ORIGINAL ARTICLE

THE EFFECT OF POLLEN VERSUS SEED FLOW ON THE MAINTENANCE OF NUCLEAR-CYTOPLASMIC GYNODIOECY

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Gynodioecy, where females co-occur with hermaphrodites, is a relatively common sexual system in plants that is often the result of a genetic conflict between maternally inherited male sterility genes in the mitochondrial genome and the biparentally inherited male fertility restorer genes in the nucleus. Previous models have shown that nuclear-cytoplasmic gynodioecy can be maintained under certain conditions by negative frequency-dependent selection, but the effect of other evolutionary processes such as genetic drift and population subdivision is only partially understood. Here, we investigate the joint effects of frequency-dependent selection, drift, and migration through either pollen or seeds on the maintenance of nuclear-cytoplasmic gynodioecy in a subdivided population. We find that the combination of drift and selection causes the loss of gynodioecy under scenarios that would maintain it under the influence of selection alone, and that both seed and, more surprisingly, pollen flow can maintain the polymorphism. In particular, although pollen flow could not avoid the loss of cytoplasmic polymorphism within demes, it allowed the maintenance of nuclear-cytoplasmic polymorphism at the metapopulation level.

KEY WORDS: Cytoplasmic male sterility (CMS), drift, gynodioecy, metapopulation, migration, nuclear-cytoplasmic polymorphism, pollen flow, seed flow.

Gynodioecy, the co-occurrence of females and hermaphrodites, is a relatively common sexual system in flowering plants (reviewed in Webb 1999). The polymorphism poses the intriguing question as to the conditions under which male-sterile individuals, which have lost one of their sexual functions, can be maintained with hermaphrodites that possess both. A great deal of theoretical analyses has been performed in addressing this question (e.g., Charlesworth and Ganders 1979; Kohn 1988; Frank 1989; Gouyon et al. 1991; Couvet et al. 1998; Bailey et al. 2003), and the predictions made by these studies have been tested in a wide range of species (e.g., Kohn 1988; Thompson and Tarayre 2000; Barrett 2002; Ramsey and Vaughton 2002; Asikainen and Mutikainen 2003; Ashman et al. 2004; Barrett and Case 2006).

A key insight is that the maintenance of gynodioecy depends critically on the details of the genetic determination of sex.

In the simplest case, gynodioecy is due to the segregation of a male-sterility allele at a single nuclear locus (Godley 1955; Kohn 1989; Ahmadi and Bringhurst 1991). Here, females need to produce at least twice as many successful progeny through seeds as hermaphrodites to compensate their loss of pollen production (Lewis 1941; Lloyd 1976; Charlesworth and Charlesworth 1978). This compensation can be achieved through the production of either more seeds (by reallocating resources from pollen to seed production; e.g., Ashman 2003), or of better progeny (obligatory outcrossed progeny that do not show the effects of inbreeding depression suffered by the progeny of partially selfing

hermaphrodites; e.g., Kohn 1989; Kohn and Biardi 1995; Weller and Sakai 2005).

More commonly, gynodioecy is the result of so-called nuclear-cytoplasmic male sterility, caused by a complex interaction between maternally inherited male-sterility genes in the mitochondrial genome and biparentally inherited male-fertility restorers in the nucleus (Cosmides and Tooby 1981; reviewed in Saumitou-Laprade et al. 1994). Theoretical studies have shown that this form of gynodioecy, which is a classic example of intergenomic genetic conflict (Frank 1989), can be maintained by frequency-dependent selection if: (1) male-sterile individuals produce more (or better-quality) seeds than hermaphrodites; and (2) carriers of nuclear restorers genes pay a fitness cost relative to those that do not (e.g., Gouyon et al. 1991; Bailey et al. 2003; Dufay et al. 2007). These predictions stem from deterministic models that account for fitness differences between genotypes in a single large population, and they potentially account for observed genotype-frequency variation among populations in terms of stable limit cycles sampled at different time points (Gouyon et al. 1991). However, they consider neither the possible influence of genetic drift, nor the effect of population subdivision and the related question of the effect of gene flow through seeds versus pollen.

Because many plant populations can be small, we might expect drift to influence genotype frequencies and the related proportion of females in the case of gynodioecious species, e.g., through the fixation or loss of either a sterility or a restorer mutation (Byers et al. 2005; Nilsson and Agren 2006; Caruso and Case 2007). Drift is likely to be particularly important in species with subdivided populations, where different genes can be fixed in different demes. In a single population, such fixation events immediately lead to the loss of nuclear-cytoplasmic gynodioecy (Frank 1989). In contrast, in population subdivided into many demes, migration among demes can restore the nuclear-cytoplasmic polymorphism after it has been lost locally (Frank 1989). Because sterility mutations are transmitted only through seeds, whereas fertility restorer genes are transmitted through both seeds and pollen, we should expect migration of genes through seeds versus pollen to affect gynodioecy differently.

To our knowledge, the maintenance of gynodioecy in a subdivided population has been analyzed by only two theoretical studies. Pannell (1997) considered the maintenance of females with hermaphrodites in a metapopulation that included both migration and colonizations after repeated extinctions, but all dispersal was by seeds and the polymorphism in his model was of a simple nuclear type. In a different model, Couvet et al. (1998) showed that a similar process of metapopulation dynamics can maintain nuclear-cytoplasmic gynodioecy without requiring a cost of fertility restoration. However, their model did not consider drift, nor did it examine the effect of pollen flow among demes.

In this study, we use computer simulations to investigate the joint effects of genetic drift and migration through either seed or pollen on the maintenance of nuclear-cytoplasmic gynodioecy in a subdivided population. Our model is similar to previous analyses that include the effects of differential seed productivity of male-sterile versus hermaphrodite individuals and the cost of fertility restoration (Dufay et al. 2007). However, it extends these earlier models by allowing for stochastic fluctuations of gene frequencies, both locally within demes and at the species level, and by assuming a simple island model of population structure in which demes are linked by seed or pollen dispersal. We verify that the combination of drift and selection causes the loss of nuclear-cytoplasmic gynodioecy under circumstances that would maintain it under the influence of selection alone, and we find that both seed dispersal and, more surprisingly, pollen flow can maintain the polymorphism.

