Spatial dynamics of cytoplasmic male sterility

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Introduction

Conflict often leads to nonequilibrium fluctuations as attackers spread, host defences increase, and counter-attack evolves. Spatial processes play a crucial role when attack types arrive from other populations and import of matching defence follows.

We focus on cytoplasmic male sterility (CMS), an ideal system in which to study the spatial dynamics of conflict. Cytoplasmically inherited genes in the mitochondria sometimes cause male sterility by interfering with pollen development. Reallocation from pollen to ovules increases the number of seeds. This reallocation to seeds benefits the mitochondrial CMS genes, which are transmitted through seeds but not through pollen.

Reproductive reallocation causes a conflict with nuclear genes, which are transmitted through both pollen and seeds. Consistent with this idea of conflict, nuclear restorers occur that counteract cytoplasmic effects and restore pollen fertility. A plant appears as a normal hermaphrodite when it has a male-sterile cytoplasm and matching nuclear restorers.

Wild populations of CMS plants maintain distinct cytoplasmic genotypes (cytotypes). Each cytotype causes male sterility by an apparently different mechanism because each responds to a particular subset of nuclear restorer alleles. Nuclear restorer alleles are typically polymorphic at several loci, with each allele specialized for restoring pollen fertility when associated with a particular cytotype. The frequencies of cytotypes, restorer alleles, and male-sterile (female) plants often vary over space.

We focus on the processes that influence spatial variation in gene frequencies. We start by summarizing the key observations and theories. With the theory as our guide, we analyse the best studies of natural populations currently available. Finally, we consider prospects for the future. The arrival of molecular tools brings great opportunity to measure the spatial distributions of genotypes and to test theories about the spatial dynamics of conflict.

In the course of our chapter, we develop a new model of CMS dynamics. We argue that the relative allocation of resources to pollen and ovules by hermaphrodites interacts with the spatial dynamics of gene frequency fluctuations driven by conflict.

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This interaction leads to an association across species between the physiological patterns of resource allocation and the population-wide frequencies of females.

Observations

Correns (1906) discovered CMS. As of 1972, CMS had been reported in 140 species of flowering plants from 47 genera and 20 families (Laser & Lersten 1972; see also Edwardson 1970; Grun 1976).

Four attributes characterize wild populations with CMS: cytotype polymorphism, nuclear restorer polymorphism, spatial variation in cytotype and restorer frequencies, and spatial variation in the frequency of male-sterile plants. Frank (1989) provides a review of these four attributes, with further data summarized in Couvet *et al.* (1990), Koelewijn and van Damme (1995a,b), Manicacci *et al.* (1996, 1997, 1998), de Haan *et al.* (1997a,b,c,d), Frank (1997, 2000), McCauley (1998), McCauley and Brock (1998), and Taylor *et al.* (1999). Here we give a brief account of the key points. We provide detailed summaries of studies on *Plantago lanceolata* and *Thymus vulgaris* in later sections.

Cytotype polymorphism

Different cytotypes appear to cause male sterility by different mechanisms because each is susceptible only to a particular subset of nuclear restorer alleles. Frank (1997) summarizes data through 1996 on the number of cytotypes per species. In most cases, only two different cytotypes had been clearly established in each wild species, with suggestive data that there may be more. Recently, de Haan *et al.* (1997a) used molecular tools to find a minimum of four different cytotypes in *P. lanceolata.* Mitochondrial genes cause the male-sterile effects of cytotypes in the cases that have been analysed (Brennicke & Kück 1993; Levings & Vasil 1995).

Restorer polymorphism

Restorer alleles typically have a dominant effect in intraspecific crosses and often appear to be specific for a particular cytotype (Edwardson 1970; Charlesworth & Laporte 1998). For some cytotypes, restorer alleles must be present at two or more nuclear loci to restore full pollen fertility (e.g. in maize; Laughnan & Gabay-Laughnan 1983). Incomplete restoration leads to partial male sterility (e.g. in *P. lanceolata*; van Damme & van Delden 1982). No male-sterile plants would be observed if restorer alleles went to fixation. Male sterility is widespread in some species, indicating polymorphism of restorers.

Spatial variation of genotypes

van Damme (1986) found high levels of spatial variation in cytotype and restorer frequencies within a single field of *P. lanceolata.* Three studies of *T. vulgaris* demonstrate spatial variation of genotypes among populations (Couvet *et al.* 1985; Manicacci *et al.* 1996, 1997). We discuss some of these studies later.

Spatial variation in phenotypes

The frequency of male-sterile (female) plants varies widely among wild populations of the same species. For example, 100 populations of *Origanum vulgare* contained 1–62% females (Kheyr-Pour 1980; similar data in Ietswaart *et al.* 1984); 110 populations of *T. vulgaris* contained 5–95% females, with the median greater than 60% (Gouyon & Couvet 1985); and 27 populations of *P. lanceolata* contained 0.4–29% females or partial male-steriles (van Damme & van Delden 1982). Later in this chapter we provide detailed summaries of spatial variation in the frequency of females in *P. lanceolata* and three species of *Thymus*.

Review of previous theories

Equilibrium models for nuclear or cytoplasmic control

Many interesting aspects of CMS follow from the conflict between nuclear and cytoplasmic genes. Matrilineally inherited cytoplasmic genes favour male sterility because females reallocate resources from pollen to ovules (Ashman 1992; Atlan *et al.* 1992; Eckhart 1992). Biparentally inherited nuclear genes favour hermaphroditism, with mixed allocation of resources to pollen and ovules. Comparison of the equilibrium frequency of females favoured by cytoplasmic and nuclear genes provides a simple way to gauge the intensity of the conflict. These equilibria also illustrate the role of traditional breeding system parameters such as selfing rate and inbreeding depression. The derivations follow Frank (1989), based on earlier work cited below.

