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Mating systems and the evolutionary transition between haploidy and diploidy

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According to the 'masking hypothesis', diploids gain an immediate fitness advantage over haploids because diploids, with two copies of every gene, are better able to survive the effects of deleterious recessive mutations. Masking in diploids is, however, a double-edged sword: it allows mutations to persist over time. In contrast, deleterious mutations are revealed in haploid individuals and are more rapidly eliminated by selection, creating genetic associations that are favourable to haploidy. We model various mating schemes and show that assortative mating, selfing, and apomixis maintain the genetic associations that favour haploidy. These results suggest that a correlation should exist between mating system and ploidy level, with outcrossing favouring diploid life cycles and inbreeding or asexual reproduction favouring haploid life cycles. This prediction can be tested in groups, such as the Chlorophyta, with extensive variation both in life cycle and in reproductive system. Confirming or rejecting this prediction in natural populations would constitute the first empirical test of the masking hypothesis as a force shaping the evolution of life cycles.

ADDITIONAL KEY WORDS: — ploidy evolution – life cycle evolution – masking hypothesis – selfing – assortative mating – asexuality – Chlorophyta – Ulvophyceae.

CONTENTS

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INTRODUCTION

The evolutionary transition between haploidy and diploidy

The haploid and diploid phases of sexual life cycles are extremely variable in length, ranging from little development in one or the other phase to equal development in both (Fowell, 1969; Raper & Flexer, 1970; Bell, 1982). The challenge for theories that describe the transition between life cycles over evolutionary time, and specifically the transition between haploidy and diploidy, is to explain this observed diversity. Predictions from these theories can then be tested in appropriate groups, such as the Chlorophyta, in which closely related species vary in the extent of the haploid and diploid phases.

Most theories concerning ploidy evolution have focussed on advantages to diploidy (reviewed in Valero *et al*., 1992), including protection against inherited mutations (the 'masking hypothesis'; Crow & Kimura, 1965), protection against somatic mutations (Efroimson, 1932), increased rates of beneficial mutations (Paquin & Adams, 1983), and the ability to repair double-stranded DNA damage (Bernstein, Byers & Michod, 1981). Some hypotheses have, however, described advantages to both haploidy and diploidy that would lead, under different circumstances, to different life cycles. We will briefly review these latter hypotheses and the predictions they make. Cavalier-Smith (1978) maintained that the distribution of ploidy levels reflects life history selection, with *r*-selection favouring small-celled haploids and *K*-selection favouring large-celled diploids (see also Weiss, Kukora & Adams, 1975). Lewis (1985) argued instead that ploidy levels evolve in response to nutrient availability, with haploid cells being favoured under nutrient poor conditions and diploid cells (which require 25 to 50% more nutrients) evolving only when nutrients are freely available (see also Adams & Hansche, 1974). Bell (1982, 1994) has argued that diploidy would be favoured when selection acts in opposing directions upon gametic and vegetative cells (because gametes produced by diploids are more likely to vary); haploidy would be favoured when there is little antagonism between selection upon gametic and vegetative cells, as when gametes are undifferentiated from somatic cells. Finally, using genetic load arguments, Kondrashov & Crow (1991) found that diploidy would be favoured when the epistasis among multiple mutations is synergistic but not when it is weak or antagonistic. They predicted that *K*-selected organisms would more often experience synergistic epistasis (favouring diploidy), while *r*-selected organisms would experience weak epistasis (favouring haploidy). Each of these theories makes different predictions about the evolution of ploidy levels, but every one of them allows the evolution of different life cycles under different conditions.

In this paper, we reevaluate the masking hypothesis, a theory which originally stated that new diploid variants would always be favoured within a haploid population because they would immediately mask deleterious recessive mutations (Crow & Kimura, 1965; Perrot, Richerd & Valero, 1991). An inherent problem with theories, such as the masking hypothesis, that always predict the evolution of diploidy is that they do not explain the maintenance of diverse life cycles and cannot explain instances where haploid life cycles are favoured (Bell, 1994). Recent models have suggested that masking deleterious mutations can be *advantageous or disadvantageous* for the evolution of diploidy, depending on the recombination rate between the relevant loci (Bengtsson, 1992; Otto & Goldstein, 1992; Jenkins & Kirkpatrick, 1994; Jenkins & Kirkpatrick, 1995). The problem with these new theoretical results is that, for organisms with many chromosomes like green algae (Bell, 1982), the average rate of recombination between randomly chosen genes is likely to be quite high, nearly one-half. With such high recombination rates and reasonable selection coefficients, diploidy is always favoured in these models; once again the theory is unable to explain comparative data. Other mechanisms can, however, act like physical linkage to reduce genetic mixing within a population and maintain genetic associations between loci. In this paper, we will examine alternative mating systems, determining which tend to favour the evolution of haploidy and which favour the evolution of diploidy in the face of recurrent deleterious recessive mutations. These conditions suggest a test of the masking hypothesis, which is outlined with reference to data from green algae.

The masking hypothesis

Simmons & Crow (1977) reviewed experimental evidence from *Drosophila* showing that the majority of mutations that affect fitness are deleterious and partially recessive. All of these mutations must be expressed in a haploid organism, whereas they will be largely unexpressed in a diploid organism due to the compensatory action of the homologous allele. The masking hypothesis states that diploid life cycles that arise in a haploid population have a fitness advantage because they 'mask' existing deleterious mutations (Crow & Kimura, 1965). Masking, however, has the negative effect of allowing the persistence of mutations (Crow & Kimura, 1965; Otto & Goldstein, 1992; see also Goff & Coleman, 1990: 69). Diploids within a population tend to accumulate more deleterious mutations, because their ancestors survived despite carrying mutations. Thus, while diploid individuals tend to mask the mutations that they carry, they can have substantially more mutations than the haploid individuals within a population. The relative advantage of diploid versus haploid life cycles depends on the exact balance reached between masking and eliminating mutations.

In a two-locus model of interbreeding haploids and diploids, the balance favours diploidy when recombination is free between the two loci and when mating is random (Perrot *et al*., 1991). In contrast, the balance favours haploidy when linkage is tight between the two loci (Otto & Goldstein, 1992). In these models, one locus is subject to deleterious mutations and one determines the life cycle. Since mutations persist longer among lineages that experience more diploid selection, a genetic

association (linkage disequilibrium) necessarily develops between deleterious mutations and diploidy. Recombination reduces the magnitude of the linkage disequilibrium, bringing the frequency of mutations among diploids nearer that of haploids. Hence, with sufficient recombination, diploids gain the benefits of masking mutations but do not suffer from many more mutations than haploids. With tighter linkage the relative frequency of mutations rises among diploids, offsetting their masking advantage, until, eventually, increased haploidy is favoured.

However, the mating system of an organism can greatly influence the extent to which genetic associations are maintained. Assortative mating, inbreeding, and asexual reproduction can all increase the levels of linkage disequilibrium between loci. Thus we would expect the evolution of life cycles to depend on the mating system of a species. Previous evaluations of the masking hypothesis have assumed random mating in large panmictic populations (Perrot *et al*., 1991; Otto & Goldstein, 1992). We generalize these models to include non-random mating, determining the influence of mating system on the evolution of life cycles. We first describe the mating systems that we model, with special reference to green and red algae (Chlorophyta and Rhodophyta).

Assortative mating

For the purpose of this paper, assortative mating means that gametes with the same allele at a single life cycle locus will unite with a higher probability than expected under random mating. This type of assortative mating will occur if individuals produce gametes at a time and place that depends on their genotype at the life cycle locus. For example, the ploidy level of an organism may affect its ability to disperse or colonize some sites (Destombe *et al*., 1992). In this case, neighbouring organisms are more likely to have the same ploidy level and the same life cycle alleles than expected based on random association. Indeed, empirical studies have shown differences in the temporal and geographical distribution of haploid and diploid forms within algal and plant species (Farrar, 1967; Bodenbender, Krause & Schnetter, 1988; DeWreede & Klinger, 1988).

Inbreeding and selfing

Assortative mating, as defined above, increases the probability that uniting gametes have the same alleles at a single locus, the life cycle locus. Inbreeding (mating between closely related individuals) affects all nuclear loci, since related gametes are more likely to carry the same allele at every locus. Inbreeding will occur in species with limited dispersal, because neighbouring individuals will be more closely related than distant individuals. Although dispersal patterns are poorly understood in algae, studies have found that gamete and spore dispersal distances are generally short, often less than 100 m (Santelices, 1990; Destombe, Godin & Remy, 1990; Destombe *et al*., 1992). Hawkes (1990) states that inbreeding may be a common phenomenon in Rhodophyta because of limited dispersal of spores and gametes.