The Model

GENETIC SYSTEM AND FITNESS PARAMETERS

We assumed the same genetic system as that analyzed by Dufay et al. (2007). This model accommodates: polymorphism at a maternally inherited cytoplasmic locus at which one allele causes cytoplasmic male sterility (CMS) and the other does not; and the segregation of alleles at a single diploid nuclear locus, a dominant "restorer" allele that restores male fertility and a "maintainer" allele that has no effect on male sterility. Thus, only genotypes carrying the CMS haplotype and two copies of the maintainer allele are females. Genotypes with the CMS mutation and carrying either one or two copies of the restorer allele are so-called "restored" hermaphrodites; we refer to genotypes with the non-sterilizing cytotype as "normal" hermaphrodites. We assumed that all hermaphrodites were self-incompatible, as in, for example, gynodioecious *Plantago lanceolata* (van Damme 1986) and *Beta vulgaris* (Cuguen et al. 1994).

We considered three fitness parameters: the female advantage, in terms of the relative number of seeds produced by females compared with non-CMS hermaphrodites (FA); a CMS cost acting on the female function (w_s); and a restorer cost (c). Female and male fitness components of the six genotypes are thus a function of these three fitness parameters. In particular, the female fitness of females is affected by two factors, the female advantage (e.g., due to resource re-allocation from pollen to seed), and the genetic cost of CMS genes, and is thus written as $FA \times (1 - w_s)$.

Our study is a development on previous work (Dufay et al. 2007) that showed that frequency-dependent selection can maintain stable nuclear-cytoplasmic gynodioecy for a large range of values for these three parameters. In particular, Dufay et al. (2007) showed that, when female advantage over-compensates the cost of CMS genes, i.e., when $FA \times (1 - w_s) > 1$, CMS genes are

selected for when they are carried by females, but counter-selected when they are carried by hermaphrodites. The CMS cost is necessary to maintain non-CMS mutations; it plays its role when a majority of CMS cytotypes are restored (i.e., when the populations contains few females), by conferring an advantage to non-CMS hermaphrodites over restored (CMS) hermaphrodites. Biologically, a CMS cost would mean that the constitutive expression of a sterilizing gene incurs a cost for the female function. The cost of the restorer allele prevents the restorer's fixation; hypothetically this cost can be of three types: constitutive (the cost occurs in any cytotype), silent (the cost occurs only in genotypes carrying a nonsterilizing cytotype), or expressed (the cost occurs only in genotypes carrying a CMS cytotype). The two first types allow selection to maintain nuclear cytoplasmic gynodioecy, whereas an expressed cost always leads to restorer extinction. Overall, Dufay et al. (2007) found that sexual polymorphism is maintained by frequency-dependent selection if (1) females have the highest female fitness [$FA \times (1 - w_s) > 1$] and restored hermaphrodites have the lowest female fitness [$(1 - w_s) < 1$]; and (2) the cost of restoration on either male or female function is of a moderate magnitude (usually lower than 0.5) so that restorers increase in frequency before CMS genes can be fixed (Dufay et al. 2007). In this study, we focused on the case of a dominant, constitutive cost of restorers acting on male function.

OVERVIEW OF THE METAPOPULATION DYNAMICS

We modeled a metapopulation of D similar demes of fixed size N (with both N and $D = 100$ for all simulations presented). Each genotype produced a set number of pollen grains and ovules according to given fitness parameters. Pollen dispersal followed an island model (Wright 1931), i.e., pollen migrants were a random subset drawn from a pool of male gametes coming from all demes in the metapopulation. After panmictic reproduction within each deme, a fixed number of genotypes were then randomly selected to form the next generation. Seed dispersal also followed an island model, with the mean number of migrants into each deme in each generation (Nm_s) held constant for the duration of each simulation.

SIMULATIONS AND SCENARIOS TESTED

We explored a wide range of parameter combinations (FA in]0;4[; CMS cost in]0;0.5[and a constitutive restoration cost acting on male function in]0;0.5[) by running 100 independent simulations for each combination. We first focused on the combined effects of drift and selection. Here, simulations were run with no migration among demes (either through pollen or seeds). We recorded the state of the metapopulation at equilibrium under the effect of selection and drift by recording the frequencies of CMS genes, restorer alleles, and females within the 100 demes, as well as their average frequencies at the metapopulation level. The state

of the metapopulation was recorded as a function of both the maintenance of a nuclear-cytoplasmic polymorphism and a sexual polymorphism at equilibrium. When at least 5% of demes contained both the CMS and the non-CMS mutation on the one hand, and both females and hermaphrodites on the other hand, we considered that nuclear cytoplasmic gynodioecy was maintained at the deme level. When all demes had fixed the CMS mutation, while maintaining a nuclear polymorphism (all demes containing a mixture of females and restored –CMS- hermaphrodites), the state of the metapopulation was recorded as "nuclear gynodioecy." Finally, when nuclear gynodioecy was maintained in demes with CMS fixed, and the CMS mutation was lost in others, so that these demes were hermaphroditic, we considered nuclear-cytoplasmic gynodioecy to be maintained at the metapopulation level, but not at the demic level.

For each of these combinations of parameter values, we studied the effect of seed migration by running simulations for increasing values of Nm_s, with all other parameters values held constant and the rate of pollen migration initially set to zero. We then performed the same analyses with increasing values of pollen migration, now with seed migration being fixed to zero. For each parameter combination, we recorded the average sex ratio, the average frequency of the restorer allele, and the average frequency of the CMS mutation; these frequencies were recorded across the whole metapopulation at equilibrium, as well as their variance among demes. Finally, because in many cases the metapopulation converged on a dynamic equilibrium, providing large undampened oscillations of genotype frequencies, we recorded the output frequencies across the metapopulation over the last 100 generations of the simulation and calculated an average value across time.

Even though our model did not explicitly simulate deme extinction, demes occasionally went extinct when they comprised only females or a single self-incompatible hermaphrodite (see Results). In these cases, two types of simulation were run for each combination of parameters: (1) empty sites received Nm_s, i.e., empty sites received the same number of colonists as extant populations received migrants ; and (2) sites were recolonized by n founders ($n = 20$ for all simulations presented). In both cases, colonists were drawn randomly from the metapopulation as a whole, corresponding to Slatkin's (1977) migrant pool model. Scenario (1) here is probably more realistic, but scenario (2) might reflect negative density-dependent colonization success, where dispersers do better when they find empty sites.

INITIAL CONDITIONS AND EQUILIBRIUM

Four sets of initial conditions were tested for all simulations, covering different situations in terms of sex-ratio and CMS frequencies (low/middle/high) and in terms of homogeneity of these ratios across demes. We ran simulations for a period of 50,000

generations for each set of initial conditions. For a given combination of parameter values, the simulations provided the same average genotype and phenotype frequencies, irrespective of the initial conditions. We thus considered these scenarios to be at equilibrium.