In most seed plants, mitochondrial genes are transmitted only through ovules (Mogensen 1996). There are two fitness components to consider (see Table 11.1 for notation).

First, the fitness of ovules in a female is proportional to $(1 + f)Cm^x$, where f measures the extra ovule production of females relative to hermaphrodites, m is the frequency of hermaphrodites in the population, and C and x are parameters that describe the availability of pollen. The term Cm^x is the proportion of ovules that successfully achieve outcrossed fertilization.

Secondly, the fitness of ovules in a hermaphrodite is proportional to $s(1 - d) + (1 - s)Cm^x$, where s is the proportion of ovules that are self-fertilized and d measures inbreeding depression. Among the ovules not self-fertilized, a proportion Cm^x successfully achieve outcrossed fertilization.

Equilibrium occurs when the fitness of ovules in a female equals the fitness of ovules in a hermaphrodite

$$(1+f)Cm^x = s(1-d)+(1-s)Cm^x \qquad (1)$$

Let $z = 1 - m$ be the frequency of females. The equilibrium frequency of females when controlled by unrestored cytoplasmic genes is

Table 11.1 Some factors influencing the dynamics of cytoplasmic male sterility (CMS).

Selfing rate, s	The probability that a hermaphrodite produces a seed by selfing when there is maximal availability of pollen from other plants
Inbreeding depression, d	One minus the fitness of a seed produced by selfing divided by a seed produced by outcrossing
Female fitness advantage, $1+f$	The total fitness of all seeds produced by a female divided by the total fitness of all seeds produced by a hermaphrodite under the assumptions that all seeds are outcrossed and pollen is not limited
Pollen limitation, $C(p/P)^x$	The fraction of non-selfed ovules than fail to receive outcrossed pollen, where C is a constant; p is the density of the pollen shadow relative to maximum density, P; and the exponent x defines the shape of the pollen limitation curve; some models set $m = p/P$, where m is the frequency of hermaphrodites
Restorer pleiotropy	The negative effect of a restorer on pollen fertility when not matched to its specific cytotype
Pollen compensation	In hermaphrodites, the enhancement of pollen fertility at the expense of seed fertility
Number of cytotypes	Alternative cytoplasmic genotypes that cause male sterility, each cytotype repressed only by a specific set of matching restorers
Restorer genetics	Number of restorers and their effects on each cytotype
Genetic drift	Loss of alleles from established populations
Demographic extinctions and colonizations	Loss of all plants from a breeding neighbourhood and subsequent recolonization
Pollen and seed shadows	Spatial scale over which pollen normally moves during outcrossing and seeds move during dispersal
Long-distance dispersal	Rare long-distance movement of pollen or seeds can have powerful consequences by introducing locally novel alleles into a breeding neighbourhood

$$z^*_{\text{cyt}} = 1 - \left(\frac{s(1-d)}{C(f+s)} \right)^{1/x} \tag{2a}$$

which is similar to a model first developed by Lloyd (1974, 1975). For no pollen limitation, $C = 1$ and $x \to 0$, $z^*_{\text{cyt}} \to 1$, as first shown by Lewis (1941). For linear pollen limitation, $C = x = 1$, the equilibrium is

$$z^*_{\text{cyt}} = \frac{f + sd}{f + s} \tag{2b}$$

To study the equilibrium favoured by nuclear control, we must also consider the pollen success of a hermaphrodite. Pollen fitness can be divided into two parts.

Pollen success in fertilizing hermaphrodites is the same as the fitness of ovules in hermaphrodites, $s(1-d)+(1-s)Cm^x$, because each successfully fertilized ovule of a hermaphrodite has obtained pollen from a hermaphrodite. Success in fertilizing females is proportional to $[(1-m)/m](1+f)Cm^x$, where $(1-m)/m$ is the number of female plants for each hermaphroditic plant, and $(1+f)Cm^x$ is proportional to the fitness of ovules per female plant.

Nuclear equilibrium occurs when the fitness of ovules in females equals the sum of ovule and pollen fitnesses in a hermaphrodite

$$(1+f)Cm^x = 2[s(1-d)+(1-s)Cm^x]+\frac{1-m}{m}(1+f)Cm^x \tag{3}$$

For no pollen limitation, $C=1$ and $x=0$, the equilibrium is

$$z^*_{\text{nuc}} = \frac{f-1+2sd}{2(f+sd)} \tag{4a}$$

as in a model presented by Charlesworth and Charlesworth (1978). For linear pollen limitation, $C=x=1$,

$$z^*_{\text{nuc}} = \frac{f-1+2sd}{2(f+s)} \tag{4b}$$

where $z^*_{\text{nuc}}=0$ when $f<1-2sd$.

When collecting data, one often does not have separate estimates for f, s, d, and the pollen limitation parameters. Instead, one obtains a measure of seed fitness in females relative to hermaphrodites, subsuming aspects of inbreeding and pollen limitation. In our notation, we can define the seed productivity of females relative to hermaphrodites as $F=(1+f)Cm^x/[s(1-d)+(1-s)Cm^x]$. Using F in Eq. 3, the nuclear equilibrium is

$$z^*_{\text{nuc}} = \frac{F-2}{2(F-1)}, \tag{5}$$

where F is measured at equilibrium. When $F>2$, the female frequency at the nuclear equilibrium is greater than zero.

Genetic model of restorer polymorphism under nuclear control

We derived the models in the previous section by equating the fitnesses of female and hermaphrodite phenotypes. Phenotypic models highlight the key processes in a simple way, but for CMS one must often understand something of the underlying genetics.