Selfing is an extreme form of inbreeding in which mating gametes are derived from the same individual. In organisms characterized by an alternation of

generations between independent gametophytic and sporophytic phases (algae, other protoctists, ferns), selfing generally occurs among the gametes of a haploid gametophyte (intragametophytically), rather than by mating of different gametophytes produced by the same sporophyte (intergametophytically, as in seed plants) (Ritland, Soltis & Soltis, 1990). In this paper, we will use a model of intragametophytic selfing to determine the effects of inbreeding on the evolution of ploidy levels. Intramagetophytic selfing is the strongest possible form of inbreeding, producing diploid progeny that are homozygous at each locus. We do not develop a model for intergametophytic selfing (which could be applied to seed plants) because such a model is very sensitive to assumptions concerning the fitness effects of gamete competition within a sporophyte.

The rate of selfing varies widely among the algae. At one extreme are organisms that produce gametes that are self-incompatible. This is true of dioecious species such as *Monostroma angicava* (Ulotrichales, Chlorophyta) or organisms with a self-incompatibility system, such as *Pandorina morum* (Volvocaceae, Chlorophyta) (Coleman, 1981). At the other extreme are organisms that exclusively self-fertilize, such as *Eudorina elegans* var *synoica*, another member of the Volvocales (Bell & Praiss, 1986). Between these extremes are organisms that self at varying frequencies (Bell, 1982; Hawkes, 1990), depending in part on the availability of other gametes.

Asexual reproduction

Asexual reproduction describes a variety of means of reproduction by which a parent begets a genetically identical offspring. Asexuality is common across all five classes (*sensu* Mattox & Stewart (1984)) of green algae (Bold & Wynne, 1985). For example, in each of the eight orders of Ulvophyceae described by Tanner (1981), asexual reproduction is known and is often common. Hawkes (1990) describes a plethora of means of asexual reproduction common in red algae, including vegetative propagation, spore production, apomeiosis, and apogamy. Each of these modes may be obligate or facultative depending on the species.

A common mode of asexual reproduction is parthenogenesis, whereby haploid parents produce haploid gametes that do not undergo syngamy but rather develop directly into haploid adults. We will discuss and extend previous results (Bengtsson, 1992) on the evolution of ploidy levels in populations that are partially parthenogenetic. Parthenogenesis can be accompanied by autodiploidization, in which haploid gametes, produced by the gametophyte, germinate without syngamy but undergo a doubling of the genome to produce a diploid sporophyte (Tanner, 1981; Bodenbender & Schnetter, 1990). Genetically, parthenogenesis with autodiploidization is identical to intragametophytic selfing because both result in a diploid that is homozygous at all loci. We will also discuss the results of a model that allows for the direct asexual reproduction of both haploids and diploids via mitotically produced zoospores, aplanospores, or fragments.

METHODS

We analyse a two-locus model that is analogous to that used by Otto & Goldstein (1992) and Perrot *et al*. (1991). As before, we consider a life cycle with non-

TABLE 1. Viability selection at the *A/a* locus

Genotype	Viability
AA	1
Aa	1-*hs*
aa	1-*s*
A	1
a	1-*s*

overlapping generations in a population large enough to ignore genetic drift. One of the two loci, the viability locus, has two alleles (*A*,*a*), with *A* mutating to *a* at a rate μ. The viability scheme is summarized in Table 1, where selection is equivalent in haploids and diploids except among heterozygous diploids (that is, no direct selection acts upon the ploidy level). Experimental studies (Simmons & Crow, 1977) indicate that *h* is generally less than 1/2 for deleterious mutations so that heterozygotes have viabilities closer to wild-type homozygotes than to mutant homozygotes. In this case, masking of mutations occurs and we would expect diploidy to be favoured by selection if it were not for the development of genetic associations. The second locus, the ploidy or life cycle locus, controls the timing of meiosis in a simple manner: meiosis either occurs early or late. The probability that meiosis is delayed is determined by which of the life cycle alleles, C_1 and/or C_2, are carried by the diploid zygote (Table 2). With early meiosis, individuals are haploid for the majority of the life cycle and experience selection as haploids. In contrast, if meiosis occurs late in the life cycle, individuals experience selection as diploids. Thus the life cycle locus is assumed to control the probability that meiosis is gametic or zygotic. While a more realistic life cycle would allow selection to occur in both haploid and diploid stages, according to the amount of time spent in each, previous work on the model with random mating indicates that the two types of life cycles lead to qualitatively similar results (Jenkins & Kirkpatrick, 1994; Otto, 1994). The rate of recombination between the two loci is *r*, which varies between 0 and 1/2.

We will focus on how different forms of non-random mating affect allele frequency changes at the ploidy locus. Specifically, we will determine when alleles that change the life cycle can invade a population and we will compare these results to the model with random mating. Note that, throughout the analysis, the mating system will be held constant and the degree of random versus non-random mating will not change. By tracking changes at the ploidy locus, we will be able to determine the conditions

TABLE 2. Genetic determination of life cycle

Genotype	Proportion diploid (at time of selection)	Proportion haploid (at time of selection)
C_1C_1	d_{11}	$1-d_{11}$
C_1C_2	d_{12}	$1-d_{12}$
C_2C_2	d_{22}	$1-d_{22}$

under which selection favours an increase or a decrease in the number of individuals experiencing diploid selection.

Model with assortative mating

Here we assume that gametes carrying the ploidy allele C_i unite exclusively with other C_i with probability, m, and mate at random with probability, $1–m$ (positive assortment). When assortative mating occurs, uniting gametes must match at the ploidy locus, but the viability alleles will be combined at random, according to their frequencies in gametes carrying the C_i allele. Note that random mating includes additional unions of C_i gametes with one another, as the mating gametes unite in proportion to their frequencies within the population. This model of assortment, illustrated in Figure 1, is standard in population genetics (O'Donald, 1960; Karlin, 1969; Roughgarden, 1979: 187–188; O'Donald, 1980: 40–43). The degree of assortment in an actual population would, presumably, depend on the temporal and spatial distribution of ploidy alleles and would reflect the dispersal patterns of the organism.

Model with selfing

Selfing is assumed to occur among the gametes produced by a haploid gametophyte (intragametophytic). With probability σ, a haploid parent produces two gametes that unite to produce a diploid zygote. The remaining gametes undergo

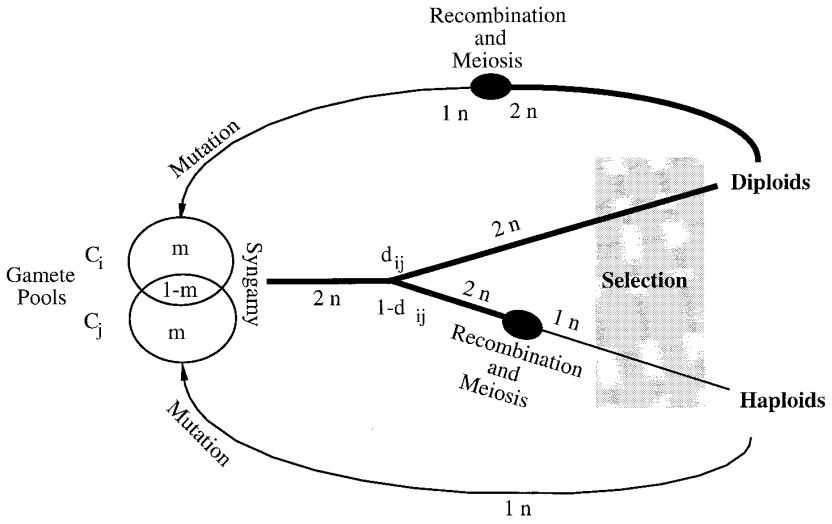

Figure 1. The life cycle with assortment. Gamete types unite assortatively on the basis of their genotype at the ploidy locus with probability m and randomly with probability $(1-m)$. Such assortment is illustrated by a displacement of the gamete pools emanating from diploids and from haploids. A proportion of these newly formed diploids (d_{ij} for C_iC_j individuals) remain diploid whereas the rest undergo recombination and meiosis becoming haploid. Selection now occurs on all individuals as in Table 1. Following selection, the remaining diploids undergo recombination and meiosis. Finally, all individuals produce gametes, some of which mutate and the cycle begins again.

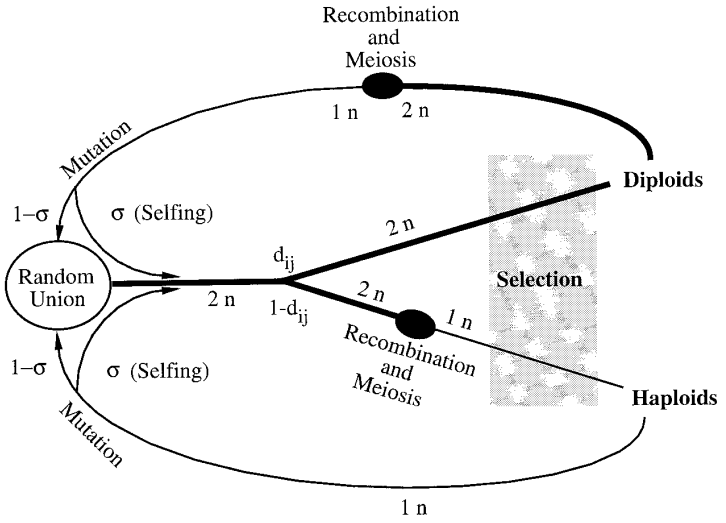

Figure 2. The life cycle with selfing. The life cycle remains unchanged except that a proportion (σ) of zygotes are produced by the self-fertilization of haploid parents. The remaining zygotes are produced by random union of gametes.

random union. This mode of selfing will produce zygotes that are homozygous at all loci (including the ploidy and viability loci) unlike the case of assortative mating (where there can be heterozygotes at the viability locus) and unlike selfing in diploid monophasic individuals (intergametophytic selfing). The life cycle is shown in Figure 2.

