing a mutation–selection model at a single locus (Ohta & Cockerham, 1974; Lande & Schemske, 1985), as well

as multilocus deterministic calculations (assuming multiplicative fitness effects among loci; Charlesworth *et al.*, 1990, 1991). Purging of deleterious alleles, i.e. the reduction of the equilibrium frequency of mutant alleles expected under consanguineous matings when compared with random mating, is the cause of this phenomenon (Wright, 1977; Lande & Schemske, 1985). This occurs because self-fertilization increases the proportion of homozygous genotypes and leads to the elimination of recessive and partially recessive deleterious alleles.

The fact that inbreeding depression is thought to play an important role in the evolution of mating systems, and an interest in its joint evolution with selfing, have motivated numerous theoretical studies (Lande & Schemske, 1985; Charlesworth *et al.*, 1990, 1991; Uyenoyama & Waller, 1991; Ronfort & Couvet, 1995). However, most of the theory has been concerned with diploid organisms (but see Bennett, 1976; Lande & Schemske, 1985). Extension of the theory to polyploid organisms would be useful, as 30–47% of the flowering plants should be polyploid

* Tel: +33 4 67 29 06 37. Fax: +33 4 67 29 39 90. e-mail: ronfort@ensam.inra.fr

(Stebbins, 1971; Grant, 1981; Masterson, 1994). Polyploid species are commonly classified into two major types according to their presumed origin: allopolyploids are thought to result from hybridization between different taxa and subsequent chromosome doubling, while autopolyploids presumably stem from the chromosome doubling of one diploid genome, primarily by fusion of unreduced gametes (Bever & Felber, 1992; Bretagnolle & Thompson, 1995). Originally thought to be rare and maladaptive compared with allopolyploidy, autopolyploidy has more recently been recognized as common and of evolutionary importance (Levin, 1983; Crawford, 1985; Soltis & Soltis, 1989; Rieseberg & Doyle, 1989). In contrast to allopolyploids, in which inheritance is disomic, autopolyploids are generally characterized by polysomic inheritance which follows from multivalent pairing of homologous chromosomes during meiosis. Multivalent formation leads to complex and somewhat variable patterns of segregation varying from random assortments of homologous chromosomes to random assortments of chromatids, so that two sister chromatids can migrate into the same gamete. This latter phenomenon, known as ‘double reduction’, is specific to autopolyploid species and may occur when there is a recombination between the centromere and the locus under consideration (see Bever & Felber, 1992).

The expected inbreeding depression in polyploids relative to diploids depends on assumptions about the polyploid type (auto- versus allopolyploid) and the selective regime (for a review see Bever & Felber, 1992). Under the selection–mutation model and multiplicative interactions among loci, Lande & Schemske (1985) pointed out that, for lethal or sublethal recessive alleles, the equilibrium inbreeding depression in tetraploids, under either disomic or tetrasomic inheritance, should be nearly half that of the diploid progenitor. For partially recessive deleterious alleles, Bennett (1976) argued that autotetraploid populations should accumulate higher frequencies of mutant alleles and hence higher magnitudes of inbreeding depression than diploids. A recent empirical study comparing inbreeding depression between an autotetraploid species and its diploid progenitor (Husband & Schemske, 1997) supports the model of Lande & Schemske (1985).

The aim of the present paper is to determine how the mutation load and inbreeding depression vary with the selfing rate under partial dominance in autotetraploid species. Throughout the paper, the terms autopolyploid (autotetraploid) and polyploid (tetraploid) are used interchangeably, unless otherwise stated, and I assume random assortment of homologous chromosomes into gametes (tetrasomic inheritance, without double reduction). Following the treatment of the diploid case by Ohta & Cockerham (1974), I first derive single locus genotypic frequencies

in partially selfing populations at equilibrium between mutation and selection. Multilocus approximations are then computed assuming multiplicative fitness interactions among loci (i.e. independence of the load due to different loci). These results are used to examine three questions related to the evolution of the selfing rate: (i) Does purging occur in autotetraploids? (ii) Is inbreeding depression higher in tetraploids than in diploids? and (iii) How does inbreeding depression vary with the selfing rate?

2. Mutation – selection equilibrium in partially selfing autotetraploid populations

(i) General model

Consider an infinite population of tetraploid, hermaphroditic individuals with discrete generations. Mutation, reproduction and selection occur successively during the life cycle. A fraction r of the individuals self-fertilize, the remaining $(1-r)$ reproducing through complete outcrossing. Individual genomes contain a large number of loci evolving under mutation and selection. At each locus, mutation from the wild-type allele A to the mutant form a occurs independently at a rate μ per generation. The selective disadvantage of heterozygotes $AAAa$, $AAaa$ and $Aaaa$ are h_1s , h_2s and h_3s respectively, where s denotes the selection coefficient against a homozygotes and h_1 , h_2 , h_3 are the dominance coefficients. Table 1 gives the notation used throughout the paper for the genotype frequencies and the associated fitness values. In order to neglect the possible occurrence of double reduction, I finally assume that there is no recombination between the centromere and the fitness determining loci.

(ii) Single-locus genotypic frequencies at equilibrium

Recurrence equations for the genotype frequencies across generations are given in the Appendix for a single locus subject to mutation and selection. I was not able to compute exact solutions for these equations. However, given the expected low frequencies of the mutant allele, terms such as $H_1(t) \cdot \mu$, $g(Aa) \cdot g(aa)$ or $g(aa)^2$ (where $g(Aa)$ and $g(aa)$ denote the relative proportion of Aa and aa gametes, respectively) can be neglected. The five equilibrium genotypic frequencies can then be approximated and computed as functions of s , h_1 , h_2 , h_3 , μ and r (Appendix). From these equilibrium solutions, the frequency of the deleterious allele (q_e) and the mutation load (L) can be inferred at equilibrium between mutation and selection. Only the main results are reported below (see Appendix for details).

Randomly mating population ($r = 0$). Under random mating, the approximate equilibrium frequency of the

Table 1. Notation used throughout the paper for fitness values and genotypic frequencies (*t* is the time in generations)

	Genotypes				
	<i>AAAA</i>	<i>AAAa</i>	<i>AAaa</i>	<i>Aaaa</i>	<i>aaaa</i>
Fitness	1	$w_1 = 1 - h_1 s$	$w_2 = 1 - h_2 s$	$w_3 = 1 - h_3 s$	$w_4 = 1 - s$
Frequency before selection	$D(t)$	$H_1(t)$	$H_2(t)$	$H_3(t)$	$R(t)$
Frequency after selection	$D'(t)$	$H'(t) = H_1(t) \cdot w_1$	$H'_2(t) = H_2(t) \cdot w_2$	$H'_3(t) = H_3(t) \cdot w_3$	$R'(t) = R(t) \cdot w_4$

deleterious allele and the equilibrium mutation load are respectively:

for $h_1 \neq 0$,

$$q_e \approx \frac{\mu}{sh_1} \tag{1}$$

and

$$L \approx 4\mu; \tag{2}$$

and for completely recessive alleles ($h_1 = h_2 = h_3 = 0$),

$$q_e \approx \sqrt[4]{\mu/s} \tag{3}$$

and

$$L \approx \mu \tag{4}$$

In the diploid case, the corresponding results for the partial dominance model are $q_e \approx \mu/(sh)$ and $L = 2\mu$ for $h \neq 0$, assuming that the fitness values associated with *AA*, *Aa* and *aa* are 1, $1 - hs$ and $1 - s$ respectively. $q_e \approx \sqrt{\mu/s}$ and $L = \mu$ for $h = 0$ (Crow & Kimura, 1970).