Later we will emphasize that as the nuclear equilibrium rises above zero, restorer alleles decline in frequency. The phenotypic models do not make this point clearly because they hide the important genetic details. In this section, we present a simple genetic model of nuclear control based on Ross and Weir (1975). The genetic model

shows the relation between the equilibrium under nuclear control and the frequency of restorer alleles at nuclear loci.

For the purposes of this model, it is best to think of the population as fixed for a single cytoplasmic genotype. This cytoplasmic genotype causes male sterility in the absence of a matching nuclear allele that restores pollen fertility and hermaphroditism. A single nuclear locus controls the frequency of females as follows: homozygotes with two copies of the dominant restorer allele are hermaphrodites, heterozygotes with one copy of the dominant restorer allele are also hermaphrodites, and homozygotes with two copies of the recessive non-restorer allele are females.

The frequencies in the population are D for the dominant homozygote, H for the heterozygote and R for the recessive homozygote. The frequency of the restorer allele is $D + H/2$, because the frequency of the restorer allele in heterozygotes is one-half. As in the previous section, we are interested in the equilibrium frequency of females. In this genetic model with nuclear control by dominant restorer alleles, the equilibrium frequency of females is $z^*_{\text{nuc}} = R^*$, the equilibrium frequency of recessive homozygotes.

To keep the model simple, we assume no selfing, $s = 0$, and no pollen limitation, $C = 1$ and $x = 0$. With these assumptions, the frequency of the restorer allele in pollen is $r = (D + H/2)/(D + H)$, where the denominator is $D + H$ because the R genotype does not produce pollen. The frequency of the restorer allele in ovules is $p = (D + H/2)/(1 + Rf)$, where the denominator corrects for the fact that the R genotype produces an extra Rf ovules.

With these gametic frequencies, we can write the three genotypic frequencies in the following generation as $D' = rp$, $H' = r(1 - p) + p(1 - r)$, and $R' = (1 - r)(1 - p)$. The equilibrium for this system can be calculated easily (Ross & Weir 1975), yielding $z^*_{\text{nuc}} = R^* = (f - 1)/2f$, which matches Eq. 4a with $s = 0$. The other genotypes have equilibrium frequencies $H^* = (1 - R^*)(1 - x)$ and $D^* = (1 - R^*)x$, where $x = f - \sqrt{f^2 - 1}$.

The genetic model produces exactly the same ratio of females to hermaphrodites as the phenotypic model, but we now also have an explicit statement about the frequencies of the restorer allele and the different genotypes. For example, $f \leq 1$ yields $R^* = 0$ and a restorer allele frequency of one, $f = 1.5$ yields $R^* = 0.17$ and a restorer allele frequency of 0.58, and $f = 2$ yields $R^* = 0.25$ and a restorer allele frequency of 0.48.

Selfing, inbreeding depression and pollen limitation change the calculations of allele frequency. But for our purposes, we only wish to emphasize the general trend mentioned above: as the nuclear equilibrium rises above zero, restorer alleles decline in frequency.

Definitions of nuclear and cytoplasmic control

We use the phrases 'nuclear control' and 'cytoplasmic control' throughout. Now that we have introduced some genetics, we can be more precise about the meaning of these phrases.

The full genetic system of CMS can be described roughly as follows. There are n different cytoplasmic genotypes (cytotypes) in the population; each individual has a haploid cytotype with one of the n different types. If an individual lacks the particular restorer alleles for its cytotype, then that individual is a female.

A cytotype can interact in a complex way with several nuclear loci. In the simplest case, each cytotype interacts with a unique, diploid nuclear locus. At the nuclear locus, the restorer allele is usually dominant to the non-restorer allele (Edwardson 1970; Charlesworth & Laporte 1998). In an individual with a particular cytotype, if the matching nuclear restorer locus has the restorer phenotype, then the individual is a hermaphrodite.

Genetic control defines the direction of evolutionary change in a local mating population (patch). If, for any of the cytotypes present in that patch, the matching restorers are absent in all individuals, then that cytotype will always cause male sterility. The unmatched cytotype will increase in frequency until the local population reaches the cytoplasmic equilibrium. Thus, cytoplasmic control drives the local population toward the cytoplasmic equilibrium.

If, for each of the local cytotypes, the matching nuclear restorer alleles are present in some individuals in the patch, then the restorers change in frequency until the population reaches the nuclear equilibrium. Thus, nuclear control drives the local population toward the nuclear equilibrium.

The CMS genetic system is sometimes referred to as joint nuclear and cytoplasmic (nucleocytoplasmic) control. This simply means that the outcome depends on the interaction between these two potentially polymorphic components of the genome. In any local population, the particular distribution of polymorphisms determines whether nuclear or cytoplasmic control reigns.

Metapopulation effects on nuclear or cytoplasmic control

This section describes the effects of variable female frequencies among patches of a metapopulation. The models show how the amount of variation among patches raises or lowers the global average frequency of females at equilibrium under either nuclear or cytoplasmic control. The following section takes up theories that analyse non-equilibrium fluctuations caused by conflict between nuclear and cytoplasmic genes.

Local mate competition and group selection

Couvet *et al.* (1998) modelled extinction and colonization of patches in a metapopulation. The number of colonizing seeds contributed by an established patch increases in proportion to the patch's frequency of females because females produce more seeds than do hermaphrodites. This colonizing advantage for female-biased patches favours an increase in the frequency of females controlled by nuclear genes. They interpreted this as a group selection process because it depends on the differential seed productivity of patches.