Model with asexual reproduction

In our first model of parthenogenesis, a certain proportion, α, of gametes develop directly into haploid adults and the remainder mate at random (Fig. 3). It can easily be shown that this model is dynamically equivalent to the previous model of selfing. This equivalence between selfing and asexuality depends on the particular viability scheme used (Table 2) in which the diploid progeny produced by selfing (*AA* or *aa*) have the same viability as haploid progeny of the same phenotype (*A* or *a*) since no heterozygotes are produced by intragametophytic selfing. Therefore, while selfing produces diploid offspring and while parthenogenesis produces haploid offspring, selection on each chromosome will be identical. In short, all the results concerning the model with selfing will apply to parthenogenesis as well.

The second model of asexual reproduction allows both diploid and haploid adults to produce offspring of their own ploidy level, through means such as budding, fragmentation, or spore production. The dynamics (available upon request) in this case are substantially more complicated, since we must keep track of both haploid and diploid adult frequencies. Nevertheless, the model exhibits very similar behavior to the model with parthenogenesis.

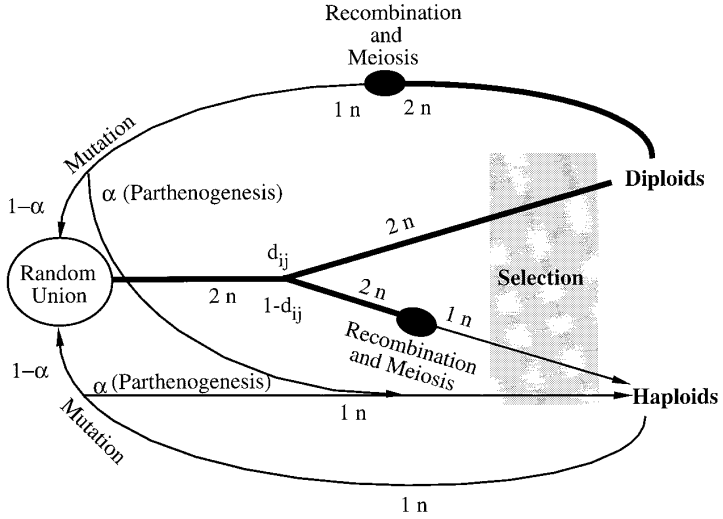

Figure 3. The life cycle with parthenogenesis. The life cycle remains unchanged except that a proportion (α) of gametes develop directly into haploid adults. The remaining gametes unite at random.

RESULTS

Ploidy evolution with assortative mating

With one ploidy allele fixed, say C_1, there will be no effect of assortative mating. Accordingly, the equilibrium frequency of the deleterious allele at the viability locus is unchanged from the model of random mating (see Otto & Goldstein, 1992):

$$\text{freq}(a) = \frac{\mu}{(1-d_{11})s + d_{11}hs} \tag{1}$$

The fate of a rare ploidy allele, C_2, introduced when the population is near this mutation-selection balance is analyzed in Appendix 1. Of special interest is whether or not *both* linkage and assortative mating reduce the probability of invasion of a new allele that increases the proportion of individuals undergoing diploid selection ($d_{12} > d_{11}$).

For arbitrary rates of assortative mating and recombination, a new ploidy allele which increases the degree of diploidy ($d_{12} > d_{11}$) will increase when rare and invade a population if and only if

$$r\,(1-m)\,(1-2h + hs - 2d_{12}hs + 2d_{12}h^2s) > hs[1-d_{12}(1-h)\,(1-m)]. \tag{2}$$

Increasing m, the amount of assortative mating, increases the right hand side of the above inequality while reducing the left hand side, both of which make the condition harder to satisfy. Therefore, the subset of the parameter space in which diploidy will increase is *reduced* by assortative mating.

Whenever condition (2) fails, an increase in the degree of haploidy is favoured (alleles with $d_{12} < d_{11}$ will increase when rare). Since the inequality (2) depends on five parameters, it is impossible to represent the inequality completely in one graph. Instead, we have chosen sample parameter sets ($d_{12} = 1/2; r = 0, 0.01, 0.1, 0.5$; m

= 0.1, 0.5, 0.9) which are used to present the results in graphical form (Fig. 4). Special cases are discussed below.

In the absence of assortative mating ($m = 0$), the recursions given in Appendix 1 reduce to those given previously (Otto & Goldstein, 1992). Thus, the discussion and figures therein apply. If new alleles that increase the rate of diploidy ($d_{12} > d_{11}$) cannot invade when $m = 0$, then they will not be able to do so when there is assortative mating ($m > 0$).

When assortative mating is complete ($m = 1$), *only* alleles that increase the proportion of haploid adults ($d_{12} < d_{11}$) will rise in frequency. The percentage of diploid individuals will decrease over time even though they are able to mask mutations. This result is exactly the same as that obtained when $r = 0$. Thus with either complete assortative mating or complete linkage, the degree of haploidy will increase over time while the degree of diploidy will decrease over time (for $h > 0$).

Ploidy evolution with selfing

As found in Appendix 2, the criterion (2) found with assortative mating applies equally well to the evolution of diploidy with selfing. New ploidy alleles that increase the proportion of diploids can invade if and only if

$$r\,(1-\sigma)\,(1-2h + hs-2d_{12}hs + 2d_{12}h^2s) \;>\; hs[1-d_{12}(1-h)\,(1-\sigma)]. \qquad (3)$$

All conclusions drawn from the analysis with assortative mating hold with selfing, including the results presented in Figure 4.

The fact that assortative mating and selfing restrict the condition under which diploidy is favoured according to equivalent formulae (compare equations (2) and (3)) can be understood as follows. Genetic associations, built up by selection, are destroyed by recombination, but recombination is only effective between two loci that are both heterozygous. Assortative mating and selfing reduce, to the same extent, the probability that both loci are heterozygous, and therefore they both reduce the effectiveness of recombination in destroying linkage disequilibrium. Whenever recombination appears in the recursions, it is multiplied by $1-m$ in the case of assortative mating and $1-\sigma$ in the case of selfing. Hence, in these two cases one can define the effective rate of recombination as $r(1-m)$ or $r(1-\sigma)$, respectively (see left hand sides of (2) and (3)). There is a further effect of assortative mating or selfing on the right hand sides of these equations. This effect is due to a reduction in the frequency that mutations are masked in diploids, since diploid homozygotes are more common and heterozygotes less common with inbreeding.

Ploidy evolution with asexual reproduction

With direct development of haploid gametes (parthenogenesis), the recursions are the same as described for selfing in Appendix 2. Thus new diploid alleles can only invade if condition (3) is met. Bengtsson (1992) has obtained similar results in his model of fusion competence. In that model, haploids are asexual with probability $(1-c)$ and are 'competent' to undergo sexual reproduction with probability c (c is equivalent to our $1-\alpha$); all sexually produced progeny remain diploid throughout selection. Making the appropriate substitutions into equation (3), we can regain