Partially self-fertilizing population ($r \neq 0$). Interestingly, both q_e and L are found to be proportional to the mutation rate:

$$q_e \approx \mu \cdot \frac{(2 + K_1 + 3K_3 + 4K_4)}{(K_1 K_{21} - K_{22})} \tag{5}$$

and

$$L \approx 4\mu K_m, \tag{6}$$

with

$$K_1 = \frac{4 \cdot \{1 - r(w_3 K_3/4 + w_2/2) - (1-r)(2K_4 w_4 + w_3 K_3 + w_2/3)\}}{rw_1},$$

$$K_3 = (4rw_2)/(18 - 2rw_3),$$

$$K_4 = [r(w_2 + 9w_3 K_3)]/[36(1 - rw_4)],$$

$$K_{21} = 1 - w_1 + (rw_1)/2,$$

$$K_{22} = (2rw_2)/9 + 1(1 - r)(4w_2/3 + w_3 K_3/2),$$

and

$$K_m = \frac{s}{K_1 K_{21} - K_{22}} (h_1 K_1 + h_2 + h_3 K_3 + K_4).$$

In the diploid case, the corresponding results are:

$$q_e = \{\mu(2 - r + 2rs - 2hs)\}/\{s[r + h(2 - 2r + rs)]\}$$

and

$$L = \mu \left[1 + \frac{2h(1 - r + rs)}{r + h(2 - 2r + rs)} \right]$$

(Ohta & Cockerham, 1974), i.e. both the frequency of the deleterious allele and the mutation load are also proportional to the mutation rate.

(iii) *Approximation for the multilocus case*

Under the assumption of independence of the load due to different loci, and assuming the same mutation rate (μ) and selective regime (h_1, h_2, h_3, s) for all loci determining fitness, multilocus approximations for the variation of inbreeding depression with selfing can be computed as the product of fitnesses across loci (for the diploid case see Charlesworth *et al.*, 1990). If n denotes the total number of loci and L_i the mutational load at the i th locus, the mean fitness of the population can be expressed as (Crow, 1970):

$$\bar{W} = \sum_{i=1}^n (1 - L_i). \tag{7}$$

Assuming independence of the load due to different loci, $L_i = L$ for all i and for large n , this equation becomes:

$$\bar{W} = (1 - L)^n \approx e^{-nL}. \tag{8}$$

Randomly mating populations. Substituting (2) and (4) into (8) gives the following mean fitness for a randomly mating population ($r = 0$) at equilibrium between mutation and selection:

for $h_1 \neq 0$,

$$\bar{W} = e^{-4n\mu}, \tag{9}$$

for $h_1 = h_2 = h_3 = 0$,

$$\bar{W} = e^{-n\mu}. \tag{10}$$

Denoting by $U_{(4\times)}$ the mutation rate per tetraploid genome per generation, i.e. $U_{(4\times)} = 4\mu n$, (9) can be rearranged as:

$$\bar{W} = e^{-U_{(4\times)}} \tag{11}$$

The multilocus mutation load expected in an outcrossing tetraploid population assuming partial dominance is determined by the mutation rate only, and is independent of both the dominance coefficients (h_1, h_2 and h_3) and selective effect (s) of the mutant allele. This is similar to the results obtained in diploids, for which $\bar{W} = e^{-U_{(2\times)}}$, with $U_{(2\times)} = 2\mu n$. Assuming equal mutation rates (μ) per allele in both diploids and tetraploids, the ratio of the mean fitness of a diploid outcrossing population ($\bar{W}_{(2\times)}$) to that of a tetraploid one ($\bar{W}_{(4\times)}$) is:

$$\frac{\bar{W}_{(2\times)}}{\bar{W}_{(4\times)}} = \frac{e^{-2n\mu}}{e^{-4\mu}} = e^{2n\mu} > 1. \tag{12}$$

Diploid outcrossing populations are therefore expected to exhibit a higher mean fitness (or lower mutation load) than tetraploid populations.

Partially selfing populations ($r \neq 0$). Using (6) and the procedure described above, we obtain:

$$\bar{W} = e^{-U_{(4\times)}K_m}. \tag{13}$$

In the diploid model, the same procedure yields:

$$\bar{W} = \exp\left\{-U_{(2\times)}\left[1 + \frac{2h(1-r+rs)}{r+h(2-2r+rs)}\right]\right\}.$$

Comparisons between the diploid and the tetraploid cases are difficult, however, given the complicated form of the parameter K_m .

(iv) *Inbreeding depression*

Using the genotype frequencies derived in the Appendix, it is possible to compute the expected fitnesses of individuals following either a self-fertilization (w_s) or a cross-fertilization (w_o) event.

Initially outcrossing populations ($r = 0$).

$$w_o \approx 1 - 4\mu. \tag{14}$$

Note that this is the equilibrium fitness in a randomly mating population, and from results in the Appendix, the equilibrium frequency of $AAAA$ genotypes in a randomly mating population is $H_1 = 4\mu/sh_1$. Then:

$$w_s \approx 1 - sh_1 H_1/2 - sh_2 H_1/4 = 1 - \mu(2 + h_2/h_1). \tag{15}$$

Expressions (14) and (15) can be used to compute the corresponding multilocus approximations of the fitness values (\bar{W}_o and \bar{W}_s):

$$\bar{W}_o = e^{-U_{(4\times)}}, \tag{16}$$

$$\bar{W}_s = \exp\left\{-\frac{U_{(4\times)}}{4} \cdot (2 + h_2/h_1)\right\}. \tag{17}$$

Inbreeding depression is usually defined as $\delta = 1 - (\bar{W}_s/\bar{W}_o)$ (Charlesworth & Charlesworth, 1987), which yields

$$\delta = 1 - \exp\left\{U_{(4\times)}\left(\frac{2h_1 - h_2}{4h_1}\right)\right\} \tag{18}$$

for $h_1 \neq 0$. Notice that when $h_2 = 2h_1$, no inbreeding depression is expected in a panmictic tetraploid population.

Partially selfing populations ($r \neq 0$). The mean fitness of products of a self-fertilization and a cross-fertilization event at a single locus are, respectively:

$$w_o \approx 1 - h_1 s(H_{1(e)} + 4H_{2(e)}/3 + H_{3(e)}) - h_2 s(2H_{1(e)} + H_{3(e)} + H_{2(e)}/3), \tag{19}$$

$$w_s = 1 - s\left\{h_1\left(\frac{H_{1(e)}}{2} + \frac{2H_{2(e)}}{9}\right) + h_2\left(\frac{H_{1(e)}}{4} + \frac{H_{2(e)}}{2} + \frac{H_{3(e)}}{4}\right) + h_3\left(\frac{2H_{2(e)}}{9} + \frac{H_{3(e)}}{2}\right) + \left(R + \frac{H_{3(e)}}{4} + \frac{H_{2(e)}}{36}\right)\right\}, \tag{20}$$

where (e) denotes the frequency at equilibrium. Using results in the Appendix (A 19–A 22) and denoting

$$K_o = \frac{4s}{K_1 K_{21} - K_{22}} \{h_1(K_1 + 4/3 + K_3) + h_2(2K_4 + K_3 + 1/3)\}$$

and

$$K_s = \frac{4s}{K_1 K_{21} - K_{22}} \left\{h_1\left(\frac{K_1}{2} + \frac{2}{9}\right) + h_2\left(\frac{K_1}{4} + \frac{1}{2} + \frac{K_3}{4}\right) + h_3\left(\frac{2}{9} + \frac{K_3}{2}\right) + K_4 + \frac{K_3}{4} + \frac{1}{36}\right\},$$