The model of Couvet *et al.* (1998) is an example of the well-known process of local mate competition from sex-ratio theory (Hamilton 1967, 1979; Charnov 1982;

Frank 1998). In local mate competition, a rise in the genetic relatedness among competing pollen causes an increase in the female bias of the sex ratio. The model requires that seeds disperse and compete over a distance longer than pollen flow. In the model of Couvet *et al.* (1998), empty patches cannot receive pollen but can be colonized by seeds, increasing the effective distance of seed flow relative to pollen flow. Frequent colonizations and extinctions at the patch level increase relatedness within patches, promoting local mate competition.

Pollen limitation

The greater the variation in female frequency among populations, the more often a female occurs within a relatively female-biased population—variation implies females are clumped. A female has difficulty obtaining pollen when in a population with mostly other females. Thus, when females are clumped, female fitness is reduced relative to hermaphrodites (McCauley & Taylor 1997).

McCauley and Taylor (1997) analysed a model of cytoplasmic control with two alternative cytotypes. One causes male sterility and the other produces hermaphrodites. Their model is similar to Eq. 1, with linear pollen limitation, $C = x = 1$, and no selfing, $s = 0$. They also accounted for variation among populations in the frequency of the two cytotypes. Such variation arises by sampling when new populations are established.

If one defines R as the variance among populations in female frequency divided by the total variance in females, then McCauley and Taylor (1997) showed that Eq. 2b with $s = 0$ becomes

$$z^*_{\text{cyt}} = \frac{f - R(1+f)}{(1-R)f} \tag{6}$$

If $R = 0$, there is no variation among populations, the frequency of females approaches 1 as in Eq. 2b, and the metapopulation is pushed toward extinction by pollen limitation. An increase in R causes a decline in the equilibrium frequency of females averaged over all populations.

Equation 6 can be understood more easily if one expresses the equilibrium as the number of females to the number of hermaphrodites, $f - R(1+f) : R$. We note that R is the coefficient of relatedness from kin selection theory (Hamilton 1970; Frank 1998). Here, R measures relatedness of cytotypes within populations. The equilibrium ratio matches the gain to an individual of becoming a female relative to the gain of becoming a hermaphrodite. Becoming a female provides an additional f ovules but incurs the loss of not being able to pollinate neighbouring females, related by R, that have $1 + f$ ovules available to receive pollen. Becoming a hermaphrodite provides pollen to neighbouring hermaphrodites, related by R, that have proportionately one ovule available to receive pollen.

McCauley and Taylor (1997) developed a similar model of pollen limitation under nuclear control. By analogy with the cytoplasmic model, under nuclear

control an increase in the variation among populations in female frequency causes a decline in the global frequency of females.

The models of local mate competition and pollen limitation appear to come to opposite conclusions. Spatial variation increases the frequency of females under local mate competition but decreases the frequency of females under pollen limitation. Couvet *et al.* (1998) assumed no pollen limitation in their model, allowing the total seed productivity of groups to rise with the local frequency of females. By contrast, pollen limitation prevents an increase in seed productivity with rising frequency of females, reversing the direction of selection on females. Both models identify important forces. The balance will fall differently in each species depending on the details of pollination ecology and demography.

Pannell (1997) focussed on an extreme form of pollen limitation, in which single females cannot colonize an empty patch but self-compatible hermaphrodites can. If single-seed colonization occurs sufficiently often, this process can bias the sex ratio away from females and toward hermaphrodites.

Nuclear–cytoplasmic conflict: three theories of non-equilibrium dynamics

The equilibrium and metapopulation models above establish the nature of nuclear-cytoplasmic conflict and provide important upper and lower bounds on female frequency. But evolutionary dynamics may often be driven by non-equilibrium fluctuations in which phenotypic control shifts back and forth between cytoplasmic and nuclear genes. The following models emphasize non-equilibrium dynamics driven by conflict.

Demographic extinctions and colonizations

Gouyon and Couvet (1985) noted two striking attributes of *T. vulgaris*. First, the frequency of females per patch varied widely over space. Female frequencies ranged from 5% to 95%, with a median greater than 60%. Secondly, *T. vulgaris* has an ephemeral, patch-structured demography. Fire or other disturbance sometimes clears a patch, followed by subsequent recolonization.

Gouyon and Couvet suggested that patch-level extinctions and recolonizations play a crucial role in CMS dynamics. During colonization of an empty patch, a cytotype may arrive without its associated restorers. An unrestored cytotype drives up the frequency of females toward the cytoplasmic equilibrium. Abundant females favour the introduction and rapid spread of restorers because restored hermaphrodites will donate pollen to the many nearby females.

In this model, patch-level extinctions and colonizations cause local bursts of females followed by return toward the nuclear optimum. Non-equilibrium demography drives spatiotemporal fluctuations in the frequency of females and in the frequency of male-sterility genotypes.

Genetic extinctions and colonizations

Frequent patch-level extinctions and recolonizations occur in some plant species.

However, spatial variation in CMS may be more widespread than such extreme demography. For example, van Damme (1986) observed spatial variation in females and in male-sterility cytotypes over a few hundred metres within a single contiguous patch of *P. lanceolata*.

Frank (1989) developed a model in which genetic extinctions and colonizations occur within established patches. Suppose, for example, that a population lacks a cytotype and its matching restorers. Immigration of that cytotype leads to its rapid increase because it will cause male sterility and increased seed fertility. A high frequency of male-sterile plants favours the introduction and increase of the matching nuclear restorer alleles.

The spread of one cytotype drives down the frequency of other cytotypes, possibly causing local loss of genotypes. Nuclear restorers that match locally extinct cytotypes no longer provide any benefit. They will be driven from the local population if they carry any negative fitness costs because such costs are no longer offset by the benefits of restoration. The local extinction of a cytotype and matching restorers leads eventually to another round of colonization. This process of genetic extinctions and colonizations maintains spatial variation among populations.