Results

COMBINED EFFECTS OF DRIFT AND SELECTION

The combined effects of drift and selection were analyzed by running simulations with 100 demes, each comprising 100 plants, with no migration among demes. Under these conditions, nuclear-cytoplasmic gynodioecy could never be maintained locally, as cytoplasmic polymorphism was quickly lost within all demes after a few hundred generations. This held for all combinations of parameter values, including those for which Dufay et al. (2007) found that frequency-dependent selection alone could maintain nuclear-cytoplasmic gynodioecy. Three different classes of results were found at the deme level: (A) extinction of both the CMS mutation and the restorer allele; (B) fixation of both the CMS and the restorer; and (C) nuclear gynodioecy, i.e., fixation of the CMS mutation with a polymorphism maintained at the restorer locus (Fig. 1).

At the metapopulation level, different combinations of these three types of demes were recorded at equilibrium as a function of fitness parameter values. The frequency of the CMS mutation (i.e., the frequency of demes in which the CMS mutation was fixed by genetic drift) and female frequency in the metapopulation at equilibrium both increased with the female fitness of female genotypes. Overall, in scenarios in which there was no female advantage to females (i.e., $FA \times (1 - w_s) < 1$), CMS genes were not selected in any deme, so that both CMS genes and restorer alleles were lost from all demes across the metapopulation (Scenario 1, Table 1).

The same outcome was observed when females benefitted from a small fitness advantage ($FA \times (1 - w_s)$ slightly larger than one). In such cases, selection alone was able to maintain nuclear-cytoplasmic gynodioecy, but it led to large frequency oscillations of both the CMS mutation and the restorer allele, with frequencies of CMS generally reaching values close to zero. Thus, in finite demes, drift essentially caused the sterility mutation to be lost (Scenario 1, Table 1).

For intermediate values of female fitness ($1 < FA \times (1 - w_s) < 2$), a cytoplasmic polymorphism could be maintained at the metapopulation level, with either cytotype, being fixed within demes. Under such parameter values, selection also led to large oscillations of the CMS mutation, with corresponding oscillations of the restorer frequency. Because the frequency of the CMS mutation reached higher values compared to scenario (1), drift could either lead to the loss or to the fixation of the sterility mutation.

For a relatively low cost of the CMS gene and/or a relatively low cost of the restorer, the fixation of the restorer allele quickly followed the fixation of the sterility mutation. Thus, a portion of the demes in the metapopulation contained normal hermaphrodites, and the remaining demes contained only restored (CMS) hermaphrodites (scenario 2, Table 1). In contrast, when the cost of the restoration was higher, or when there was an elevated cost of the CMS genes themselves, the restorer allele was maintained at a relatively low frequency. Simultaneous fixation of CMS and restorers was thus unlikely in this case, and nuclear gynodioecy could be maintained in some demes (scenario 3, Table 1).

Finally, for a large fitness advantage for females ($FA \times (1 - w_s) > 2$), the frequency of the CMS mutation increased quickly, relatively to frequency of restorer allele, and this led to nuclear gynodioecy in all demes at equilibrium (scenario 4, Table 1).

EFFECT OF GENE MIGRATION: GENERAL PATTERNS

For some of the fitness parameter values, gene migration among demes did not affect the final state of the metapopulation, compared to the model with no migration. First, when females enjoyed no fitness advantage (i.e., $FA \times (1 - w_s) < 1$), the CMS mutation was lost from all demes, leading to a purely hermaphroditic metapopulation. Second, when females enjoyed a strong fitness advantage (i.e., $FA \times (1 - w_s) > 2$), the CMS mutation was, as above, quickly fixed in all demes, leading to nuclear gynodioecy at the metapopulation level. In both cases, the outcome in terms of sexual and cytoplasmic polymorphism was the same in a large panmictic population, in a metapopulation with genetically isolated demes (selection and drift), and in a metapopulation including either seed or pollen dispersal (selection, drift and gene migration).

For intermediate values of female fitness ($1 < FA \times (1 - w_s) < 2$; scenarios 1, 2, and 3), gene migration among demes had a significant effect on the cytoplasmic and sexual polymorphism within demes and at the metapopulation level. For all possible sets of parameter values within each scenario, simulations showed the same qualitative effect of gene migration on the final state of the metapopulation. We thus present in the following two sections, respectively, the effect of (1) seed migration and (2) pollen migration for three typical situations, corresponding to scenarios 1, 2, and 3 in Table 1. The exact values of fitness parameters that were used to present the detailed effects of gene migration are provided in Table 2.

EFFECTS OF SEED MIGRATION

In all three scenarios, seed flow allowed nuclear-cytoplasmic gynodioecy to be maintained within demes under certain conditions (Fig. 2). When the number of migrants was lower than 1, a large portion of demes evolved toward pure hermaphroditism as a result
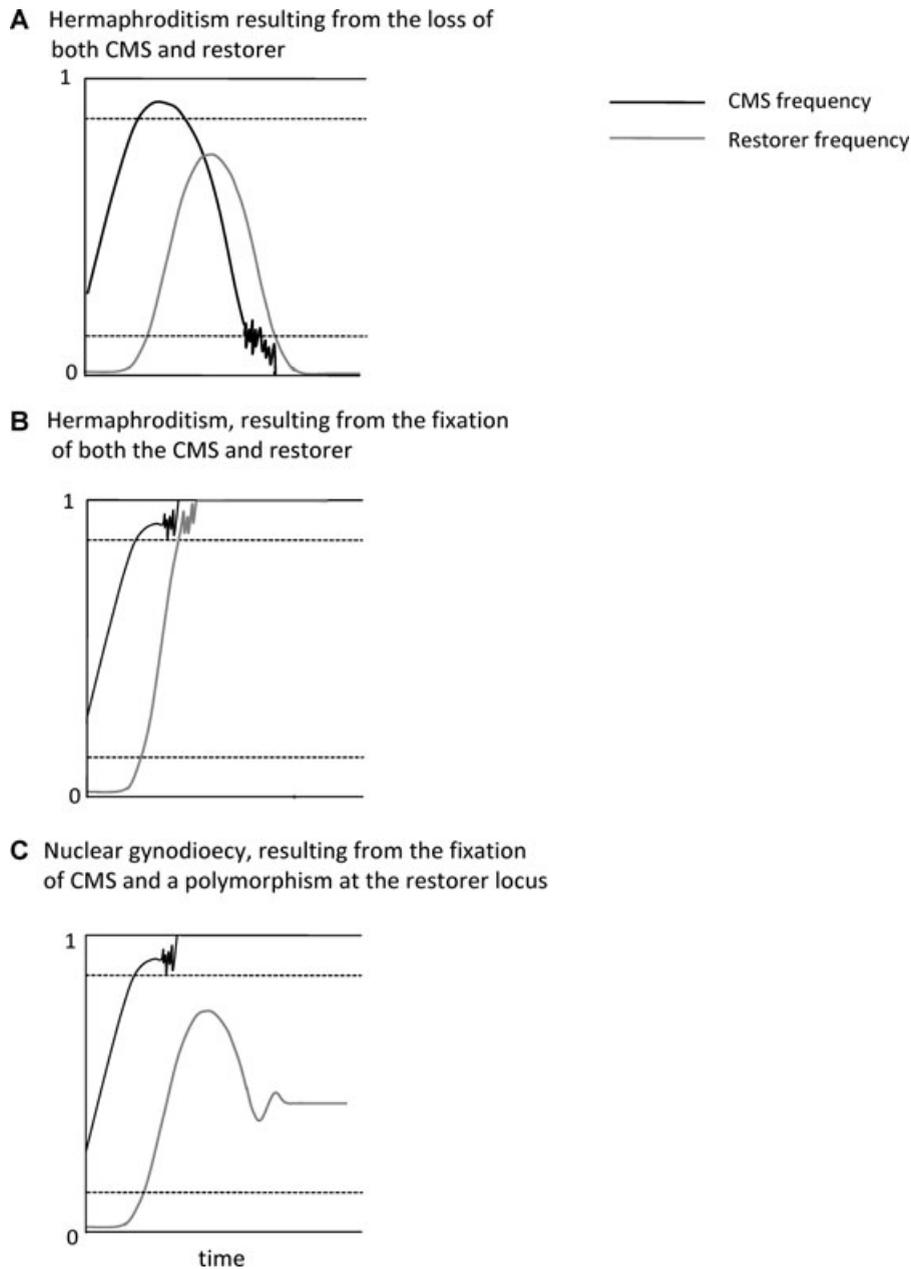
A Hermaphroditism resulting from the loss of both CMS and restorer