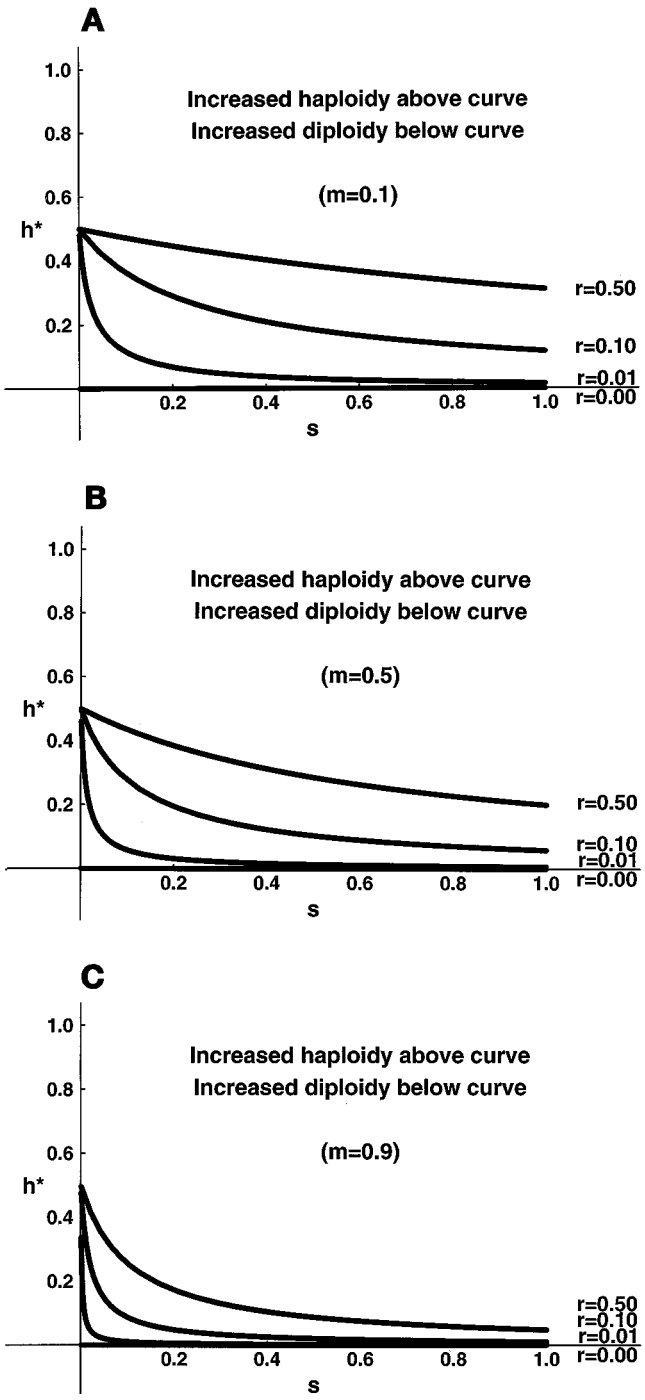

Figure 4. Assortment and recombination both influence the evolution of diploidy. Condition (2) is depicted graphically for four different rates of recombination 0, 0.01, 0.1, 0.5 and three different rates of positive assortative mating (A) 0.1, (B) 0.5, (C) 0.9 assuming that $d_{12} = 1/2$. In each graph, we show the curve below which only an increase in the level of diploidy is favoured ($d_{12} > d_{11}$ for invasion) and above which only an increase in the level of haploidy is favoured ($d_{12} < d_{11}$ for invasion).

Bengtsson's equation (2). This indicates the close similarity between models of ploidy evolution and models of the evolution of sex.

Our second model of apomixis, which allows both haploid and diploid individuals to reproduce asexually at arbitrary rates, leads to a different quantitative result. Letting both haploids and diploids reproduce asexually at the same rate, α, diploidy will be favoured whenever

$$r\,(1-\alpha)\,[(1-2h+hs)(1-\alpha+\alpha hs)-2d_{12}(1-h)hs] > hs[(1-d_{12})\,(1-h)\,(1-\alpha)+h\,(1-\alpha+\alpha s)]. \quad (4)$$

While this condition is different than that derived with only haploid parthenogenesis, equation (3), it is not qualitatively different. When $r = 1/2$ or $r = 0$, the equations coincide exactly. For other values of r, the difference between the conditions is fairly trivial. Thus, while the exact condition for the evolution of ploidy levels depends on the exact model of asexual reproduction, the general results do not.

The conditions under which diploidy is able to evolve are summarized in Table 3.

Genetic associations favour haploids

It can be shown using a disequilibrium analysis (Uyenoyama & Bengtsson, 1989) that genetic associations develop to couple a C_i allele with the most fit viability allele (A) when and only when C_i leads to an increase in the proportion of haploids. In words, deleterious mutations are more common on chromosomes carried by a diploid individual then on those carried by a haploid. Essentially, diploid selection enables mutations to persist over time because these mutations are partially masked. Conversely, haploid selection 'purges' the genome of deleterious mutations in a manner similar to the purging of mutations that occurs during the evolution of selfing (Jarne & Charlesworth, 1993). Consequently, haploids are less likely to inherit a mutation, even though when they do so they have a lower fitness than diploids

TABLE 3. Summary of the analytical results. Diploidy is 'favoured' if the new allele can invade a population when it increases the degree of diploidy in the population ($d_{12}>d_{11}$). Haploidy is favoured whenever diploidy is not favoured. See Appendices

Case	Diploidy favoured when:
Assortative Mating (m)	$r(1-m)(1-2h+hs-2d_{12}hs+2d_{12}h^2s) > hs[1-d_{12}(1-h)(1-m)]$
Selfing (σ)	$r(1-\sigma)(1-2h+hs-2d_{12}hs+2d_{12}h^2s) > hs[1-d_{12}(1-h)(1-\sigma)]$
Parthenogenesis (α)	$r(1-\alpha)(1-2h+hs-2d_{12}hs+2d_{12}h^2s) > hs[1-d_{12}(1-h)(1-\alpha)]$
Haploid and diploid apomixis (α)	$r(1-\alpha)[(1-2h+hs)(1-\alpha+\alpha hs)-2d_{12}(1-h)hs] > hs[(1-d_{12})(1-h)(1-\alpha)+h(1-\alpha+\alpha s)]$
$m=\sigma=\alpha=0$	$r(1-2h+hs-2d_{12}hs+2d_{12}h^2s) > hs[1-d_{12}(1-h)]$
m, σ, or α near 1	Never

bearing a mutation ($1-s$ compared to $1-hs$). Each mating system that we have examined increases the magnitude of the linkage disequilibrium between the two loci when a new ploidy allele is introduced into a population. It is this association that favours haploidy, outweighing the masking advantage that diploids gain whenever the conditions are right (r small enough; m, σ, or α large enough).

These results also suggest that mating systems might influence the evolution of haploid males within a diploid species (arrhenotoky). Goldstein (1994) recently showed that low rates of recombination would aid in the transition from diploidy to haplo-diploidy. We suspect that inbreeding might have a similar effect, maintaining genetic associations that favour male haploids.

An interesting point recently made by Bell (1994) is that the genetic associations that develop during selection on the adult phase can actually work against haploidy if the opposite allele, *a*, is strongly favoured by selection among gametes. Gametic selection, in this case, acts upon the haploid gametes of all individuals, regardless of their life cycle type. In Figure 5, we show the influence of gametic selection on the evolution of ploidy levels for recombination rates of $r = 0.1$ and $r = 0.5$ (see model of Bell, 1994 and Fig. 5, therein). For all recombination rates, the very disequilibrium that favours haploidy in the adult phase works against haploidy if *a* is advantageous in gametes (negative pleiotropy). Conversely, these genetic associations provide an additional advantage to haploidy whenever selection continues to act against the *a* allele among gametes (positive pleiotropy). It would appear, however, far more likely that alleles deleterious among adults would remain deleterious among gametes. Thus, we expect that gametic selection will act, primarily, to augment the importance of linkage disequilibrium, favouring the evolution of haploid adult phases.

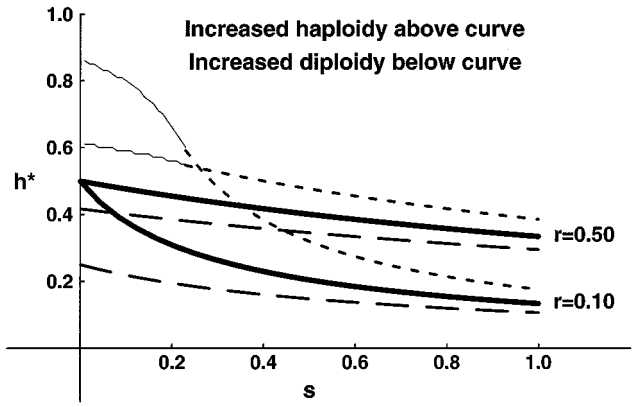

Figure 5. Gametic selection and the evolution of ploidy levels. Selection acts upon all gametes, with *A* gametes having a relative fitness of $1-v$ compared to *a* gametes. In the absence of gametic selection ($v = 0$) and with random mating, haploidy will evolve below the thick solid curves (shown for $r = 0.1$ and $r = 0.5$). With selection acting against alleles *A* in gametes but favouring it in adults ($v = 0.2$), the curves become raised (short dashed lines), such that diploidy is favoured over a larger parameter range. With selection favouring the *A* allele in both gametes and adults ($v = -0.2$), the curves lower (long dashed lines) and haploidy is favoured over a larger parameter range. The light solid line represents simulation results performed when a local stability analysis was inappropriate (the mutation-selection balance becomes unstable).

A PROPOSAL FOR TESTING THE THEORY

Based on the above results, if life cycles evolve in response to deleterious mutations, there should be a correlation between the extent of the diploid phase of an organism and the degree to which it mates at random. One can test whether such a correlation exists in a group of organisms by calculating the association between life cycles and reproductive systems on a phylogenetic tree (Brooks & McLennan, 1991; Harvey & Pagel, 1991). Of course, a significant correlation would not prove that changes in reproduction *cause* changes in life cycles. Such a correlation would also arise if life cycle evolution *causes* mating systems to evolve. For example, we expect that the evolution of haploidy would ease the evolutionary transition to selfing, since haploids purge their genome of deleterious mutations, lessening the amount of inbreeding depression that would be observed upon selfing (Charlesworth & Charlesworth, 1992). Methods have been developed which attempt to discern the direction of causality by estimating which traits tend to change first on a phylogeny and which traits change secondarily (Pagel, 1994). In theory, such a method could be used to determine whether changes in the reproductive system of a lineage cause life cycle evolution rather than the reverse (but see Read & Nee, 1995). We therefore propose that the masking hypothesis be tested by determining whether life cycles and mating systems co-evolve in the expected manner.