(19) and (20) can be rearranged as:

$$w_o = 1 - \mu K_o, \tag{21}$$

$$w_s = 1 - \mu K_s, \tag{22}$$

where K_o and K_s are functions only of s, h_1, h_2, h_3 and r . The multilocus fitness approximations then become

$$\bar{W}_o \approx \exp(-U_{(4\times)} K_o), \tag{23}$$

$$\bar{W}_s \approx \exp(-U_{(4\times)} K_s), \tag{24}$$

and the expected inbreeding depression in such a population becomes:

$$\delta \sim 1 - \exp\{U_{(4\times)}(K_s - K_o)\}. \tag{25}$$

3. Numerical examples

Equations (2), (6), (18) and (25) were used to compute the variation of both the mutation load and inbreeding depression with selfing assuming various sets of

parameters (h_1, h_2, h_3, s and μ). Depending on the values of h_1, h_2 and h_3 , the behaviour of inbreeding depression was very different: inbreeding depression either increased or decreased with increasing selfing rates. It is not always a monotonic function of r . Given this variation and the lack of empirical data concerning the values of h_1, h_2 and h_3 , only two striking cases are presented below: (i) The simple case of ‘dosage-like’ dominance effect, i.e. $h_2 = 2h_1$ and $h_3 = 3h_1$ (Lande & Schemske, 1985). This model is close to the physiological theory of dominance proposed by Wright (1934), which states that the relationship between gene activity and phenotypic effect follows a hyperbolic function. (ii) $h_1 \ll h_2 < h_3$, i.e. a mutation has a marked deleterious effect when the deleterious allele occurs as at least two copies (this case will be referred to as the ‘duplex-mutation effect’ in what follows).

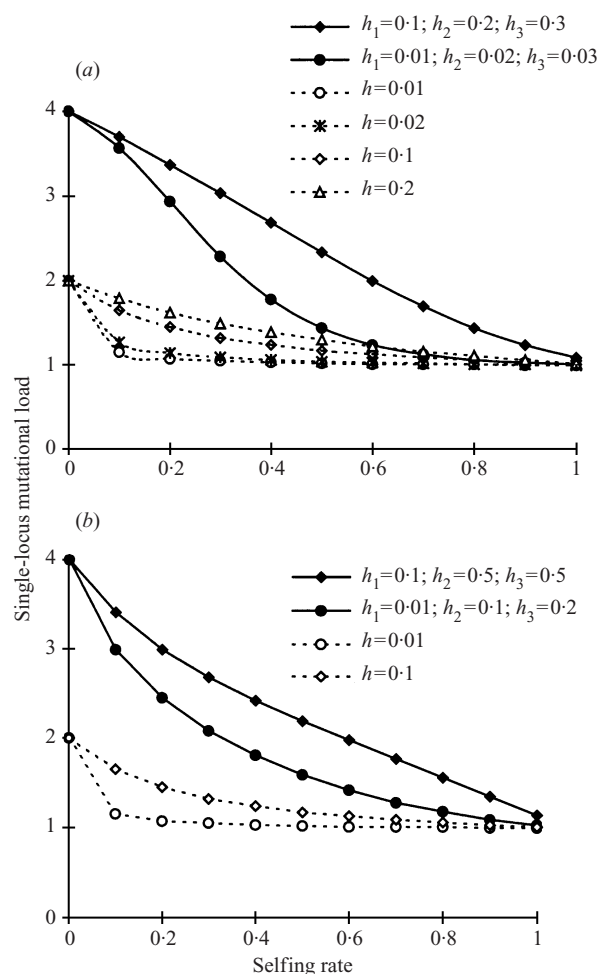


Fig. 1. Single-locus mutation load expected at equilibrium between mutation and selection in partially selfing populations as a function of the population selfing rate. Open and filled symbols refer to diploid and tetraploid populations respectively. (a) ‘dosage-like’ dominance; (b) ‘duplex-mutation’ effect. The mutation load is to be multiplied by 10^{-6} . $\mu = 10^{-6}$ and $s = 0.05$ in all situations. Results were computed using equations (2) and (6).

Table 2. Variation of the single-locus mutation load ($L; \times 10^6$), the multilocus fitness value (W) and multilocus inbreeding depression (δ) with the selfing rate for various dominance and selection coefficients

$h_1 = 0.2; h_2 = 0.4; h_3 = 0.6$						
Selfing rate	$s = 0.2$			$s = 0.05$		
	L	W	$\delta (\times 10^2)$	L	W	$\delta (\times 10^2)$
0	4	0.135	0	4	0.135	0
0.1	3.72	0.155	0.23	3.71	0.157	0.25
0.2	3.45	0.178	0.54	3.41	0.182	0.60
0.3	3.18	0.204	0.94	3.11	0.211	1.06
0.4	2.92	0.234	1.44	2.81	0.245	1.66
0.5	2.66	0.267	2.03	2.51	0.284	2.40
0.6	2.41	0.304	2.72	2.22	0.329	3.30
0.7	2.17	0.346	3.51	1.93	0.386	4.37
0.8	1.94	0.390	4.39	1.66	0.437	5.61
0.9	1.73	0.437	5.34	1.40	0.497	6.99
1	1.55	0.486	6.34	1.17	0.558	8.51

$h_1 = 0.05; h_2 = 0.25; h_3 = 0.5$						
Selfing rate	$s = 0.2$			$s = 0.05$		
	L	W	δ	L	W	δ
0	4	0.135	0.777	4	0.135	0.777
0.1	3.39	0.183	0.699	3.37	0.185	0.696
0.2	2.94	0.230	0.627	2.90	0.234	0.621
0.3	2.59	0.274	0.563	2.54	0.281	0.553
0.4	2.30	0.316	0.506	2.23	0.327	0.492
0.5	2.06	0.356	0.456	1.97	0.373	0.437
0.6	1.86	0.394	0.412	1.75	0.417	0.388
0.7	1.68	0.431	0.374	1.55	0.460	0.344
0.8	1.53	0.465	0.340	1.38	0.502	0.305
0.9	1.40	0.497	0.311	1.12	0.543	0.270
1	1.28	0.527	0.285	1.08	0.582	0.240

Variation of the mutation load with selfing. Fig. 1 gives the variation of the equilibrium ‘single-locus’ mutation load with the selfing rate, for different sets of parameters (r, h_1, h_2, h_3, s and μ). As in the diploid situation, the mutation load always decreases with increasing selfing rates, and more rapidly with decreasing dominance coefficients. For a given selfing rate, and assuming similar dominance coefficients in diploids and in tetraploids ($h = h_1$), the mutation load is always higher in tetraploids than in diploids. Table 2 shows that the selection coefficient has only a slight effect on the efficiency of the purging process, as previously shown for diploids (Ohta & Cockerham, 1974; Charlesworth *et al.*, 1990).