CMS frequency
Restorer frequency

B Hermaphroditism, resulting from the fixation of both the CMS and restorer

C Nuclear gynodioecy, resulting from the fixation of CMS and a polymorphism at the restorer locus

time

Figure 1. Schematic representation of three classes of results for temporal variation of allele frequencies under the combined effects of genetic drift and frequency-dependent selection at the deme level. Frequency-dependent selection leads to wide oscillations in frequency of both CMS (black lines) and restorer alleles (gray lines). Dotted lines indicate the values for which genetic drift tends to drive alleles to loss or fixation.

of the loss of both the cytoplasmic sterility mutation and the restorer allele. In contrast, the other demes converged toward a population exclusively composed of females, subsequently leading to local extinction. These extinctions were due to the combination of two events: the fixation of the cytoplasmic sterility mutation; and the loss of the fertility restorer allele within those demes. For the same combinations of parameter values, we ran a second type of simulation in which empty demes were recolonized just after extinction by $n = 20$ founders. In these simulations, for low values

of Nm_s, a portion of the demes still experienced local extinctions, but they were filled with colonists in the next generation. As a result, at equilibrium, the metapopulation contained a mixture of hermaphroditic and nuclear-cytoplasmic gynodioecious demes (data not shown).

When Nm_s exceeded 1 (Fig. 2), nuclear-cytoplasmic gynodioecy could be maintained at the deme level. Each deme followed a cycling dynamic, showing temporal oscillations in the sex ratio and oscillations in the frequencies of the CMS

Table 1. Description of the four possible states of the metapopulation under the effects of selection and genetic drift, in terms of sexual system and occurrence of the CMS mutation and the restorer (R) allele in demes of the metapopulation. In scenarios 2 and 3, two types of demes co-occur (separated by dashed lines) within the metapopulation. In all scenarios, cytoplasmic polymorphism was lost at the deme level, with CMS genes being either lost or fixed within demes, following one of the three dynamics pictured in Figure 1. The fourth column shows for which range of values of fitness parameters (*FA*, female advantage; w_s, CMS cost; *c*, restorer cost) each of these scenarios was found.

Scenario	Sexual system	Genetic system	Fitness parameter values
1	Hermaphroditism	Loss of both CMS and R	$FA \times (1-w_s) < 1$ or slightly > 1
2	Hermaphroditism	Loss of both CMS and R	$1 < FA \times (1-w_s) < 2$;
	Hermaphroditism	Fixation of both CMS and R	low w_s and/or low c
3	Hermaphroditism	Loss of both CMS and R	$1 < FA \times (1-w_s) < 2$;
	Nuclear gynodioecy	Fixation of CMS with R polymorphism	high w_s and/or high c
4	Nuclear gynodioecy	Fixation of CMS with R polymorphism	$FA \times (1-w_s) > 2$

cytotype and the restorer allele. Drift regularly enhanced the loss of polymorphism within demes, but this polymorphism could be restored by seed migration. Despite the cyclical dynamics, all simulations converged toward the same average frequencies at the metapopulation level. For all values of Nm_s above the threshold value, the average frequency of genotypes and the sex ratio depended only poorly on the magnitude of seed migration (Fig. 2). In contrast, the variance of these frequencies among demes showed a decrease when seed migration increased. The average sex ratios and frequencies of the CMS mutation recorded for $Nm_s = 100$ were similar to those predicted for a large panmictic population.

EFFECTS OF POLLEN MIGRATION

Simulations including pollen flow but no seed flow showed maintenance of nuclear gynodioecy within some demes, whereas others converged on pure hermaphroditism (Fig. 3). Consequently nuclear-cytoplasmic gynodioecy was always maintained at the metapopulation level. The consequence of pollen flow for the maintenance of gynodioecy thus differed among the three scenarios outlined above. Whereas it did not qualitatively change the fate of the metapopulation in scenario (3), in which nuclear

gynodioecy was already maintained within demes with no migration, it had a much greater impact on the sexual system for the first two scenarios. In scenario (2), pollen flow allowed a stable maintenance of a polymorphism at the restorer locus in all demes, whether they had fixed or lost the sterility mutation. The most remarkable result held for scenario (1), in which pollen flow allowed the sterility mutation to become fixed in some of the demes even under conditions that had caused its loss in the absence of pollen flow. Similar to the other scenarios, a polymorphism was also maintained at the restorer locus, both within and across demes (Fig. 3).