The greater the number of alternative cytotypes, the more opportunity for dynamics to be driven by local extinction and colonization processes (Frank 1989, 1997, 2000). Drift can also play an important role—the loss of rare cytotypes and restorers from local populations sets the stage for the next rounds of reintroductions and local genetic turnover.

The model of Gouyon and Couvet (1985) depends on an extreme form of drift, in which severe demographic fluctuations cause local loss of cytotypes and restorers. The model of Frank (1989) combines drift and selection by emphasizing that the spread of a cytotype in a local population deterministically drives down the frequency of alternative cytotypes. Such deterministic selection combined with drift can maintain spatial variation in CMS without frequent demographic extinctions, although such extinctions certainly enhance spatial variation.

Deterministic fluctuations within patches

The previous models depend on stochastic perturbations. Gouyon and Couvet (1985) emphasize local demographic extinctions and recolonizations; Frank (1989) emphasizes genetic extinctions in extant populations followed by subsequent colonizations. Spatial variation in both models increases as the number of alternative cytotypes rises—more cytotypes increase the chance that a particular cytotype can shake free of its matching restorers (Frank 1989).

Studies of *P. lanceolata* in the 1980s found only two alternative male-sterile cytotypes (van Damme & van Delden 1982). In addition, many *P. lanceolata* populations are large and probably not subject to frequent demographic extinctions. In spite of the apparently small number of cytotypes and relative demographic stability, van Damme and van Delden (1982) did observe some spatial variation in the frequency of females.

Gouyon *et al.* (1991) formulated a deterministic model to explain these observa-

tions of *P. lanceolata* (see Charlesworth 1981 for a similar model). Gouyon and colleagues assumed two alternative male-sterile cytotypes, each with its own matching, specific nuclear restorer locus. For their parameters, the restorers go to fixation and there are no females unless some process opposes the increase in the restorer allele frequencies.

To oppose fixation of restorers, Gouyon and colleagues assumed that restorers not matched to the resident cytotype reduce pollen fertility when in hermaphrodites. They found that the severity of the fluctuations in gene frequencies and female frequencies depends mainly on the pleiotropic cost of the restorer alleles. When the cost is high, restorer frequency drops as its matching cytotype frequency declines, setting up a strong rise in that cytotype and recovery of the restorers. Thus, high pleiotropic costs lead to a steady cycle of rising and falling frequencies—the classical limit cycle.

Fluctuations within each local population would probably be out of phase with fluctuations in other populations. Asynchronous dynamics lead to spatial variation in females and in gene frequencies. This model requires only two cytotypes and does not depend on stochastic processes or colonizations over space.

This model's simplicity makes it attractive. But we will not discuss it further for three reasons. First, restorer costs needed to drive the fluctuations seem too high to be a widespread explanation for the observed spatial variations. With the most common assumptions about genetic interactions, the model requires restorer costs of about 30%, that is, unmatched restorers in a hermaphrodite lower pollen fertility by about 30%. It has generally been difficult to detect any costs, and when detected such costs tend to be small (de Haan *et al.* 1997d). Because detecting small fitness effects is difficult, costs might be widespread but probably are not very large.

Secondly, Gouyon *et al.* (1991) were compelled to develop their model because the evidence available at that time suggested only two alternative cytotypes in *P. lanceolata*. Recent studies have found additional cytotypes (de Haan *et al.* 1997a), making more plausible the models that work best with higher numbers of cytotypes.

Finally, some level of deterministic fluctuations may occur, but the case studies below on natural populations call attention to spatial processes and to stochastic events.

Runaway allocation: a new theory

We complete our discussion of models by proposing a new theory. We developed this theory as a novel explanation for comparative patterns among species based on the data we will discuss in the second half of this paper. In particular, Manicacci *et al.* (1998) recently studied three species of *Thymus*. Across species, they found that the frequency of females rose with F, the seed productivity of females relative to hermaphrodites. Our theory of runaway allocation explains how this positive association may arise.

Our runaway theory follows a cycle. We briefly list each phase and then expand our explanation.

1 A rise in F causes a decline in the frequency of restorer alleles and an increase in the frequency of females.
2 A decline in the frequency of restorers increases the probability in a local population that at least one cytotype will exist for which the matching restorers are absent. An unmatched cytotype establishes cytoplasmic control and causes a rapid rise in the frequency of females.
3 When the frequency of females rises above the nuclear equilibrium, hermaphrodites have a higher fitness through pollen than through seeds. This asymmetry favours hermaphrodites to reallocate resources from ovules to pollen, a process we call pollen compensation.
4 Pollen compensation lowers the seed productivity of hermaphrodites, raising F, the ratio of seed productivity of females relative to hermaphrodites.
5 A rise in F feeds back into the first phase of the cycle, continuing the process and causing a runaway increase in both F and the frequency of females. Across species, the theory predicts a positive association between F and the frequency of females.

We now provide additional explanation for each phase of the cycle.
1 A rise in F causes a decline in the equilibrium frequency of restorers when $F > 2$. We established this in an earlier section in which we presented a genetic model of restorer polymorphism under nuclear control. Note that restorer polymorphism does not require any costs of restoration when $F > 2$. If $F < 2$, a rise in F reduces the selective pressure to increase restorer frequency toward fixation. Thus, rising F may reduce the frequency of restorers under non-equilibrium fluctuations when $F < 2$. In general, a lower frequency of restorers raises the frequency of females.
2 When restorer frequencies decline, the probability rises that a particular restorer allele will be locally extinct in some patches of a metapopulation. This enhances the probability of cytoplasmic control in a local population. Under cytoplasmic control, the frequency of females rises to a high level.
3 Under cytoplasmic control, a locally high frequency of females favours hermaphrodites to increase pollen productivity at the expense of seed productivity (Charlesworth & Charlesworth 1978; Charlesworth 1989; Maurice *et al.* 1993). Pollen compensation is favoured because the excess females in the local population provide additional ovules for the hermaphrodites to fertilize via pollen, which enhances the value of pollen relative to ovules.
4 Pollen compensation lowers the seed productivity of hermaphrodites, raising F, the ratio of seed productivity of females relative to hermaphrodites. Pollen compensation cannot easily be measured. Suppose, for example, that hermaphrodites reduced their seed production by one-half and doubled their pollen production. The outcome would be a doubling of F, without any indication that the doubling was caused by pollen compensation rather than inbreeding depression or some other process.
5 A higher value of F reduces the selective pressure to raise the frequency of restorers. The lower the frequency of restorers, the more often cytoplasmic control occurs because of an increased probability of local extinction of restorers. Cytoplasmic