Multilocus approximation of inbreeding depression. Fig. 2 and Table 2 show how inbreeding depression is expected to vary with the selfing rates, depending on the dominance coefficients. As can be seen, the variation of inbreeding depression depends not only on the relationships between h_1, h_2 and h_3 but also on the mean degree of dominance of the deleterious

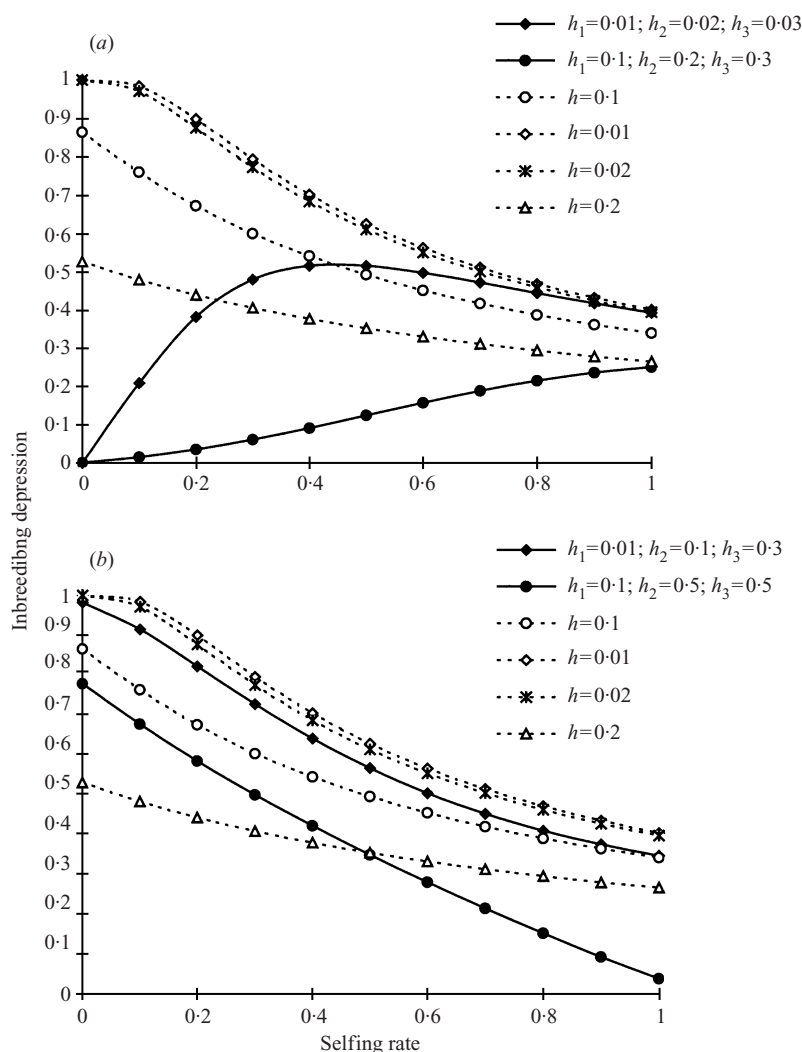


Fig. 2. Expected inbreeding depression in partially selfing diploid (open symbols) and tetraploid (filled symbols) populations assuming multiplicative fitness interactions among loci under (a) ‘dosage-like’ dominance and (b) ‘duplex-mutation’ effect. $\mu = 10^{-6}$, $s = 0.05$ in both cases. Results were computed using equations (18) and (25).

alleles. Under ‘dosage-like’ dominance, inbreeding depression is an increasing function of the selfing rate for moderate levels of dominance ($h_1 = 0.1$, Fig. 2a). For highly recessive mutations ($h_1 = 0.01$, Fig. 2a), however, inbreeding depression increases with the selfing rate up to a maximum and then decreases. Whatever the dominance coefficient, this differs from the continuous decrease of inbreeding depression with the selfing rate in diploids. On the contrary, for ‘duplex-mutation effect’, inbreeding depression decreases monotonically with increasing selfing rates, which is similar to what is seen in diploids (Fig. 2b).

The change in inbreeding depression with selfing in diploids can be understood in terms of a decreased frequency of the (single) heterozygote class with increasing selfing rates. In tetraploid populations, three classes of heterozygotes have to be considered. Thus, in order to explain the results described above, the changes in the equilibrium frequencies of the genotypes with selfing were computed. Results from

these computations are given in Table 3 for ‘dosage-like’ dominance, and compared with expectations for diploids. This shows that while the frequency of the single heterozygote class (Aa) always decreases with increasing selfing rates in diploids, only the genotype $AAaa$ shows a similar monotonic decrease in frequency in tetraploids; the frequency of both $AAaa$ and of $Aaaa$ first increase up to a maximum and then decrease. Another way to explain changes in inbreeding depression with selfing rates is to compare the frequency of the different genotypes expected after a cross versus a self-fertilization event. As shown in Fig. 3 for the ‘dosage-like’ dominance model, the difference between the proportion of homozygotes for the mutant allele expected after a self-fertilization versus a cross-fertilization event strictly parallels the magnitude of inbreeding depression (Fig. 2). The decrease in inbreeding depression with selfing under the duplex mutation effect model (Fig. 2) is better explained by the decrease in the $AAaa$ heterozygotes for small

Table 3. Equilibrium single-locus genotype frequencies expected under partial selfing in autotetraploid populations with 'dosage-like' dominance

		Genotype frequencies							
		Tetraploid					Diploid		
$h_1 = 0.01$	Selfing rate	AAAA	AA ³ A ($\times 10^3$)	A ² aa ($\times 10^4$)	Aaaa ($\times 10^6$)	aaaa ($\times 10^6$)	AA	Aa ($\times 10^4$)	aa ($\times 10^5$)
	0	0.9920	8.00	0	0	0	0.9960	4.00	0
	0.1	0.9932	6.51	2.61	6.11	0.97	0.9994	6.13	1.69
	0.2	0.9949	4.63	4.01	19.8	3.97	0.9997	2.99	1.85
	0.3	0.9967	2.87	4.06	31.8	8.05	0.9998	1.82	1.91
	0.4	0.9980	1.60	3.31	36.8	11.9	0.9999	1.20	1.94
	0.5	0.9989	0.83	2.39	35.4	14.7	0.9999	0.82	1.96
	0.6	0.9994	0.41	1.59	30.3	16.7	0.9999	0.56	1.97
	0.7	0.9997	0.18	0.99	23.6	13.2	0.9999	0.38	1.98
	0.8	0.9998	0.08	0.56	16.5	18.9	0.9999	0.24	1.99
	0.9	0.9999	0.03	0.26	9.61	19.4	0.9999	0.13	1.99
	1	0.9999	0.01	0.07	3.18	19.8	0.9999	0.04	1.99
$h_1 = 0.1$	0	0.9992	8.00	0	0	0	0.9996	4.00	0
	0.1	0.9993	6.82	0.27	0.63	0.10	0.9997	2.58	0.71
	0.2	0.9994	5.66	0.48	2.36	0.47	0.9998	1.79	1.10
	0.3	0.9995	4.53	0.63	4.90	1.24	0.9998	1.29	1.35
	0.4	0.9996	3.47	0.71	7.76	2.49	0.9999	0.95	1.52
	0.5	0.9997	2.51	0.71	10.4	4.30	0.9999	0.70	1.65
	0.6	0.9997	1.69	0.64	12.7	6.62	0.9999	0.50	1.75
	0.7	0.9998	1.03	0.52	12.3	9.38	0.9999	0.35	1.82
	0.8	0.9999	0.56	0.37	10.9	12.4	0.9999	0.23	1.88
	0.9	0.9999	0.26	0.22	7.69	15.4	0.9999	0.12	1.94
	1	0.9999	0.11	0.07	2.97	18.4	0.9999	0.04	1.98

Values are given for the tetraploid (left-hand part) and diploid (right-hand part for $h_1 = h$) situations for two values of h_1 ; $s = 0.05$.

selfing rates, and by the Aaaa genotype for higher selfing rates (Fig. 4).