As shown in Figure 3, an increase of the migration rate led to an increase in the frequency of the CMS mutation, for all three scenarios. More precisely, under relatively high rates of pollen flow, a larger portion of demes could fix the CMS mutation at equilibrium. This led to an increase in both the sterility haplotype and females at the level of the subdivided population. Finally, for all scenarios, simulations showed a decrease in the CMS frequency at equilibrium for very high pollen migration (when pollen migration rate increased from 0.1 to 1 in scenario 1, the CMS mutation first increased and then decreased in frequency; data not shown). In these simulations, the metapopulation

Table 2. Values of fitness parameters chosen for the study of gene migration in scenarios 1, 2, and 3. Each scenario is defined by the values of three fitness parameters: female advantage (*FA*), CMS cost (w_s), and restorer cost. The combination of the first two parameters provides the female fitness of females [$FA \times (1-w_s)$]. For each set of parameter values, the exact state of the metapopulation under the combined effects of selection and drift is indicated as CMS frequency in the metapopulation (i.e., frequency of demes in which CMS genes have been fixed), restorer frequency, and sex ratio at the metapopulation level.

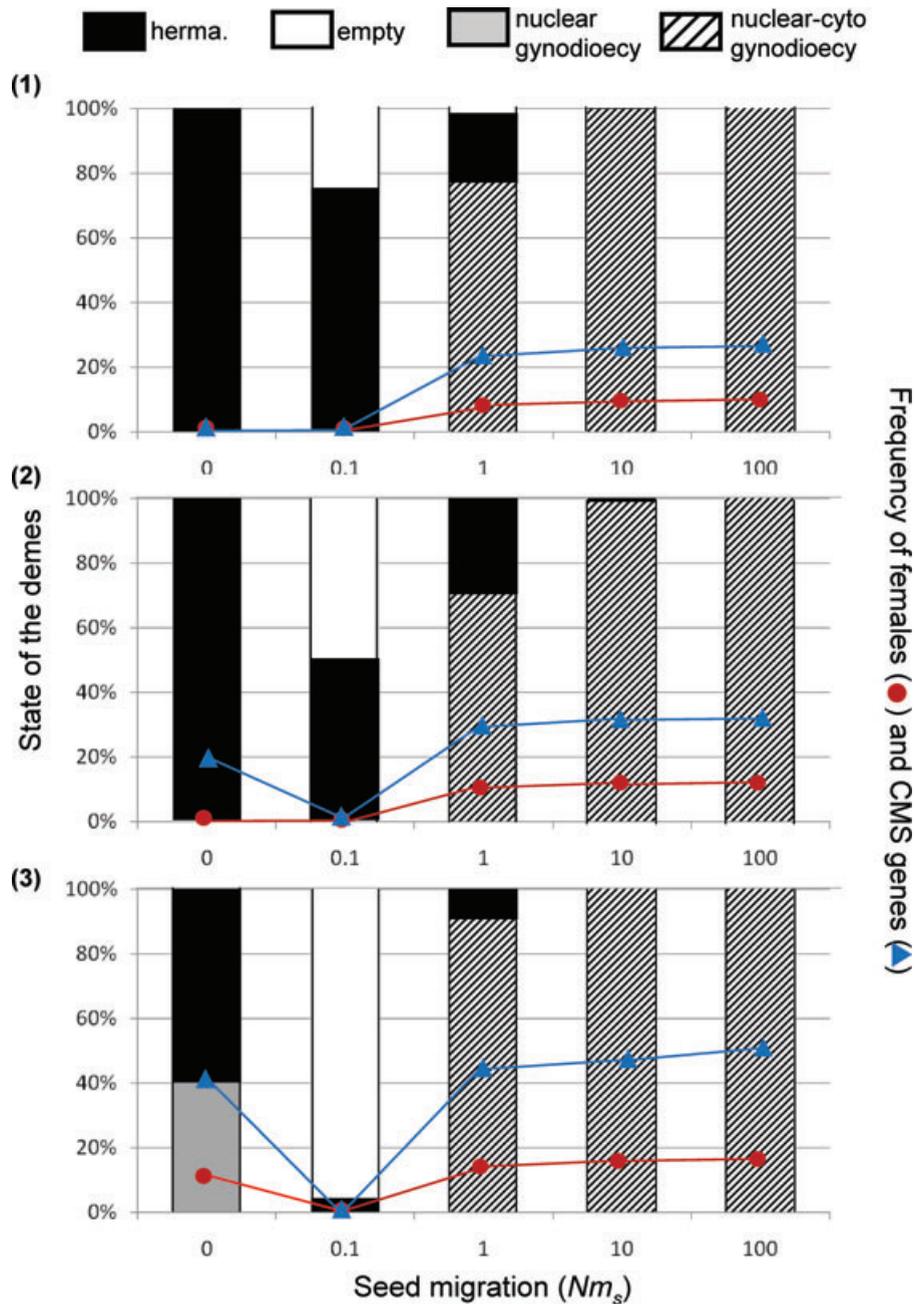| Scenario	Female advantage *(FA)*	CMS cost *(ws)*	Female fitness of females	Restorer cost *(c)*	Frequency of CMS genes	Frequency of restorer alleles	Frequency of females
1	2.2	0.4	1.32	0.2	0	0	0
2	2	0.2	1.6	0.2	0.21	0.21	0
3	3	0.4	1.8	0.2	0.42	0.25	0.1

Figure 2. Effect of seed migration on the state reached by demes (bars; left vertical axis) and the frequency (curves; right vertical axis) of females (red circles) and CMS cytotypes (blue triangles) in a metapopulations at equilibrium for scenarios 1, 2, and 3. Restorer frequency can be deduced from blue and red curves: when CMS frequency was higher than 0, restorer fixation lead to an extinction of females, whereas restorer loss lead to the same frequency for females and CMS. Other situations mean that there is a polymorphism at the nuclear locus. Deme states are: empty, due to a former invasion of females (white); fully hermaphrodite (black); CMS fixed and nuclear polymorphism at the restorer locus, i.e., nuclear gynodioecy (gray); and nuclear-cytoplasmic gynodioecy (dashed).

functioned almost as a single panmictic unit: as oscillating dynamics usually began with an almost simultaneous increase in the frequencies of the CMS mutation and the restorer allele, they caused a strong increase of restorer frequency over the whole metapopulation, decreasing the probability that CMS was fixed in local demes.