control favours pollen compensation, which in turn raises *F*. This causes a further decline in the frequency of the restorers and a rise in the frequency of females, and another turn of the cycle.

Self-compatible species may be particularly prone to this runaway cycle. Selfing and inbreeding depression raise *F*, and may do so sufficiently such that the initial value of *F* is high enough to get the cycle started.

Various processes such as pollen limitation may oppose the continued increase of *F* and the frequency of females. Such processes will stop the runaway increase at different points for different species. Thus, the model predicts a positive association between *F* and the frequency of females across species.

The first step in our cycle establishes a positive association between *F* and the frequency of females. We developed our runaway model to explain the wide variations in *F* observed among *Thymus* species, which we suggest have been driven by pollen compensation.

Case studies

Only a few CMS species have been studied intensively in natural populations. We briefly summarize the key observations. Because these studies guided the development of our runaway allocation theory, we cannot test our theory from available data. Rather, we use our theory along with the other models described above to organize what has been observed and to highlight the kinds of problems that can be studied in the future.

Plantago lanceolata

van Damme and van Delden (1984) measured the seed productivity of female and hermaphroditic plants. In two different plots, females produced 38% and 20% more seeds, respectively. Seeds of females were 20% heavier than those of hermaphrodites. Overall, it appeared that the total seed fitness of females was less than twice that of hermaphrodites. In our notation, $f < 1$, and because this species is self-incompatible, $s = 0$. According to simple models of sex expression in Eq. 4, the nuclear genes would favour a complete absence of females in this species.

Studies by van Damme and van Delden (1982) demonstrated nuclear–cytoplasmic inheritance with two distinct cytotypes, each with its own set of nuclear restorers. The cytotype *R* causes the male-sterile phenotype MS1 when unrestored and IN1 when partially restored. The cytotype *P* causes MS2 when unrestored and IN2 when partially restored. All four types are morphologically distinct and can be scored by direct examination. Restored cytoplasms of either type are hermaphroditic, *H*. The cytoplasmic type of a hermaphrodite can be determined only by crossing until the cytoplasm is exposed in an unrestored nuclear background.

van Damme and van Delden (1982) studied spatial variation of phenotypes. Table 11.2 shows phenotypic frequencies in 12 populations in two habitat groups; the original paper lists data for 27 populations in five categories. The labels for each population are abbreviations for locations.

Table 11.2 Phenotype percentages in natural populations of *Plantago lanceolata.* (From van Damme & van Delden 1982.)

Population	MS1	IN1	MS2	IN2	H	Sample size
Hayfield						
Dr	0	0	0.2	0.2	99.6	811
Ze	0	0	5.0	0.8	94.1	742
An	0	0	8.2	1.3	90.5	754
Re	0	0	5.0	3.6	91.4	695
Me1	0	0	3.9	5.5	90.6	688
Ve	12.2	2.2	0	0.6	85.0	623
Br	23.0	7.0	0.3	0.2	69.5	601
Pasture						
Wd	4.6	0.6	0.5	0.9	93.4	6902
Bm2	7.3	3.9	0	1.3	87.5	386
Pa	7.6	7.8	0.5	0.9	83.2	437
Ac2	11.8	10.8	0	1.0	76.4	305
Ju	21.5	7.0	0	0.5	71.0	414

Table 11.3 Percentage of phenotypes of *Plantago lanceolate* at a Westduinen field.* (Data from van Damme 1986.)

Description	MS1	IN1	MS2	IN2	MS3	H	Sample size
Total field	4.6	0.6	0.5	0.9	0.5	92.9	6902
p1	28.6	5.4	0	0	0	66.1	112
p2	25.6	1.6	0	0	0	72.8	188
p3	21.3	3.0	0.3	1.2	0	74.3	695
p4	22.7	1.6	0	4.7	0	71.1	688
Remainder	1.9	0.4	0.6	0.9	0.5	95.7	6140

* MS3 is a rare phenotype controlled by variation at nuclear loci. The locations of populations p1–p4 are shown in Figure 11.1.

The two population groups shown in Table 11.2 are the most differentiated of the five groups listed in the original paper. Five of the hayfield populations either lacked the *R* cytoplasm or were fixed for the *R* restorers. In the pasture populations, either the *P* cytoplasm was very rare or the *P*-specific restorers were common. The other three population groups were relatively more mixed for MS1 and MS2 phenotypes.

van Damme (1986) made an intensive study of spatial variation within the Westduinen (*Wd*) population listed in Table 11.2. A picture of the field at Westduinen is shown in Figure 11.1, with some of the data listed in Table 11.3. Females were rare over the whole population, with MS1 more common than MS2. However, in a few locations the frequency of MS1 was high (Figure 11.1). Within the larger clusters of MS1, p1–p4, the frequency of MS1 phenotypes was close to zero at the borders and rose to 60% near the centre.