The Chlorophyta are an ideal group to determine whether such a correlation exists, since life cycles and mating systems are highly variable. To explore life cycle diversity within this group, we developed an index (Table 4) that was designed to encompass the entire spectrum of life cycles. Among the Chlorophyta, the Ulvophycea, in particular, have undergone a large amount of life cycle evolution as illustrated in Figure 6 using a composite phylogeny based on morphological and molecular data (Mishler *et al*., 1994; Zechman *et al*., 1990; Kantz *et al*., 1990). With ancestral character states inferred on this phylogeny by MacClade (Maddison & Maddison, 1992), transitions both to increased haploidy and to increased diploidy are observed within this group. For example, there is a transition to diploidy in the Caulerpales clade then a transition back to alternating generations in the Bryopsidae clade. In the Ulvales/Ulotrichales clade there is another transition from alternation of generations to haploidy. Within each of the Ulvophycean orders (with the

TABLE 4. Index of life cycles

	Life cycle	Description
1	Haploid	Haploid adult. No mitoses within diploid (zygotic meiosis).
2	Codiolum-like	Haploid adult. No mitoses within diploid, but some development.
3	Heteromorphic (H)	Heteromorphic alternation of generations with haploid more developed.
	3a	Both haploid and diploid phases are independent.
	3b	Haploid develops on diploid stage.
	3c	Diploid develops on haploid stage.
4	Heteromorphic	Heteromorphic alternation of generations with equal development.
5	Isomorphic	Isomorphic alternation of generations.
6	Heteromorphic (D)	Heteromorphic alternation of generations with diploid more developed.
	6a	Both haploid and diploid phases are independent.
	6b	Haploid develops on diploid stage.
	6c	Diploid develops on haploid stage.
7	Acetabularia-like	Diploid organism that undergoes meiosis to produce haploid soma.
8	Diploid	Diploid adult. No mitoses within haploid (gametic meiosis).

exception of Dasycladales) *and even within genera*, further life cycle variation is known (Table 5). These results indicate that life cycles can evolve remarkably rapidly.

We searched the existing literature for details concerning the reproductive systems of the Ulvophyceae, but found insufficient data to perform a test of our hypothesis. Mating systems under natural conditions are poorly understood for these taxa, with essentially no quantitative data on the extent of various forms of reproduction. For example, asexual reproduction is often known or even common within algal taxa (see Table 5), but its frequency is essentially unmeasured. Rather than collecting extensive data on reproductive systems in these groups, an alternative approach, which would be more practical for algae, would be to collect molecular data on the degree of inbreeding and the degree of genetic associations observed at marker loci. A test could then be performed to determine, for instance, whether more inbred species tend to have longer haploid phases, as we expect. Further work within the Ulvophyceae and similar taxa, gathering genetic and natural history data, promises to provide rich evidence by which to test theories concerning the evolution of ploidy levels.

CONCLUSIONS

We have examined the influence of masking deleterious mutations on the evolution of ploidy levels in models that allow for non-random mating. In these models, a life cycle locus controls the probability that an organism will undergo

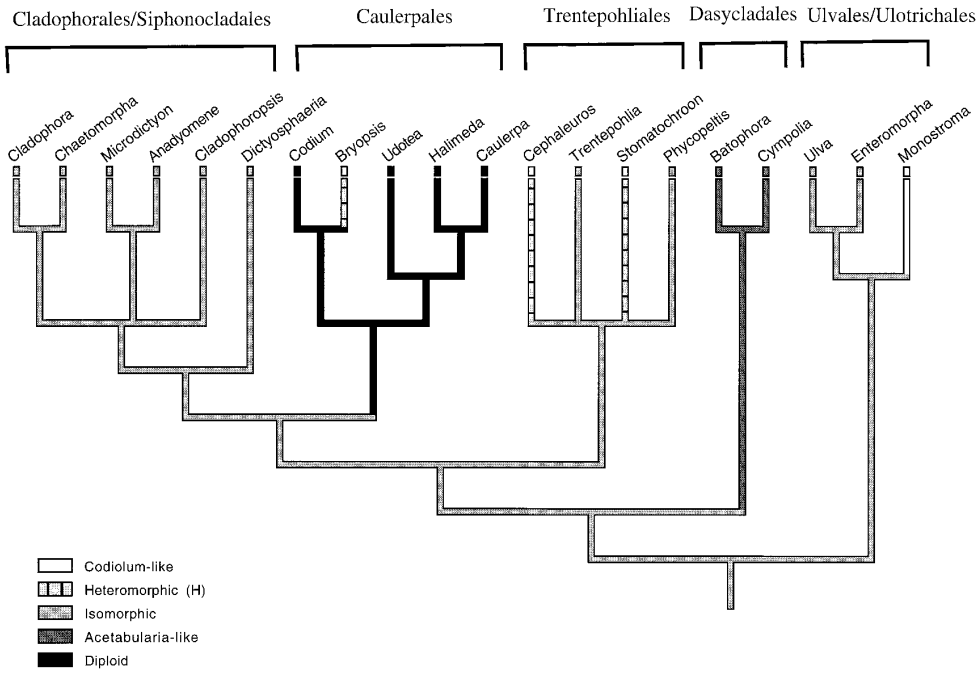

Figure 6. Phylogeny and life cycles of the Ulvophyceae. Phylogeny is based on Mishler *et al*. (1994), Zechman *et al*. (1990), and Floyd & O'Kelly (1984). Life cycle evolution is depicted by shading on the tree, where the legend is described more fully in Table 4. MacClade (Maddison & Maddison, 1992) was used to draw characters upon the tree and to infer ancestral states.

meiosis either early or late within the life cycle. If meiosis occurs early (zygotic meiosis), the organism will be dominated by the haploid stage of the life cycle, while if meiosis is late (gametic meiosis), the diploid stage will dominate. By examining when new alleles can invade the life cycle locus, we were able to determine how ploidy levels would evolve. The masking hypothesis predicts that increases in the diploid phase should occur whenever deleterious alleles are masked ($h < 1/2$). This prediction ignores, however, the development of genetic associations (Otto & Goldstein, 1992). Generally, alleles that extend the diploid phase become coupled with higher numbers of mutations, because mutations are concealed from selection in diploid heterozygotes and tend to persist. Conversely, alleles that extend the haploid phase become coupled with non-mutant alleles, because mutations are revealed to selection and are eliminated from the population. Because revealing and eliminating mutations can be more advantageous than masking them, we suggest the replacement of the masking hypothesis with a 'masking/revealing hypothesis', which takes into account both the masking advantage to diploids and the associative advantage to haploids.

Under the 'masking/revealing hypothesis', the evolution of ploidy levels depends on the extent to which genetic associations develop in a population, since the level of association determines the advantage that haploids gain from revealing mutations. Tight genetic linkage favours the evolution of haploidy while loose linkage favours diploidy (Otto & Goldstein, 1992). We have found that assortative mating,

TABLE 5. Life cycle variation within the Ulvophyceae. Question marks indicate missing information. [1]Bodenbender & Schnetter (1990). [2]Graham (1982). [3]Calderon-Saenz & Schnetter (1989). [4]Tanner (1981). [5]Churchill & Moeller (1972). [6]Smith (1955). [7]Floyd & O'Kelly (1984). [8]Tatewaki (1972). [9]Leonardi & Caceres (1988). [10]Bliding (1963)

Clade	Species	Life Cycle	Asexuality	Gametes
SCC	*Chaetomorpha pachynema*[1]	5	Common	Isogamous
SCC	*Chaetomorpha antennina*[1]	Asexual	Exclusive	(NA)
SCC	*Cladophora glomerata*[2]	Asexual	Exclusive	(NA)
SCC	*Cladophora pellucida*[1]	5	Known	Isogamous
SCC	*Ernodesmis verticillata* (Caribbean)[1]	8	Known	Isogamous
SCC	*Ernodesmis verticillata* (Canary)[1]	5	Known	Isogamous
SCC	*Struvea anastomosans*[1]	6a	Known	Isogamous
Caulerpales	*Pedobesia clavaeformis*[3,4]	6c? 8?	Known	Anisogamous
Caulerpales	*Derbesia tenuissima*[3,4]	6a	Known	Anisogamous
Caulerpales	*Bryopsidella ostreobiformis*[3]	4	Known	Anisogamous
Caulerpales	*Bryopsidella neglecta*[3,4]	3a	Known	Anisogamous
Caulerpales	*Bryopsis plumosa*[3,4]	3b	?	Anisogamous
Caulerpales	*Codium fragile* (Atlantic)[5]	Asexual	Exclusive	(NA)
Caulerpales	*Codium fragile* (Pacific)[5,6]	8	Known	Anisogamous
Ulotrichales	*Acrosiphonia spinecens*[4,7]	2	Common	Isogamous
Ulotrichales	*Acrosiphonia arcta*[4,7]	1	Common	Isogamous
Ulotrichales	*Eugomontia sacculata*[7]	5	Common	?
Ulotrichales	*Monostroma angicava*[8]	2	Known	Anisogamous
Ulotrichales	*Monostroma groenlandicum*[8]	2	Known	Anisogamous
Ulotrichales	*Monostroma undulatum*[8]	Asexual(2)	Exclusive	(NA)
Ulotrichales	*Monostroma zostericola*[8]	6a	?	Anisogamous
Ulotrichales	*Monostroma fuscum* var. *splendens*[8]	4	?	Anisogamous
Ulvales	*Ulva mutabilis*[4]	4	Common	Anisogamous
Ulvales	*Enteromorpha flecuosa*[9]	1	Almost complete	(NA)
Ulvales	*Enteromorpha prolifera*[10]	4	Known	Anisogamous
Dasycladales	*Acetabularia mediterranea*[4]	7	Known	Isogamous