Discussion

Our examination of the behavior of nuclear-cytoplasmic gynodioecy in a subdivided population has revealed complex and surprising effects of drift and gene flow. To understand these results, it is useful to begin by considering the simpler case of the evolutionary dynamics of gynodioecy in the absence of drift and
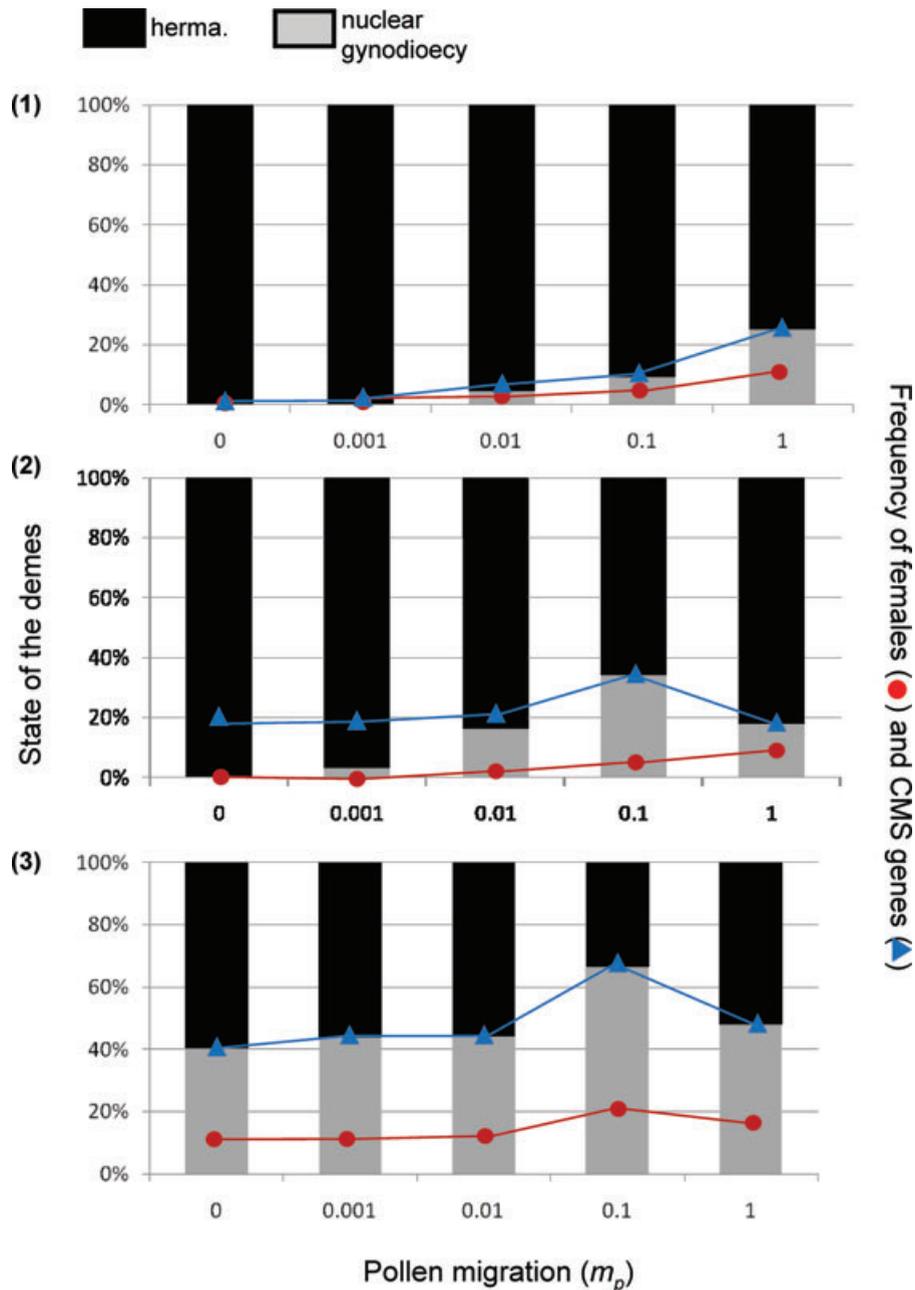
Figure 3. Effect of pollen migration on the state reached by demes (bars; left vertical axis) and the frequency (curves; right vertical axis) of females (red circles) and CMS cytotypes (blue triangles) in a metapopulations at equilibrium, for scenarios 1, 2, and 3. See legend of Figure 3 for details.

population structure. Figure 4 presents a diagram of the evolutionary dynamics of nuclear-cytoplasmic gynodioecy in a single population. As is evident in the figure, the frequencies of both phenotypes and genotypes are expected to cycle widely as a result of phase-dependent and negative frequency-dependent selection (Gouyon et al. 1991; Dufay et al. 2007). In an infinite population, the cycles are expected often to be dampened over time, so that a single equilibrium is reached (Dufay et al. 2007). This scenario would apply in any of the demes of our model under deterministic conditions. However, genetic drift in finite populations, as in our simulations, prevents them from reaching their deterministic equilibrium. More importantly, drift can cause the fixation of different alleles, when their frequency is driven close to 0 or 1 by this cyclical selection (illustrated by the shaded areas in Fig. 4 and dotted lines in Fig. 1). Below, we refer to these ideas in our discussion of (1) the effects of genetic drift on its own, (2) its effect when interacting with seed flow, and (3) its interactive effect with pollen flow.
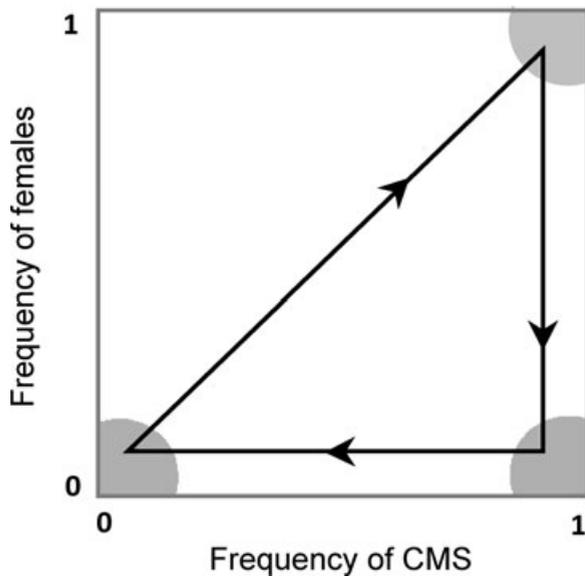
Figure 4. Schematic representation of sex-ratio and CMS-frequency evolution in a gynodioecious population, under the effects of frequency-dependent selection. When the nuclear restorer allele is rare, CMS cytotypes are mainly carried by females, which experience higher female fitness than hermaphrodites and are thus selected. Consequently, during the first phase, both CMS and females increase in frequency. When CMS is frequent, the restorer allele becomes selected over the nonrestorer allele, as it is transmitted through both male and female function. During this second phase, the proportion of females decreases. Finally, because CMS enhances the cost of the female function, the frequency of CMS decreases during a third phase. The shaded areas represent situations in which alleles can be driven to loss or fixation by genetic drift. The bottom left-hand and the bottom right-hand corners of the graph depict the loss of both CMS and restorers, and the fixation of both CMS and restorers, respectively. Both situations lead to pure hermaphroditism in the population. The upper right corner depicts the fixation of CMS whereas the restorer allele is lost, leading to a pure female population and population extinction.