4. Discussion

(i) Evidence for purging in autotetraploids

As in diploids, the frequency of a partially dominant deleterious allele maintained at equilibrium under mutation and selection in an autotetraploid population decreases with increasing levels of self-fertilization. Indeed, the proportion of deleterious mutations expressed in the homozygous form (aaaa) increases with increasing selfing rates, so that selection against deleterious mutations becomes more efficient. As in diploid populations, purging occurs and the population fitness increases. At a single locus, the mutation load decreases from 4μ in a random mating population to μ in a selfing population. Expectations in the diploid case are 2μ and μ respectively (Crow, 1970; Ohta & Cockerham, 1974). In other words, the mutation load for a given selfing rate is always higher in tetraploids than in diploids, except in purely selfing populations (Fig. 1). However, purging is also more efficient in tetraploids than in diploids. Note also that purging is more efficient with low dominance co-

efficients and, to a lesser extent, with low selection coefficients. In the particular case of completely recessive alleles, although the frequency of deleterious mutations still decreases with increasing selfing rates, the load remains constant over the whole range of selfing rates, in accordance with what is found in diploids (Ohta & Cockerham, 1974; Charlesworth & Charlesworth, 1990).

(ii) Lower inbreeding depression in tetraploids than in diploids

Under complete recessivity ($h_1 = h_2 = h_3 = 0$), the theoretical inbreeding depression maintained in tetraploids and in diploids is identical (data not shown). This result disagrees with predictions from Lande & Schemske (1985; see the appendix of the paper), who stated that the equilibrium inbreeding depression due to completely recessive lethal and sublethal mutations in both auto- and allotetraploid outcrossing populations ($r = 0$) is expected to be half that expected for completely recessive mutations in diploids,

For partially recessive mutations, diploid and tetraploid expectations are not easy to compare since those comparisons depend upon assumptions concerning the dominance coefficients. Following our

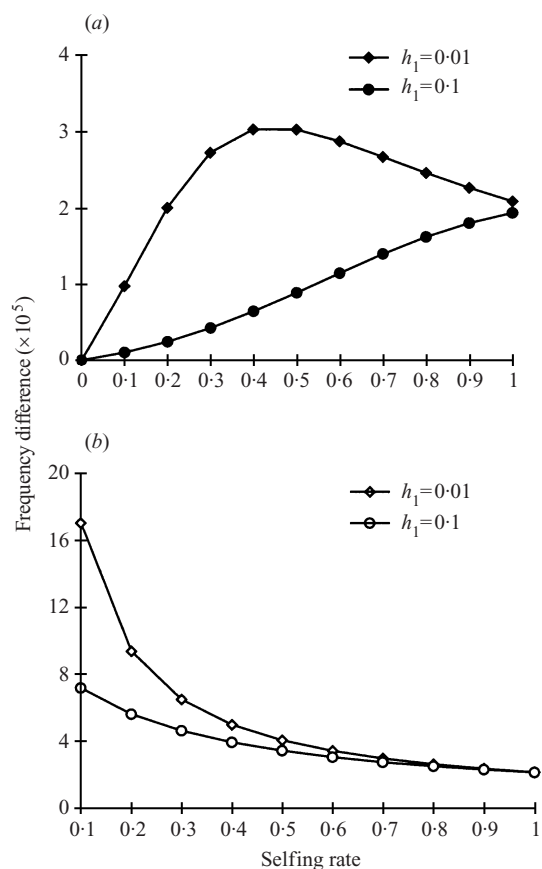


Fig. 3. Expected difference between the frequency of the homozygote mutant genotype ($aaaa$ for autotetraploids; aa for diploids) after a self-fertilization event and after a cross-fertilization event, in populations initially at equilibrium for different selfing rates. (a) Autotetraploid populations and 'dosage-like' dominance ($h_2 = 2h_1$; $h_3 = 3h_1$; computations are based on equations (A 19–A 22); (b) diploid populations for $h = h_1$ (computations follow Ohta & Cockerham, 1974). $\mu = 10^{-6}$, $s = 0.05$.

derivations, a general trend is, however, that the inbreeding depression maintained for a given selfing rate is lower in tetraploid than in diploid populations (assuming identical mutation rate (μ), selection (s) and dominance coefficients: $h_{(2\times)} = h_{1(4\times)}$ or $h_{(2\times)} = h_{2(4)}$). Given the larger equilibrium mutation load expected in tetraploids compared with diploids, higher inbreeding depression would be expected when $h \neq 0$. This counterintuitive result, already shown for partially recessive lethal and sublethal mutations, in the particular case of randomly mating populations (Lande & Schemske, 1985), can be explained by the relative increase of homozygosity under self-fertilization in tetraploids compared with diploids. Homozygosity increases by 50% after one generation of selfing in a diploid population, while its increase is only about 17% under tetrasomic inheritance (see also Husband & Schemske, 1997).

The variation of inbreeding depression with ploidy levels has been addressed empirically in only a few

studies. Husband & Schemske (1997) showed that inbreeding depression was, on average, 29% lower in three tetraploid populations of *Epilobium angustifolium* than in diploid populations of the same species showing similar selfing rates. This is in agreement with our prediction. Results in ferns are also consistent with this prediction (Hedrick, 1987; see also references in Husband & Schemske, 1997). Studies indicating higher inbreeding depression in polyploids than in diploids mostly concern crop plants (Dewey, 1966; Bingham, 1980). However, the measurements were performed after several generations of artificial self-fertilization and are therefore difficult to compare with the equilibrium expectations computed in the present study.

(iii) Inbreeding depression may increase with increasing selfing rates

When mutations are partially recessive, the equilibrium inbreeding depression in partially selfing diploid populations decreases monotonically when the selfing rate increases (Lande & Schemske, 1985; Charlesworth *et al.*, 1990). A similar variation was observed in tetraploids when we assumed that the mutant allele has a marked deleterious effect only if present in two or more copies (duplex mutation). The decrease of inbreeding depression is mostly attributable to the variation in frequency of heterozygotes $AAaa$ and $Aaaa$ with increasing selfing rates (Fig. 4). The difference between the frequency of $AAaa$ genotypes after a self- versus a cross-fertilization event accounts for the decrease in inbreeding depression when the selfing rate lies between 0 and 0.6; for higher selfing rates, the trend is due to $Aaaa$ genotypes (Fig. 4).

On the other hand, inbreeding depression may either increase or vary non-monotonically with increasing selfing rates under dosage-like dominance (Fig. 2). This is unexpected since the genetic load decreases when the selfing rate increases, but can be understood by considering the equilibrium frequencies of the heterozygotes and their variation after an additional self-fertilization event. In tetraploid populations, one has to consider three classes of heterozygotes, and after a self-fertilization event the frequency of mutant homozygotes ($aaaa$) mainly depends on the equilibrium frequencies of $Aaaa$ and $AAaa$ in the initial population. For $h_1 = 0.1$, the difference between the frequency of the homozygote $aaaa$ following self- and cross-fertilization events increases with increasing selfing rates (Fig. 3a). This explains why inbreeding depression increases with increasing selfing rates in tetraploid populations. When the dominance coefficient is low ($h_1 = 0$), inbreeding depression increases up to a maximum value at an intermediate selfing rate, and then decreases: this is