intragametophytic selfing and asexual reproduction also act to maintain the genetic associations that favour haploidy. Each of these mechanisms reduce the effective recombination rate: tight linkage reduces the probability of a cross-over event; assortative mating and selfing reduce the frequency of double heterozygotes (the only genotypes affected by recombination); and parthenogenesis reduces the number of recombination events that occur per generation. With fewer recombination events in a population, the associations that develop between certain ploidy and viability alleles (measured by linkage disequilibria) are more likely to be maintained over time. Consequently, when the mating scheme of a population is marked by extreme non-random mating of the forms considered, increased haploid phases are expected to evolve (see Fig. 4). Thus the 'masking/revealing hypothesis' predicts that haplonty should evolve in organisms with extensive non-random mating whereas diplonty should evolve when mating is fairly random.

The importance of the 'masking/revealing hypothesis' to the evolution of ploidy levels can therefore be tested by a comparative study that determines whether or not a correlation exists between ploidy level and the mechanisms which maintain genetic associations. An ideal group for such a test would be the Chlorophyta, especially the Ulvophyceae. This group is characterized by a wide variety of different life cycles and mating systems. The appropriate test cannot be completed, however, until more detailed knowledge is gathered on the reproductive systems of the green algae. Additional studies of the natural history and genetics of algae are sorely needed to further our understanding of the evolution of life cycles and to test theories such as the masking hypothesis.

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REFERENCES

Adams J, Hansche PE. 1974. Population studies in microorganisms I. Evolution of diploidy in *Saccharomyces cerevisiae*. *Genetics* **76:** 327–338.

Aoki K, Feldman MW. 1991. Recessive hereditary deafness, assortative mating, and persistence of a sign language. *Theoretical Population Biology* **39:** 358–372.

Bell G. 1982. *The Masterpiece of Nature: The Evolution and Genetics of Sexuality*. Berkeley: University of California Press.

Bell G. 1994. The comparative biology of the alternation of generations. In: Kirkpatrick M, ed. *Lectures on Mathematics in the Life Sciences: Theories for the Evolution of Haploid-Diploid Life Cycles*. Providence, RI: American Mathematical Association, 1–26.

Bell G, Praiss M. 1986. Optimality and constraint in a self-fertilized alga. *Evolution* **40:** 194–198.

Bengtsson B. 1992. Deleterious mutations and the origin of the meiotic ploidy cycle. *Genetics* **131:** 741–744.

Bernstein H, Byers G, Michod R. 1981. Evolution of sexual reproduction: Importance of DNA repair, complementation, and variation. *American Naturalist* **117:** 537–549.

Bliding C. 1963. A critical survey of European taxa in Ulvales. I. *Capsosiphon, Percursaria, Blidingia, Enteromorpha. Opera Botanica* **8:** 1–160.

Bodenbender S, Krause UR, Schnetter R. 1988. Notes on life cycles in Colombian isolates of *Ernodesmis* and *Boodlea* (Siphonocladales, Chlorophyta). *Cryptogamie, Algologie* **9:** 279–287.

Bodenbender S, Schnetter R. 1990. Nuclear behaviour during the life cycles of *Chaetomorpha, Ernodesmis* and *Struvea* (Ulvophyceae, Chlorophyta) under culture conditions. *Cryptogamic Botany* **1:** 340–354.

Bold HC, Wynne MJ. 1985. *Introduction to the Algae: Structure and Reproduction, 2nd Ed.* Englewood Cliffs, NJ: Prentice-Hall Inc.

Brooks DR, McLennan DA. 1991. *Phylogeny, Ecology, and Behavior: A Research Program in Comparative Biology.* Chicago: University of Chicago Press.

Calderón-Sáenz E, Schnetter R. 1989. Life cycle and morphology of *Bryopsidella ostreobiformis* spec. nov. (Bryopsidaceae, Chlorophyta) from the Mediterranean, under culture conditions, with comments on the phylogeny of the *Bryopsis/Derbesia* Complex. *Botanica Acta* **102:** 249–260.

Cavalier-Smith T. 1978. Nuclear volume control by nucleoskeletal DNA, selection for cell volume and cell growth rate, and the solution of the DNA C-value paradox. *Journal of Cell Science* **34:** 247–278.

Charlesworth D, Charlesworth B. 1992. The effects of selection in the gametophytic stage on mutational load. *Evolution* **46:** 703–720.

Churchill AC, Moeller HW. 1972. Seasonal patterns in New York. Populations of *Codium fragile* (Sur.) Hariot subsp. *tomentosoides* (van Goor) Silva. *Journal of Phycology* **8:** 147–152.

Coleman AW. 1981. The use of natural genetic markers to study colonization and sexuality in nature. *American Naturalist* **118:** 761–769.

Crow J, Kimura M. 1965. Evolution in sexual and asexual populations. *American Naturalist* **99:** 439–450.

Destombe C, Godin J, Lefebvre C, Dehorter O, Vernet P. 1992. Differences in dispersal abilities of haploid and diploid spores of *Gracilaria verrucosa* (Gracilariales, Rhodophyta). *Botanica Marina* **35:** 93–98.

Destombe C, Godin J, Remy J-M. (1990). Viability and dissemination of spermatia of *Gracilaria verrucosa* (Gracilariales, Rhodophyta). *Hydrobiologia* **204/205:** 219–223.

DeWreede RE, Klinger T. 1988. Reproductive strategies in algae. In: Doust JL, Doust LL, eds. *Plant Reproductive Ecology: Patterns and Strategies.* New York: Oxford University Press, 267–284.

Efroimson V. 1932. On some problems of the accumulation of lethals. *Journal de Biologie* **1:** 87–102.

Farrar DR. 1967. Gametophytes of four tropical genera reproducing independently of their sporophytes in the southern Appalachians. *Science* **155:** 1266–1267.

Floyd GL, O'Kelly CJ. 1984. Motile cell ultrastructure and the circumscription of the orders Ulotrichales and Ulvales (Ulvophyceae, Chlorophyta). *American Journal of Botany* **71:** 111–120.

Fowell RR. 1969. Life cycles in yeast. In: Rose AH, Harrison JS, eds. *The Yeasts.* New York: Academic Press, 461–471.

Goff LJ, Coleman AW. 1990. DNA: Microspectrofluorometric studies. In: Cole KM, Sheath RG, eds. *Biology of the Red Algae.* Cambridge: Cambridge University Press, 43–71.

Goldstein DB. 1994. Deleterious mutations and the evolution of male haploidy. *American Naturalist* **144:** 176–183.

Graham LE. 1982. Cytology, ultrastructure, taxonomy, and phylogenetic relationships of great lakes filamentous algae. *Journal of Great Lakes Research* **8:** 3–9.

Harvey PH, Pagel MD. 1991. *The Comparative Method in Evolutionary Biology.* Oxford: Oxford University Press.

Hawkes MW. 1990. Reproductive strategies. In: Cole KM, Sheath RG, eds. *Biology of the Red Algae.* Cambridge: Cambridge University Press, 455–476.

Jarne P, Charlesworth D. 1993. The evolution of the selfing rate in functionally hermaphrodite plants and animals. *Annual Reviews of Ecology and Systematics* **24:** 441–466.

Jenkins CD, Kirkpatrick M. 1994. Deleterious mutations and ecological selection in the evolution of life cycles. In: Kirkpatrick M, ed. *Lectures on Mathematics in the Life Sciences: Theories for the Evolution of Haploid-Diploid Life Cycles.* Providence, RI: American Mathematical Association, 53–68.

Jenkins CD, Kirkpatrick M. 1995. Deleterious mutations and the evolution of genetic life cycles. *Evolution* **49:** 512–520.

Kantz TS, Theriot EC, Zimmer EA, Chapman RL. 1990. The Pleurastrophyceae and Micromonadophyceae: A cladistic analysis of nuclear rRNA sequence data. *Journal of Phycology* **26:** 711–721.

Karlin S. 1969. *Equilibrium Behaviour of Population Genetic Models with Non-random Mating.* New York: Gordon and Breach.

Kondrashov A, Crow J. 1991. Haploidy or diploidy: which is better? *Nature* **351:** 314–315.