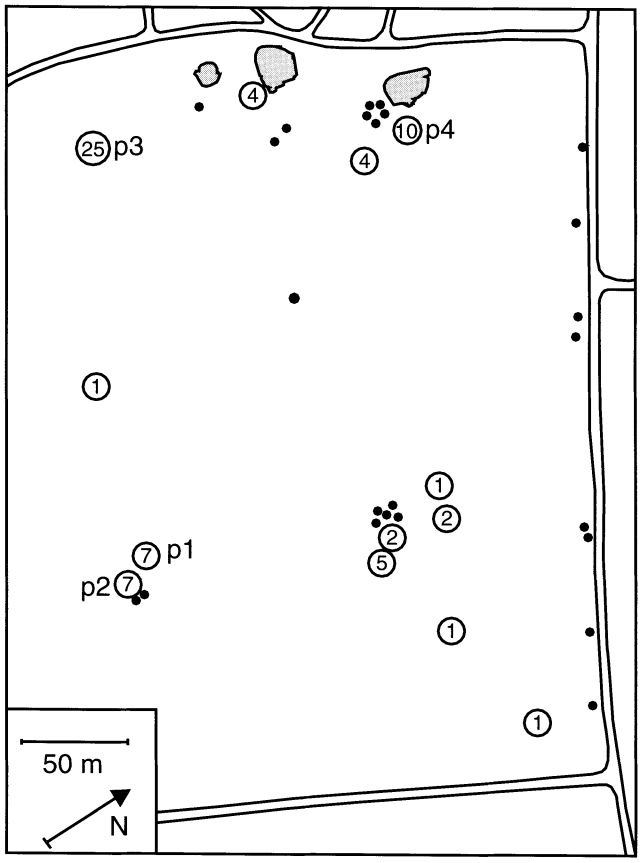

Figure 11.1 Distribution in a pasture of areas with MS1 and IN1 plants of *Plantago lanceolata*. Single plants are represented by dots and groups of plants by circles. The number within each circle is the area in square metres covered by the local group of plants. The four largest groups are labelled p1–p4. The shaded areas are pools that cattle use for water. (From van Damme 1986.)

The field as a whole was dominated by the *P* (MS2) cytoplasm, with an overall frequency of 0.94. The frequencies of the *P*-specific restorer alleles were also high. Thus, most plants were hermaphrodites with a *P* cytoplasm and *P* restorers. The overall frequency of the *R* cytoplasm was 0.06, and the *R*-specific restorers at the two restorer loci had frequencies of 0.02 and 0.08.

Genotypic composition was very different in those few areas that had high frequencies of the MS1 phenotype (Figure 11.1 and Table 11.3). The *R* (MS1) cytoplasm, rare in the population as a whole, had frequencies ranging between 26% and 39% in populations p1–p4. The *R*-specific restorers, also rare in the whole field, were more frequent in the MS1 clusters, although the exact frequencies were difficult to estimate.

van Damme's interpretation suggests colonization by a locally rare cytotype into an established patch. Initially, most of the field was dominated by *P* cytoplasms and *P*-specific restorers. *R*-bearing colonists founded the MS1 spots and, since the *R*-specific restorers were initially rare, the MS1 females spread from colonizing foci. MS1 plants produce more seeds that are larger and survive better than seeds from hermaphrodites (van Damme & van Delden 1984), so the females have a competitive advantage locally. Seeds disperse at a slow rate (about 0.1 m per generation; Bos *et al.* 1986), thus well-defined patches can form. As the frequency of unrestored *R* cytoplasms rises in an area, selection favours an increase in *R*-specific restorers. In an area with a high concentration of *R* cytoplasms, the main pollen donors will be *R*-restored hermaphrodites.

The low frequency of the *R*-specific restorers in the overall population suggests that these alleles are at a selective disadvantage when the *R* cytoplasm is absent. If so, then a population dominated by the *P* cytoplasm is likely to have a low frequency of the *R* restorers, as at Westduinen. That genotypic composition is susceptible to invasion by *R* cytoplasms, followed by a subsequent change in genotypic composition.

van Damme's (1986) study of a single field calls attention to differing spatial scales. Spatially contiguous populations define physical patches of habitat. Studies often focus on those spatial patches as the unit of study. But key processes may occur on different length scales from the structure of the physical patches.

The field in Figure 11.1 has spatial dimensions on the order of hundreds of metres. By contrast, typical seed flow occurs on the order of 0.1 m per generation and pollen flow at less than 1 m per generation (Bos *et al.* 1986). Thus, the spatial variation that drives the dynamics can occur within a single field. Measures of variation among large patches may hide much of the interesting spatial dynamics that occur within patches.

de Haan (1996) measured the spatial distribution of females in several populations. She found that blocks of length 10–20 m maximized the variation in the frequency of females among spatial units, suggesting that differentiation occurred on a relatively short spatial scale within large aggregations of plants.

The number of different cytotypes can influence CMS dynamics. van Damme and van Delden (1982; van Damme 1983) conducted a series of extensive crossing experiments over many populations and several years. They established the identity of two cytotypes, for each of which they found matching nuclear restorer genotypes.

de Haan *et al.* (1997a) used molecular tools to study the diversity of *P. lanceolata* mitochondria. They sampled 528 plants from 12 populations in the Netherlands and 13 plants from seven European and North American populations. Their tools did not directly identify genes involved in CMS. Instead, they established characteristic restriction fragment length polymorphism (RFLP) patterns for over 20 mitochondrial types, of which nine were relatively common.

de Haan *et al.* (1997a) used the nine common RFLP types as candidates for new CMS specificities, tested by segregation analysis. They clearly established two new mitochondrial CMS specificities and matching nuclear restorer sets—a total of four types have now been identified. Other candidates within their sample may be new

CMS types, but the segregation analyses were not sufficient to identify them unambiguously. This study demonstrates the specificity that had gone undetected in previous, extensive analyses. Given the limited sampling in this first major molecular study of *P. lanceolata*, perhaps more diversity remains to be discovered.