THE EFFECT OF DRIFT

Our simulations revealed two important effects of drift: first, nuclear-cytoplasmic polymorphism was always lost from individual populations, due to the fixation or loss of the CMS mutation; and second, the fixation of a sterility mutation in some populations and its loss in others could cause the global maintenance of a cytoplasmic polymorphism at the metapopulation level, even though each population was monomorphic.

In all of our simulations of genetically isolated populations, we observed the loss of a nuclear-cytoplasmic polymorphism, regardless of the parameter combinations investigated and including scenarios that would maintain the polymorphism indefinitely in the absence of drift. These results can be explained by the stochastic fixation within a population of one or other of the two cytoplasmic haplotypes (Fig. 1). Selection in the model studied

here is known to lead to wide oscillations of gene and genotype frequencies over time (Dufay et al. 2007). These oscillations may eventually be dampened, but for a long period of time (typically hundreds of generations; Dufay et al. 2007), they take gene frequencies close to fixation or loss (Fig. 1, and see Pannell et al. 2005). Under all such circumstances, drift quickly causes the loss of the cytoplasmic polymorphism when it reaches a high or low frequency. In some cases, in which the CMS mutation was fixed, we found that the fertility restorer mutation was first taken to a high frequency by selection and then either drifted to fixation (leading to hermaphroditism; scenario 2 and Fig. 1B). An important implication is that many populations of apparently hermaphroditic species may carry the signature of past CMS, as has indeed been found in several studies (e.g., Tiffin et al. 2001; Fishman and Willis 2006). In other cases, when the CMS mutation was fixed, the fertility restorer was drifted back to an intermediate frequency where it could be maintained by negative frequency-dependent selection (scenario 3 and Fig. 1C). This scenario represents a case of simple nuclear gynodioecy, in which females are maintained at a frequency less than 0.5 (Lewis 1941; Lloyd 1976).

When demes in a spatially subdivided population are poorly linked by gene flow (in our simulations, when there was no migration through seeds or pollen), the cytoplasmic polymorphism could be maintained globally despite its loss within all demes. This result is due to the simple fact that restricted migration among geographically isolated demes increases the genetic effective size of the entire metapopulation by slowing or preventing the coalescence of lineages sampled from different demes (Whitlock and Barton 1997; Wakeley 1998). Although our assumption of no migration between demes is extreme and perhaps unrealistic for any real metapopulation, our result should apply to cases in which the migration rate is much smaller than the reciprocal of the inbreeding effective size of individual demes (i.e., $N_e \, m \ll 1$). The fact that very high levels of genetic differentiation have typically been found for mitochondrial haplotypes in gynodioecious species (McCauley 1998; Laporte et al. 2001; Olson and McCauley 2002; Klaas and Olson 2006; Olson et al. 2006) suggests that seed flow between them is indeed low.

SEED MIGRATION

Results of our simulations of a metapopulation with demes linked by seed flow differed in important ways from those discussed above, where demes were genetically completely isolated from one another. Broadly, two different patterns were evident. In the case of relatively high levels of seed migration, the nuclear-cytoplasmic polymorphism was generally maintained within all demes. In the case of very low migration, we found that many demes suffered local extinction and gynodioecy was lost in the remaining populations. These results can be understood, on the one hand, in terms of a shift in the action of drift on variation

within demes to its diminished effects on that at the level of the metapopulation, and, on the other hand, by the evolution of strong pollen limitation caused by sex-ratio bias.

The maintenance of a nuclear-cytoplasmic polymorphism in all demes linked by strong migration is a reasonably trivial outcome of diminishing the effect of drift on the fixation of either of the cytotypes. As noted above, when drift is important, cytotypes are easily fixed when their frequency is taken to high values by selection in the recurrent oscillations. In principle, this potential remains in a large metapopulation, but the risk of loss or fixation is reduced when demes are linked by strong migration because drift is now governed by the reciprocal of the product of the number of demes and the population size (Whitlock and Barton 1997). Indeed, we found that a metapopulation with a small number of demes occasionally became fixed for one or other of the two haplotypes (results not shown).

Note that the mean frequency of genotypes was fairly invariant to the migration rate above about $Nm = 1$. This indicates that, with strong migration, genotype frequencies depend largely on their relative (frequency-dependent) fitness, rather than on the details of the migration matrix. Nevertheless, for intermediate migration rates ($Nm \sim 1$), local frequencies varied widely among demes, even though the frequency of genotypes averaged across the metapopulation was more or less invariant (Fig. 2). This result can be attributed to both the effect of drift, as discussed above, as well as to the asynchronous nature of frequency oscillations among demes. Our explanation is consistent with that given by previous authors (e.g., Frank 1989; Gouyon et al. 1991) who invoked frequency oscillations driven by selection to explain the common observation in gynodioecious species of between-population variation in the frequency of females (i.e., Kawakubo 1994; Koelewijn and Van Damme et al. 1996; Barr 2004; Murayama et al. 2004; Byers et al. 2005; Cuevas et al. 2006; Nilsson and Agren 2006; Caruso and Case 2007; Dufay et al. 2009).

The effect of seed flow on the evolutionary dynamics of the metapopulation was more interesting for weak migration (i.e., $0 < Nm < 1$). Here, the most striking finding was a high frequency of local population extinction across the metapopulation. This result is easily understood in terms of the effect of high female frequencies on the seed production within demes due to pollen limitation. In particular, under weak migration, the evolutionary dynamics of local demes become almost independent of one another. First, there can be a long waiting time until a seed immigrates into a population. During such a period of isolation, the nuclear-cytoplasmic polymorphism is typically lost (see previous section). Importantly, if the CMS mutation is lost, so is the fertility restorer (Fig. 1A). The upshot is a monomorphic population of hermaphrodites, i.e., one with no fertility restorer alleles segregating.

Second, because females produce more seeds than hermaphrodites, they are more likely to be the source of immigrants when they finally arrive in such a population of hermaphrodites. In this case, the CMS mutation will quickly increase in frequency. When migration rates are high, the population is likely to be rescued by the arrival of an individual carrying a restorer allele. However, under low migration, as the frequency of females approaches 1, the population may be driven to extinction before this can happen. Under the realistic scenario that empty demes continue to receive an average of Nm_s migrants per generation, in the case of low m_s, this implies either zero or one migrant under Poisson-distributed migration. Because a single migrant may be either a female or a self-incompatible hermaphrodite, such a deme would stay empty.