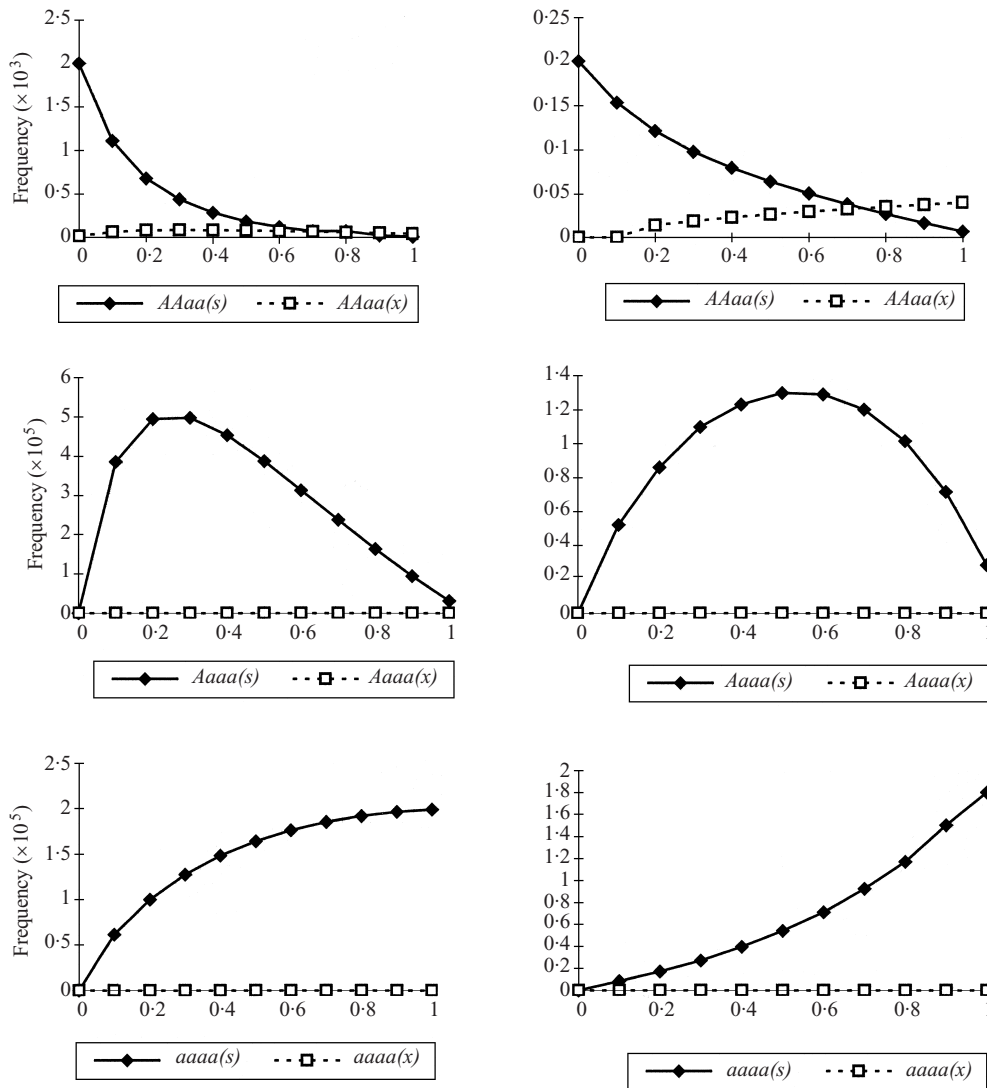


Fig. 4. Expected frequencies of genotypes $AAAa$, $Aaaa$ and $aaaa$ after either a self-fertilization (filled symbols) or a cross-fertilization (open symbols) event, in tetraploid populations initially at equilibrium for different selfing rates with ‘duplex mutation’. $\mu = 10^{-6}$, $s = 0.05$, with (a) $h_1 = 0.01$, $h_2 = 0.1$, $h_3 = 0.3$ and (b) $h_1 = 0.1$, $h_2 = 0.5$, $h_3 = 0.5$. Computations are based on equations (A 19)–(A 22).

strictly paralleled by the expected frequency of the homozygous mutant after one generation of selfing (Fig. 3a).

(iv) *Evolutionary consequences*

The simplest model for the evolution of selfing in diploids implies that the intrinsic advantage to selfing (the cost of outcrossing; Fisher, 1941) is opposed by inbreeding depression. In this model, an inbreeding depression of 50% is a critical value, above (under) which outcrossing (selfing) is favoured. Under this simple model, there are therefore only two stable states: pure selfing and pure outcrossing (Lande & Schemske, 1985; Charlesworth & Charlesworth, 1987; for more complex models explaining the maintenance of intermediate selfing rates in diploids see, however, Holsinger, 1991; Charlesworth *et al.*, 1991; Charles-

worth & Charlesworth, 1990; Ronfort & Couvet, 1995). This is due to the decreasing relationship between the equilibrium inbreeding depression and the selfing rate. As a population becomes sufficiently inbred, inbreeding declines, so that alleles increasing the selfing rate are increasingly favoured. Conversely, alleles promoting increasing selfing rates are counter-selected in outcrossing populations. This reasoning applies only to tetraploid populations under the ‘duplex-mutation’ model of dominance or for completely recessive mutations. With ‘dosage-like’ dominance, and for an intermediate dominance coefficient ($h_1 = 0.1$), inbreeding depression is always lower than 0.5, so that pure selfing should be the unique stable state. For almost completely recessive mutations, inbreeding depression does not vary monotonically with the selfing rate (Fig. 2a). For $h_1 = 0.01$ the inbreeding depression increases with the selfing rates, reaching

values higher than 0.5 when $r > 0.4$. When the selfing rate is 0.3, an allele causing selfing to increase is thus expected to be counter-selected (since inbreeding depression exceeds 0.5). On the other hand, when $r > 0.4$, alleles promoting outcrossing are expected to spread. Thus, this intermediate selfing rate would appear to be a stable state. No such situation is expected under the same model (large population, multiplicative fitness interactions among loci and partial dominance) in diploids (for review see, however, Waller & Uyenoyama, 1993; Charlesworth & Charlesworth, 1998).

Few data are available to elucidate the relationship between ploidy levels and selfing rates in natural populations. In the genus *Medicago*, autotetraploid forms are all predominantly outcrossing, but diploids vary between high and low selfing rates (Olivieri *et al.*, 1991). More generally, most naturally occurring autopolyploid angiosperms are outcrossers (Stebbins, 1957), and selfing autopolyploid crop plants are generally not successful (Bingham, 1980). Evidence for higher selfing rates in polyploids than in diploids exists only in ferns (Watano & Maruyama, 1991).

(v) *The need for further empirical studies and future theoretical work*

Due to the restricted assumptions and approximations used in the above derivations (independence of the loads due to different loci; use of specific and somewhat arbitrarily chosen sets of both dominance and selective coefficients), the study presented here is only a preliminary approach to how inbreeding depression may vary with the rate of self-fertilization in autotetraploid species. Several further empirical and theoretical investigations are required before general and undubious conclusions can be drawn. First, deterministic models involving more than one locus would be welcome in order to check the accuracy of the multilocus approximation that assumes independence of the loads due to different loci. In diploids, it has been shown that such approximations are only accurate when the total inbreeding depression is not very high (Lande *et al.*, 1994). An extension of the method developed by Kondrashov (1985, see also Charlesworth *et al.*, 1990, 1991) is currently being investigated to determine whether this approximation is reasonable in the case of tetraploidy. Secondly, data on the effects of mutation on fitness-determining loci in autotetraploid species are required as no data are currently available concerning the relative and absolute values of the dominance and selection coefficients. Thirdly, the possible occurrence of double reduction in autopolyploids has been neglected because the frequency of double reduction differs among loci (depending on position relative to the centromere), so that no multilocus approximations can be done.

Intuitive predictions can, however, be made. Under this phenomenon (two sister chromatids migrate into the same gamete), single mutants can produce double mutant gametes, that is a higher frequency of homozygous genotypes. This should increase the efficiency of the purging process and should further reduce the magnitude of inbreeding depression compared with results based on the assumption of no double reduction (see also predictions from Lande & Schemske, 1985). Simulations are, however, necessary to check these predictions and to quantify the effect of double reduction on the variation of both the mutational load and inbreeding depression. Finally, models involving modifier loci of the selfing rate and the analysis of their behaviour in conjunction with that of selected loci (see Campbell, 1986; Charlesworth *et al.*, 1990) are required in order to examine more accurately the joint evolution of inbreeding depression and self-fertilization expected in autotetraploid species.