Leonardi PI, Caceres EJ. 1988. Contribución al conocimiento del ciclo biologicó de *Enteromorpha flexuosa* subsp. *pilifera* (Chlorophyceae). *PHYSIS (Buenos Aires), Seccion B* **46:** 29–39.

Lewis WM. 1985. Nutrient scarcity as an evolutionary cause of haploidy. *American Naturalist* **125:** 692–701.

Maddison WP, Maddison DR. 1992. *MacClade, Version 3.* Sunderland, MA: Sinauer Associates, Inc.

Mattox KR, Stewart KD. 1984. Classification of the green algae: A concept based on comparative cytology. In: Irvine DEG, John DM, eds. *Systematics of the Green Algae.* New York: Academic Press, 29–72.

Mishler BD, Lewis LA, Buchheim MA, Renzaglia KS, Garbary DJ, Delwiche CF, Zechman FW, Kantz TS, Chapman RL. 1994. Phylogenetic relationships of the green algae and bryophytes. *Annals of the Missouri Botanical Garden* **81:** 451–483.

O'Donald P. 1960. Assortative mating in a population in which two alleles are segregating. *Heredity* **15:** 389–396.

O'Donald P. 1980. *Genetic Models of Sexual Selection.* Cambridge: Cambridge University Press.

Otto S. 1994. The role of deleterious and beneficial mutations in the evolution of ploidy levels. In: Kirkpatrick M, ed. *Lectures on Mathematics in the Life Sciences: Theories for the Evolution of Haploid-Diploid Life Cycles* volume 25. Providence, RI: American Mathematical Association, 69–96.

Otto S, Goldstein D. 1992. Recombination and the evolution of diploidy. *Genetics* **131:** 745–751.

Pagel M. 1994. Detecting correlated evolution on phylogenies — a general method for the comparative analysis of discrete characters. *Proceedings of the Royal Society, London B* **255:** 37–45.

Paquin C, Adams J. 1983. Frequency of fixation of adaptive mutations is higher in evolving diploid than haploid yeast populations. *Nature* **302:** 495–500.

Perrot V, Richerd S, Valero M. 1991. Transition from haploidy to diploidy. *Nature* **351:** 315–317.

Raper JR, Flexer AS. 1970. The road to diploidy with emphasis on a detour. *Symposia of the Society for General Microbiology* **20:** 401–432.

Read AF, Nee S. 1995. Inference from binary comparative data. *Journal of Theoretical Biology* **173:** 99–108.

Ritland K, Soltis DE, Soltis PS. 1990. A two-locus model for the joint estimation of intergametophytic and intragametophytic selfing rates. *Heredity* **65:** 289–296.

Roughgarden J. 1979. *Theory of Population Genetics and Evolutionary Ecology: An Introduction*. New York: Macmillan Publishing Co.

Santelices B. 1990. Patterns of reproduction, dispersal and recruitment in seaweeds. *Oceanography and Marine Biology: An Annual Review* **28:** 177–276.

Simmons MJ, Crow JF. 1977. Mutations affecting fitness in *Drosophila* populations. *Annual Review of Genetics* **11:** 49–78.

Smith GM. 1955. *Cryptogamic Botany. I. Algae and Fungi*. New York: McGraw-Hill Book Company, Inc.

Tanner CE. 1981. Chlorophyta: Life histories. In: Lobban CS, Wynne MJ, eds. *Botanical Monographs: The Biology of Seaweeds.* Berkeley CA: University of California Press, 218–247.

Tatewaki M. 1972. Life history and systematics in *Monostroma*. In: Abbot IA, Kurogi M, eds. *Contributions to the Systematics of the Benthic Marine Algae of the North Pacific*. Kobe: Japanese Society of Phycology, 1–15.

Uyenoyama M, Bengtsson B. 1989. On the origin of meiotic reproduction: A genetic modifier model. *Genetics* **123:** 873–885.

Valero M, Richerd S, Perrot V, Destombe C. 1992. Evolution of alternation of haploid and diploid phases in life cycles. *Trends in Ecology and Evolution* **7:** 25–29.

Weiss RL, Kukora JR, Adams J. 1975. The relationship between enzyme activity, cell geometry, and fitness in *Saccharomyces cerevisiae. Proceedings of the National Academy of Science, USA* **72:** 794–798.

Zechman FW, Theriot EC, Zimmer EA, Chapman RL. 1990. Phylogeny of the Ulvophyceae (Chlorophyta): Cladistic analysis of nuclear-encoded rRNA sequence data. *Journal of Phycology* **26:** 700–710.

APPENDIX 1. ANALYSIS WITH ASSORTMENT

Recursions

We census at the gamete stage and keep the same definitions used in Otto & Goldstein (1992):

$$x_1 = \text{frequency } (AC_1) \quad x_2 = \text{frequency } (aC_1)$$
$$x_3 = \text{frequency } (AC_2) \quad x_4 = \text{frequency } (aC_2),$$

where $x_1 + x_2 + x_3 + x_4 = 1$. Mutation occurs from allele A to a with frequency μ as gametes are produced. A fraction of the gametes unite assortatively (m) on the basis of their ploidy genotype (assortment occurs by randomly choosing two gametes among those with the same ploidy allele). The remaining gametes unite at random following the recursions in Otto & Goldstein (1992). The recursions with assortative mating are thus:

$$Tx_1' = (1-m)(1-\mu)\{x_1^2 + x_1x_2(1-hsd_{11}) + x_1x_3 + (1-r)x_1x_4(1-hsd_{12}) + rx_2x_3(1-hsd_{12})\}$$

$$+ m(1-\mu)\frac{x_1^2 + (1-hsd_{11})x_1x_2}{x_1 + x_2}$$

$$Tx_2' = (1-m)[\mu\{x_1x_1 + x_1x_2(1-hsd_{11}) + x_1x_3 + (1-r)x_1x_4(1-hsd_{12}) + rx_2x_3(1-hsd_{12})\}$$
$$+ x_1x_2(1-s + sd_{11}-hsd_{11}) + rx_1x_4(1-s + sd_{12}-hsd_{12}) + x_2^2(1-s) + (1-r)x_2x_3(1-s + sd_{12}-hsd_{12}) + x_2x_4(1-s)]$$

$$+ m[\mu\frac{x_1^2 + (1-hsd_{11})x_1x_2}{x_1 + x_2} + \frac{x_2^2(1-s) + (1-s + sd_{11}-hsd_{11})x_1x_2}{x_1 + x_2}]$$

$$Tx_3' = (1-m)(1-\mu)\{x_1x_3 + rx_1x_4(1-hsd_{12}) + (1-r)x_2x_3(1-hsd_{12}) + x_3x_3 + x_3x_4(1-hsd_{22})\}$$

$$+ m(1-\mu)\frac{x_3^2 + (1-hsd_{22})x_3x_4}{x_3 + x_4}$$

$$\begin{aligned}Tx_4' = (1-m)[&\mu\{x_1x_3 + rx_1x_4(1-hsd_{12}) + (1-r)x_2x_3(1-hsd_{12}) + x_3x_3 + x_3x_4(1-hsd_{22})\}\\ &+ (1-r)x_1x_4(1-hsd_{12}-s + sd_{12}) + rx_2x_3(1-hsd_{12}-s + d_{12}s) + x_2x_4(1-s) + x_3x_4(1-s + sd_{22}-hsd_{22}) + x_4x_4(1-s)]\end{aligned}$$

$$+ m[\mu\frac{x_3^2 + (1-hsd_{22})x_3x_4}{x_3 + x_4} + \frac{x_4^2(1-s) + (1-s + sd_{22}-hsd_{22})x_3x_4}{x_3 + x_4}]$$

where T is the sum of the right hand sides and is equal to the mean fitness. With the C_1 allele fixed ($\hat{x}_3 = \hat{x}_4 = 0$), the equilibrium frequencies are:

$$\hat{x}_1 = 1-\hat{x}_2$$
$$\hat{x}_2 = \frac{\mu}{s(1-d_{11}) + hsd_{11}}. \tag{5}$$

As expected, assortative mating on the basis of ploidy does not alter the population composition when there is no variation at the ploidy locus. Note that equation (5) is invalid when ($h = 0$) and ($d_{11} = 1$). This special case may easily be analyzed; the local stability analysis given below concentrates, however, on the stability of equilibrium (5) when it is valid.