The impact of high local female frequencies on seed production by both females and hermaphrodites via pollen limitation has been documented in a number of studies (Graff 1999; McCauley et al. 2000; Lopez-Villavicencio et al. 2005; Alonso et al. 2007). Whether natural populations are ever driven to the extinction by this process, as implied by our simulation results, is not known. However, we might expect such populations to be more susceptible to extinction as result of demographic stochasticity, particularly in self-incompatible species. In cases in which hermaphrodites can produce progeny by selfing, reproductive assurance confers a fitness benefit greater than that enjoyed by females due to their greater seed productivity, particularly when the local female frequency is high (McCauley and Taylor 1997). In such a situation, the local invasion of CMS genes, potentially causing local extinction, is less likely. Similarly, further studies including simultaneous dispersal of both pollen and seeds might show that such local extinctions due to severe pollen limitation are not likely to happen if some pollen flow occurs among demes.

POLLEN MIGRATION

The results of our simulation of a metapopulation with demes linked by pollen flow were strikingly different from those of a metapopulation in which demes were linked by seed flow. Although our model assumes that cytoplasmic genes are transmitted only through seeds, we found that pollen flow actually contributed to the maintenance of cytoplasmic polymorphism across the metapopulation (among demes) under conditions in which the polymorphism would otherwise have been lost. This surprising result can be explained by the interaction between selection on sterility mutations and fertility restorers in different genotypic contexts.

As we saw above, in some populations the CMS mutation is fixed by drift, and this often leads to the subsequent fixation by selection of the restorer allele in the same deme (Fig. 1B); such populations comprise restored hermaphrodites that nonetheless carry an unexpressed CMS mutation. In contrast, populations

in which the CMS mutation is lost by drift experience a corresponding selective loss of the restorer allele (because of its cost; Fig. 1A); these populations are also hermaphroditic, but they do not contain the CMS mutation. Under some scenarios of a metapopulation with no gene flow among demes (e.g., scenario 3), some populations comprise only hermaphrodites whereas others comprise both hermaphrodites and females; in contrast, under other scenarios (e.g., scenarios 1 and 2, Table 1), all populations may comprise only hermaphrodites, and gynodioecy is thus lost. However, with pollen flow, we found that gynodioecy was maintained in the metapopulation in all cases investigated (Fig. 3). This is because migration among demes that have been fixed in different directions maintains the polymorphism both within demes and globally. Essentially, our result provides an example of the classic scenario in which polymorphism is maintained in two different genetic contexts in a balance between selection and migration (Levene 1953). Thus, ongoing migration maintains restorers in populations that have lost the CMS mutation, even though they are deleterious. Similarly, migration maintains the nonrestoring allele in populations that have fixed the CMS mutation, despite the selective advantage of the restorer.

Another striking result of pollen flow is that the cytoplasmic polymorphism at the metapopulation level and, correspondingly, gynodioecy can be maintained where it would otherwise be entirely lost (see scenario 1, Fig. 3). Additionally, pollen flow may actually increase the frequency of the cytoplasmic sterility mutation at the metapopulation level, relative to that expected in its absence, so that it is maintained at an intermediate frequency (see CMS frequency at pollen migration 0.1 compared to no migration for all scenarios, Fig. 3). We propose the following two-phase explanation for this interesting result.

First, in a metapopulation of finite demes, some will lose the CMS mutation as a result of genetic drift. We have already seen that these demes then also lose their fertility restorers. Critically, this reduces the frequency of restorer genes in the pollen pool and, consequently, it increases the proportion of nonrestorer pollen entering into other demes. This affects the second phase of the process.

In the second phase, demes that are still polymorphic for CMS receive extra nonrestorer pollen, and this immediately gives an advantage to the cytoplasmic sterility mutation, which increases in frequency by selection. As before, when the mutation reaches a high frequency, it may be fixed by drift. The result is that the loss by drift of the sterility mutation in one class of demes indirectly drives its fixation in a second class in a frequency-dependent process that acts at the interdemic level (i.e., dependent on the frequency of demes fixed for one or other of the cytoplasmic types). This result represents an instance of negative frequency-dependent selection operating in a system above the individual level.

In essence, our simulations suggest that pollen flow is able to maintain nuclear-cytoplasmic gynodioecy in a subdivided population. We assumed a simple island model structure, but our results ought to apply to a spatially explicit scenario at a range of different spatial scales. For example, we might expect that nuclear-cytoplasmic gynodioecy could be maintained within a patchy (meta)population in which each patch is monomorphic for a cytoplasmic haplotype, but the population as a whole is polymorphic and patches exchange genes through pollen flow. This type of structure has been observed in several gynodioecious species (e.g., McCauley 1998; Laporte et al. 2001; Olson and McCauley 2002; Klaas and Olson 2006; Olson et al. 2006) and may be relatively common in plants generally (Freckleton and Watkinson 2002). The extent to which patch-level dynamics and the relative importance of pollen versus seed migration at different spatial scales for the evolutionary dynamics of gynodioecious species warrants more empirical attention (Pannell and Obbard 2003).

Conclusions and Perspectives

Our simulations of a simple model of nuclear-cytoplasmic gynodioecy in a subdivided population have yielded several insights that would not have been obvious from analyses of single panmictic population under deterministic dynamics. Even this simple model, however, included a relatively large number of parameters, so that necessarily we were able to explore only a small part of the possible parameter space. More importantly, we have ignored several factors that are known or suspected to be important in the evolutionary dynamics of gynodioecy and the demography of subdivided plant populations. These would include: further details concerning the mode of sex determination (e.g., multiple CMS genes co-occurring in the same population, dominance of restorer alleles, more complex genetics of restoration, or partial paternal inheritance of cytoplasmic genes [McCauley and Olson 2008]); variation in the mating system (e.g., partial selfing of hermaphrodites in self-compatible species); the effect of the accumulation or purging of genetic load, and the expression of the resulting inbreeding depression (Mutikainen and Delph 1998; Glaettli and Goudet 2006; Chang 2007); details of the pollination biology (Alonso 2005); and demographic fluctuations that might include local extinctions and recolonizations, or the effects of seed-bank dynamics (Couvet et al. 1998). Some of these factors may be worth further investigation. In particular, we expect that population turnover in a metapopulation would cause interesting effects through its influence on the genetic effective size of the species and the related increase in drift. It would be interesting to know, for instance, whether the dependency of our results on gene flow, even in the absence of externally imposed extinctions, could be understood simply in terms of its impact on the genetic

effective size of the relevant genomic compartments involved in nuclear-cytoplasmic gynodioecy (Whitlock and Barton 1997).

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