Appendix

In what follows, the genotypic frequencies expected at equilibrium in a partially selfing populations subjected to mutation and selection are derived. t denotes the time in generations; r , the selfing rate; μ , the mutation rate from allele A towards a ; s , the coefficient of selection; and h_1, h_2 and h_3 the dominance coefficients associated with heterozygotes $AAAA, AAAa$ and $Aaaa$ respectively. We first derive recurrence equations for the genotypic frequencies across generations (see Table 1 for notation). Mutation from $AAAA, AAAa, AAaa, Aaaa$ to $AAAa, AAaa, Aaaa$ and $aaaa$ respectively occurs at rate $4\mu, 3\mu, 2\mu$ and μ respectively (terms of second and higher orders, i.e. μ^2, μ^3 , etc., are neglected).

$$H_{1(t+1)} = r \cdot \{(H'_{1(t)}/2) + (2H'_{2(t)}/9)\} + (1-r) \cdot \{2g(AA) \cdot g(Aa) + 4\mu D'_{(t)}\}, \tag{A 1}$$

$$H_{2(t+1)} = r \cdot \{(H'_{1(t)}/4) + (H'_{2(t)}/2 + (H'_{3(t)}/4)\} + (1-r) \cdot \{2 \cdot g(AA) \cdot g(aa) + g(Aa)^2\} + 3\mu \cdot H'_{1(t)}, \tag{A 2}$$

$$H_{3(t+1)} = r \cdot \{(H'_{3(t)}/2) + (2H'_{2(t)}/9)\} + (1-r) \cdot \{2 \cdot g(aa) \cdot g(Aa)\} + 2\mu \cdot H'_{2(t)}, \tag{A 3}$$

$$R_{(t+1)} = r \cdot \{R'_{(t)} + (H'_{3(t)}/4) + (H'_{2(t)}/36)\} + (1-r) \cdot g(aa)^2 + \mu \cdot H'_{3(t)}, \tag{A 4}$$

where

$$g(AA) = D'_{(t)} + (H'_{1(t)}/2) + (H'_{2(t)}/6),$$

$$g(Aa) = (2H'_{2(t)}/3) + (H'_{1(t)}/2) + (H'_{3(t)}/2),$$

$$g(aa) = R_{(t)} + (H'_{3(t)}/2) + (H'_{2(t)}/6),$$

$$D'_{(t)} = 1 - H'_{1(t)} - H'_{2(t)} - H'_{3(t)} - R'_{(t)}.$$

I was not able to derive exact equilibrium solutions to these equations (either by hand or using *Mathematica*, Wolfram, 1996). However, it was possible to

iterate these equations using a computer for selection coefficients (s) ranging between 0.05 and 0.5, and dominance coefficient ranging from 0.01 to 0.5 ($h_1 < h_2 < h_3$) and single-locus mutation rate lower than 10^{-5} ; this showed that the frequency of the mutant allele remains sufficiently low to make the following approximations:

$$g(AA).g(Aa) \approx g(Aa) \text{ and } g(AA).g(aa) \approx g(aa)$$

$$\text{since } g(AA) \approx 1,$$

$$g(Aa)^2 \approx g(aa)^2 \approx 0,$$

$$g(Aa).g(aa) \approx 0,$$

$$\mu.H'_{1(t)} \approx 0, \mu.H'_{2(t)} \approx 0, \mu.H'_{3(t)} \approx 0$$

$$\text{and } \mu.R'_{(t)} \approx 0.$$

Using these approximations, (A 1)–(A 4) simplified to:

$$H_{1(t+1)} = r.\{(H'_{1(t)}/2) + (2H'_{2(t)}/9)\} + 2.(1-r).\{(2H'_{2(t)}/3) + (H'_{1(t)} + H'_{3(t)})/2\} + 4\mu, \tag{A 5}$$

$$H_{2(t+1)} = \frac{r}{4}.\{H'_{1(t)} + H'_{2(t)} + H'_{3(t)}\} + 2.(1-r).\{R'_{(t)} + (H'_{3(t)}/2) + (H'_{2(t)}/6)\}, \tag{A 6}$$

$$H_{3(t+1)} = r.\{(H'_{3(t)}/2) + (2H'_{2(t)}/9)\}, \tag{A 7}$$

$$R_{(t+1)} = r.\{R'_{(t)} + (H'_{3(t)}/4) + (H'_{2(t)}/36)\}. \tag{A 8}$$

Consider first purely outcrossing populations ($r = 0$). Equations (A 5)–(A 8) can be further simplified as:

$$H_{2(t+1)} \approx 0,$$

$$H_{3(t+1)} \approx 0,$$

$$R_{(t+1)} \approx 0,$$

$$H_{1(t+1)} \approx H_{1(t)}.w_1 + 4\mu.$$

Using ‘(e)’ to denote equilibrium frequencies, and assuming $h_1 \neq 0$, we then obtain:

$$H_{2(e)} \approx 0, \tag{A 9}$$

$$H_{3(e)} \approx 0, \tag{A 10}$$

$$R_{(e)} \approx 0, \tag{A 11}$$

$$H_{1(e)} \approx 4\mu/h_1. \tag{A 12}$$

The equilibrium frequency of the mutant allele, the mean fitness of the population and the corresponding mutation load are, respectively:

$$q_e \approx H_1/4 = \mu/(sh_1), \tag{A 13}$$

$$w = 1 - sh_1 H_{1(e)} = 1 - 4\mu \tag{A 14}$$

and

$$L = 4\mu. \tag{A 15}$$

For complete recessivity ($h_1 = h_2 = h_3 = 0$), it is not necessary to compute the genotype frequencies to derive the equilibrium frequency of the deleterious allele and the mutation load expected in a randomly mating population ($r = 0$). This derivation simply follows the equilibrium principle, i.e. ‘in the long run,

the rate at which new deleterious mutations occur must equal that at which mutations are eliminated by selection’ (Maynard Smith, 1989). In a large random-mating population of size N , the number of deleterious genes lost by selection is equal to $4Nq^4s$, while the number of new deleterious genes arising by mutation is $4Np\mu$. Equalling these values gives the equilibrium frequency of the deleterious allele (noting that $p_e \approx 1$):

$$q_e \approx \sqrt[4]{(\mu/s)}. \tag{A 16}$$

The fitness value and the mutation load are:

$$w = 1 - sR_e \approx 1 - s.q_e^4 = 1 - \mu \tag{A 17}$$

$$L \approx \mu. \tag{A 18}$$

Consider now partially selfing populations ($r \neq 0$). Equation (A 3) gives at equilibrium:

$$H_{3(e)} \approx r.\left\{\frac{H_{3(e)}w_3}{2} + \frac{2H_{2(e)}w_2}{9}\right\},$$

so that, denoting $K_3 = (4r.w_2)/(18 - 2r.w_3)$,

$$H_{3(e)} = K_3.H_{2(e)}. \tag{A 19}$$

Similar reasoning with equations (A 4) and (A 1) and using (A 19) gives:

$$H_{4(e)} \approx K_4.H_{2(e)}, \tag{A 20}$$

$$H_{1(e)} \approx K_1.H_{2(e)}, \tag{A 21}$$

with

$$K_4 = (K.(w_2 + 9w_3K_3))/(36.(1 - r.w_4))$$

and

$$K_1 =$$

$$\frac{4.\{1 - r.(w_3K_3/4 + w_2/2) - (1 - r).(2K_4w_4 + w_3K_3 + w_2/3)\}}{r.w_1}.$$

The equilibrium frequency of H_2 is then obtained using (A 2), (A 19), (A 20) and (A 21):

$$H_{2(e)} = \frac{4\mu}{K_1K_{21} - K_{22}}, \tag{A 22}$$

where

$$K_{21} = 1 - w_1 + (r.w_1)/2$$

and

$$K_{22} = (2.r.w_2)/9 + (1 - r).(4w_2/3 + w_3K_3/2).$$

The equilibrium frequency of the deleterious allele and the mutation load are:

$$q_e = (H_{1(e)} + 2H_{2(e)} + 3H_{3(e)} + 4R_e)/4 \approx \mu.(2 + K_1 + 3K_3 + 4K_4)/(K_1K_{21} - K_{22}) \tag{A 23}$$

$$L_e = sh_1 H_{1(e)} + sh_2 H_{2(e)} + sh_3 H_{3(e)} + sR_{(e)} \approx \frac{4\mu.s}{K_1K_{21} - K_{22}}(h_1K_1 + h_2 + h_3K_3 + K_4). \tag{A 24}$$

We are grateful to M. Kirkpatrick, P. Jarne, D. Couvet, T. Bataillon, E. Jenczewski and J. M. Prosperi for their critical comments on the manuscript and for helpful discussions concerning the analytical developments and the results of this work. This work was supported by the French Bureau des Ressources Génétiques.