Stability analysis

When a population is near the equilibrium described by equation (5), we follow the fate of a rare ploidy allele, C_2. To linear order in x_3 and x_4, the recursions become:

$$x_3' = (1-m)(1-\mu)\frac{\{\hat{x}_1x_3 + \hat{x}_2x_3(1-hsd_{12}) + rD(1-hsd_{12})\}}{T} \tag{6}$$

$$+ m(1-\mu)\frac{x_3^2 + (1-hsd_{22})x_3x_4}{T(x_3 + x_4)}$$

$$\begin{aligned}x_4' = (1-m)[&\mu\frac{\{\hat{x}_1x_3 + \hat{x}_2x_3(1-hsd_{12}) + rD(1-hsd_{12})\}}{T}\\ &+ \frac{\hat{x}_1x_4(1-hsd_{12}-s + sd_{12}) + \hat{x}_2x_4(1-s) + rD(1-hsd_{12}-s + d_{12}s)}{T}]\\ &+ m\frac{\mu\{x_3^2 + (1-hsd_{22})x_3x_4\} + x_4^2(1-s) + (1-s + sd_{22}-hsd_{22})x_3x_4}{T(x_3 + x_4)}\end{aligned} \tag{7}$$

where $\hat{x}_1$ and $\hat{x}_2$ are given in equation (5),

$$D = \hat{x}_1x_4-\hat{x}_2x_3$$

and

$$T = 1-s\hat{x}_2 - 2\hat{x}_1\hat{x}_2hsd_{11} + \hat{x}_1\hat{x}_2sd_{11}.$$

Notice that quadratic terms such as x_3^2 cannot be ignored when they are divided by ($x_3 + x_4$). Therefore, the recursions are not linear functions even though they are of linear order. In order to obtain recursions that are linear functions of x_3 and x_4, we determined the constant ($\hat{w}$) which approximates the ratio of x_4 to x_3 in the vicinity of the equilibrium (see Aoki & Feldman, 1991, for details of the method). Essentially, equation (7) is divided by equation (6) to obtain w' ($= x_4'/x_3'$) as a function of w ($= x_4/x_3$). We assumed that the aC_2 chromosome was much less common than the AC_2 chromosome (reflecting the low frequency of mutations) so that quadratic and higher terms

in w could be ignored [$(x_4/x_3)^2 \approx 0$]. In this case, it is straightforward to show that w tends monotonically towards a constant ratio, $\hat{w}$, which may easily be found:

$$\hat{w} = \mu\frac{(1-m)d_{12}r(1-h)s + (1-m)r(1-s)-d_{11}(1-h)s + s}{(1 + d_{11}h-d_{11})sK_1}$$

where

$$K_1 = (1-d_{12})(1-r)(1-h)(1-m)s + (1-d_{22})ms(1-h) + (1-m)r + hs(1-r) + rmhs.$$

Using $\hat{w}$ for x_4/x_3 within the recursions, we have

$$x_3' = (1-m)(1-\mu)\frac{\{\hat{x}_1x_3 + \hat{x}_2x_3(1-hsd_{12}) + rD(1-hsd_{12})\}}{T}$$

$$+ m(1-\mu)\frac{x_3 + (1-hsd_{22})x_4}{T(1+\hat{w})}$$

$$x_4' = (1-m)[\mu\frac{\{\hat{x}_1x_3 + \hat{x}_2x_3(1-hsd_{12}) + rD(1-hsd_{12})\}}{T}$$

$$+ \frac{\hat{x}_1x_4(1-hsd_{12}-s + sd_{12}) + \hat{x}_2x_4(1-s) + rD(1-hsd_{12}-s + d_{12}s)}{T}]$$

$$+ m\frac{\mu\{x_3 + (1-hsd_{22})x_4\} + x_4\hat{w}(1-s) + (1-s + sd_{22}-hsd_{22})x_4}{T(1+\hat{w})}.$$

From these recursions, which assume that $x_4/x_3 \approx \hat{w}$, one can construct a local stability matrix and determine the leading eigenvalue (λ_L):

$$\lambda_L = 1 + \frac{\mu\{m(d_{22}-d_{12}) + (d_{12}-d_{11})\}K_2}{(1-d_{11} + hd_{11})K_1} + O(\mu^2)$$

where

$$K_2 = (1-m)(r-2hr + hd_{12}s-h^2d_{12}s + hrs-2hd_{12}rs + 2h^2d_{12}rs)-hs.$$

When the leading eigenvalue is greater than unity in magnitude, the new allele, C_2, increases in frequency over time; otherwise, C_2 decreases in frequency. We shall assume that the determination of ploidy is directional so that $\{m(d_{22} - d_{12}) + (d_{12} - d_{11})\}$ has the sign of $(d_{12} - d_{11})$. In this case, λ_L is greater than one when K_2 is positive and the new ploidy allele increases the amount of diploidy ($d_{12} > d_{11}$) or when K_2 is negative and the new allele decreases the degree of diploidy ($d_{12} < d_{11}$). K_2 is positive (diploidy favoured) whenever:

$$r(1-m)K_3 > hs-hs(1-m)(1-h)d_{12}$$

where

$$K_3 = 1-2h + hs-2d_{12}hs + 2d_{12}h^2s$$

This condition reduces to that given in Otto & Goldstein (1992) when m is equal to zero. Increases in the rate of assortative mating limit the parameter range in which the above inequality is satisfied. Therefore, assortative mating hinders the evolution of diploidy and favours the increase of haploid life cycles.

APPENDIX 2. ANALYSIS WITH SELFING

In this appendix, we analyse the evolution of a population in which haploid gametophytes self at a rate σ. All other parameters are the same as in Appendix 1. The recursions that describe the change in the composition of the population after one generation are:

$$Tx_1' = (1-\sigma)(1-\mu)\{x_1^2 + x_1x_2(1-hsd_{11}) + x_1x_3 + (1-r)x_1x_4(1-hsd_{12}) + rx_2x_3(1-hsd_{12})\} + \sigma(1-\mu)x_1$$

$$Tx_2' = (1-\sigma)[\mu\{x_1x_1 + x_1x_2(1-hsd_{11}) + x_1x_3 + (1-r)x_1x_4(1-hsd_{12}) + rx_2x_3(1-hsd_{12})\} + x_1x_2(1-s+sd_{11}-hsd_{11}) + rx_1x_4(1-s+sd_{12}-hsd_{12}) + x_2^2(1-s) + (1-r)x_2x_3(1-s+sd_{12}-hsd_{12}) + x_2x_4(1-s)] + \sigma[\mu x_1 + x_2(1-s)]$$

$$Tx_3' = (1-\sigma)(1-\mu)\{x_1x_3 + rx_1x_4(1-hsd_{12}) + (1-r)x_2x_3(1-hsd_{12}) + x_3x_3 + x_3x_4(1-hsd_{22})\} + \sigma(1-\mu)x_3$$

$$Tx_4' = (1-\sigma)[\mu\{x_1x_3 + rx_1x_4(1-hsd_{12}) + (1-r)x_2x_3(1-hsd_{12}) + x_3x_3 + x_3x_4(1-hsd_{22})\} + (1-r)x_1x_4(1-hsd_{12}-s+sd_{12}) + rx_2x_3(1-hsd_{12}-s+d_{12}s) + x_2x_4(1-s) + x_3x_4(1-s+sd_{22}-hsd_{22}) + x_4x_4(1-s)] + \sigma[\mu x_3 + x_4(1-s)]$$

where T is the sum of the right hand sides and is equal to the mean fitness. The equilibrium frequencies in the absence of the C_2 allele ($\hat{x}_3 = \hat{x}_4 = 0$) are:

$$\hat{x}_1 = 1-\hat{x}_2$$
$$\hat{x}_2 = \frac{\mu}{s[1-(1-\sigma)(1-h)d_{11}]}. \quad (8)$$

Selfing, unlike assortative mating, does change the equilibrium configuration of a population even when the ploidy locus is fixed on one allele. Specifically, selfing reduces the frequency of mutations at equilibrium (when $h < 1$). Equation (8) is valid whenever there is selfing (only invalid when $\sigma = 0$, $h = 0$ and $d_{11} = 1$).

We will not present the linearized recursions in this case, as they can be obtained in the standard manner. The leading eigenvalue in a partially selfing population equals:

$$\lambda_L = 1 + \frac{\mu(1-\sigma)(d_{12}-d_{11})K_4}{[1-(1-\sigma)(1-h)d_{11}]K_5} + O(\mu^2)$$

where

$$K_4 = (1-\sigma)(r-2hr + hd_{12}s - h^2d_{12}s + hrs - 2hd_{12}rs + 2h^2d_{12}rs) - hs$$
$$K_5 = (1-d_{12})(1-r)(1-h)(1-\sigma)s + (1-\sigma)(1-r)hs + s\sigma + r(1-\sigma).$$

Invasion ($\lambda_L > 1$) of a new ploidy allele that increases the proportion of diploids in the population ($d_{12} > d_{11}$) will occur if K_4 is positive. If K_4 is negative, only by increasing the degree of *haploidy* can the new allele invade. The only caveat is that σ (the selfing rate) must be less than one in magnitude. If σ is exactly one, no heterozygotes exist in the population and a chromosome has the same chance of surviving whether it appears in a haploid or a diploid at the time of selection (evolution of ploidy levels become neutral; $\lambda_L = 1 + O(\mu^2)$). Note that K_4 is equivalent to K_2 with σ replaced by m, and the same condition must be satisfied for diploidy to be favoured:

$$r(1-\sigma)K_3 > hs - hs(1-\sigma)(1-h)d_{12}.$$

Selfing, like assortative mating and linkage, makes this condition harder to satisfy, thereby aiding the evolution of haploidy and hindering the evolution of diploidy. See Table 3 for a summary of the invasion criteria.