References

- Bennett, J. H. (1976). Expectations for inbreeding depression on self-fertilization of tetraploids. *Biometrics* **32**, 449–452.
- Bever, J. D. & Felber, F. (1992). The theoretical population genetics of autopolyploidy. *Oxford Surveys on Evolutionary Biology* **8**, 185–217.
- Bingham, E. T. (1980). Maximising heterozygosity in autopolyploids. In *Polyploidy: Biological Relevance* (ed. W. H. Lewis), pp. 269–273. New York: Plenum Press.
- Bretagnolle, F. & Thompson, J. D. (1995). Gametes with somatic chromosome number: mechanisms of their formation and role in the evolution of autopolyploid plants. *New Phytologist* **129**, 1–22.
- Campbell, R. B. (1986). The interdependence of mating structure and inbreeding depression. *Theoretical Population Biology* **30**, 232–244.
- Charlesworth, B. & Charlesworth, D. (1998). Some evolutionary consequences of deleterious mutations. *Genetica*, 102/103, 3–19.
- Charlesworth, D. & Charlesworth, B. (1987). Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* **18**, 237–268.
- Charlesworth, D. & Charlesworth, B. (1990). Inbreeding depression with heterozygote advantage and its effect on selection for modifiers changing the outcrossing rate. *Evolution* **44**, 870–888.
- Charlesworth, D., Morgan, M. T. & Charlesworth, B. (1990). Inbreeding depression, genetic load, and the evolution of outcrossing rates in a multilocus system with no linkage. *Evolution* **44**, 1469–1489.
- Charlesworth, D., Morgan, M. T. & Charlesworth, B. (1991). Multilocus models of inbreeding depression with synergistic selection and partial self-fertilization. *Genetical Research* **57**, 177–194.
- Crawford, D. J. (1985). Electrophoretic data and plant speciation. *Systematic Botany* **10**, 405–416.
- Crow, J. F. (1970). Genetic loads and the cost of natural selection. In *Mathematical Models in Population Genetics* (ed. K. I. Kojima), pp. 128–177. Berlin: Springer.
- Crow, J. F. & Kimura, M. (1970). *An Introduction to Population Genetics Theory*. New York: Harper & Row.
- Dewey, D. R. (1966). Inbreeding depression in diploid, tetraploid, and hexaploid crested wheatgrass. *Crop Science* **6**, 144–147.
- Fisher, R. A. (1941). Average excess and average effect of a gene substitution. *Annals of Eugenics* **11**, 53–63.
- Grant, V. (1981). *Plant Speciation*, 2nd edn. New York: Columbia University Press.
- Hedrick, P. W. (1987). Genetic load and the mating system in homosporous ferns. *Evolution* **41**, 1282–1289.
- Holsinger, K. E. (1991). Mass-action models of plant mating systems: the evolutionary stability of mixed mating systems. *American Naturalist* **138**, 606–622.
- Husband, B. C. & Schemske, D. W. (1997). The effect of inbreeding in diploid and tetraploid populations of *Epilobium* (Onagraceae): implications for the basis of inbreeding depression. *Evolution* **51**, 737–746.
- Jarne, P. & Charlesworth, D. (1993). The evolution of the selfing rate in functionally hermaphrodite plants and animals. *Annual Review of Ecology and Systematics* **24**, 441–466.
- Kondrashov, A. S. (1985). Deleterious mutation as an evolutionary factor. II. Facultative apomixis and selfing. *Genetics* **111**, 635–653.
- Lande, R. & Schemske, D. W. (1985). The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* **39**, 24–40.
- Lande, R., Schemske, D. W. & Schultz, S. T. (1994). High inbreeding depression, selective interference among loci and the threshold selfing rate for purging recessive lethal mutations. *Evolution* **48**, 965–978.
- Levin, D. A. (1983). Polyploidy and novelty in flowering plants. *American Naturalist* **122**, 1–25.
- Masterson, J. (1994). Stomatal size in fossil plants: evidence for polyploidy in the majority of angiosperms. *Science* **264**, 421–424.
- Maynard-Smith, J. (1989). *Evolutionary Genetics*. Oxford: Oxford University Press.
- Ohta, T. & Cockerham, C. C. (1974). Detrimental genes with partial selfing and effects on a neutral locus. *Genetical Research* **23**, 191–200.
- Olivieri, I., Gouyon, P. H. & Prosperi, J. M. (1991). Life cycles of some Mediterranean invasive plants. In *Biogeography of Mediterranean Invasions* (ed. R. H. Groves & F. Di Castri), pp. 145–157. Cambridge: Cambridge University Press.
- Riesberg, L. H. & Doyle, M. F. (1989). Tetrasomic segregation in the naturally occurring autotetraploid *Allium nevilii* (Alliaceae). *Hereditas* **111**, 31–36.
- Ronfort, J. & Couvet, D. (1995). A stochastic model of selection on selfing rates in structured populations. *Genetical Research* **65**, 209–222.
- Soltis, D. E. & Soltis, P. S. (1989). Genetic consequences of autopolyploidy in *Tolmiea* (Saxifragaceae). *Evolution* **43**, 586–594.
- Stebbins, G. L. (1957). Self-fertilization and populations variability in higher plants. *American Naturalist* **91**, 337–354.
- Stebbins, G. L. (1971). *Chromosomal Evolution in Higher Plants*. London: Edward Arnold.
- Thornhill, N. W. (1993). *The Natural History of Inbreeding and Outbreeding*. Chicago: University of Chicago Press.
- Uyenoyama, M. K. & Waller, D. M. (1991). Coevolution of self-fertilization and inbreeding depression. I. Mutation–selection balance at one and two loci. *Theoretical Population Biology* **40**, 14–46.
- Uyenoyama, M. K., Holsinger, K. E. & Waller, D. M. (1993). Ecological and genetical factors directing the evolution of self-fertilization. *Oxford Surveys on Evolutionary Biology* **9**, 327–381.
- Watano, Y. & Maruyama, S. (1991). Inbreeding in natural populations of the annual polyploid fern *Ceratopteris thalictroides* (Parkeriaceae). *Systematic Botany* **16**, 705–714.
- Wolfram, S. (1996). *The Mathematica Book*, 3rd edn. Cambridge: Wolfram Media/Cambridge University Press.
- Wright, S. (1934). Physiological and evolutionary theories of dominance. *American Naturalist* **68**, 25–53.
- Wright, S. (1977). *Evolution and the Genetics of Populations*, vol. 3. *Experimental Results and Evolutionary Deductions*. Chicago: University of Chicago Press.