Thymus vulgaris

We describe the biology of *T. vulgaris* by developing contrasts with *P. lanceolata* and other *Thymus* species.

The net ovule fitness advantage of females compared to hermaphrodites is greater in T. vulgaris *than in* P. lanceolata

Figure 11.2(a) shows the relative seed fitness of females compared with hermaphrodites for three *Thymus* species compared with data for *P. lanceolata*. The plot shows a trend of increasing relative seed fitness for females with a rise in the frequency of females.

Thymus vulgaris is self-compatible whereas *P. lanceolata* is self-incompatible. Thus, measures of the relative ovule fitness advantage of females compared with hermaphrodites in natural populations of *T. vulgaris* confound inbreeding depression, *d*, and the outcrossed ovule fitness advantage of females, *f*. We can instead use the combined measure, *F*, for the seed fitness in females relative to hermaphrodites, which led to the nuclear equilibrium in Eq. 5 as $z^*_{\text{nuc}} = (F-2)/2(F-1)$.

The nuclear equilibrium predicts that increases in *F* raise the lower bound on the frequency of females. Using the values of *F* in Figure 11.2(a), the nuclear equilibria of female frequency are: 0 for *P. lanceolata*, 0 for *T. zygis*, 0.14 for *T. vulgaris*, and 0.28 for *T. mastichina*. The value of *F* for *P. lanceolata* is less than for *T. zygis*, thus the intensity of selection on nuclear genes to push down the frequency of females toward zero is greatest in *P. lanceolata*.

Figure 11.3 shows the distribution of female frequencies among patches in each of the four species. The locations of the lower bounds and the medians for female frequency follow the trend predicted by the values of *F*.

Pollen compensation appears to increase with female frequency among three species of Thymus

Figure 11.2(b) shows that across three *Thymus* species, as female frequency increases, the relative pollen investment rises. Manicacci *et al.* (1998) calculated the relative male investment in Figure 11.2(b) as full pollen grains divided by germinating seeds per flower. It could be that higher frequencies of females raise this ratio by reducing the seeds per flower because higher female frequency increases pollen limitation. However, Figure 11.2(a) shows that an increase in female frequency correlates with a rise in the relative seed productivity of females compared with hermaphrodites. This argues against severe pollen limitation at high female frequency, because pollen limitation would reduce the seed productivity of females relative to hermaphrodites.

Our runaway model predicts a positive association of *F*, relative pollen invest-

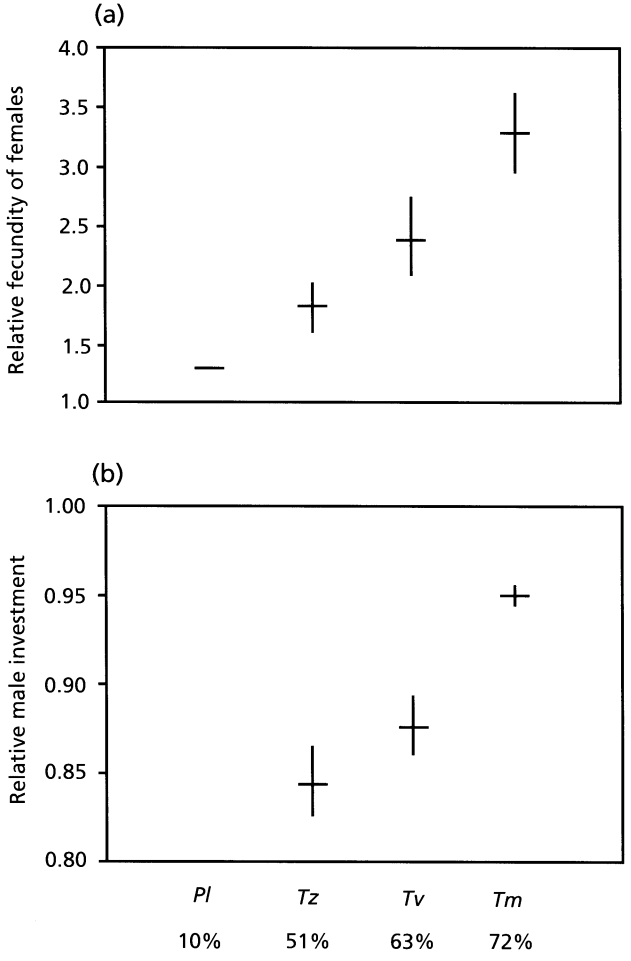

Figure 11.2 Relative female and male investment of sex types. Species abbreviations are: *Pl* for *Plantago lanceolata*, *Tz* for *Thymus zygis*, *Tv* for *T. vulgaris* and *Tm* for *T. mastichina*. The percentages below each species label give the average frequency of females observed in that species. Horizontal lines mark the mean and vertical lines mark ± 1 SE of the mean. (a) Relative seed fecundity of females to hermaphrodites. For the three *Thymus* species, Manicacci *et al.* (1998) measured the number of germinating seeds per fruit. No measures were taken of the number of fruits, differential survival of females and hermaphrodites, or differential survival of seedlings beyond germination. For *Plantago*, van Damme and van Delden (1984) measured total seed production per plant over 2–3 years. Thus, their results include components of adult survival but no measures of seedling fitness. Female frequency taken from van Damme and van Delden (1982). (b) Relative male investment measured as the number of full pollen grains per flower (with stainable cytoplasm) relative to the germinating seeds per flower. (From Manicacci *et al.* 1998.)

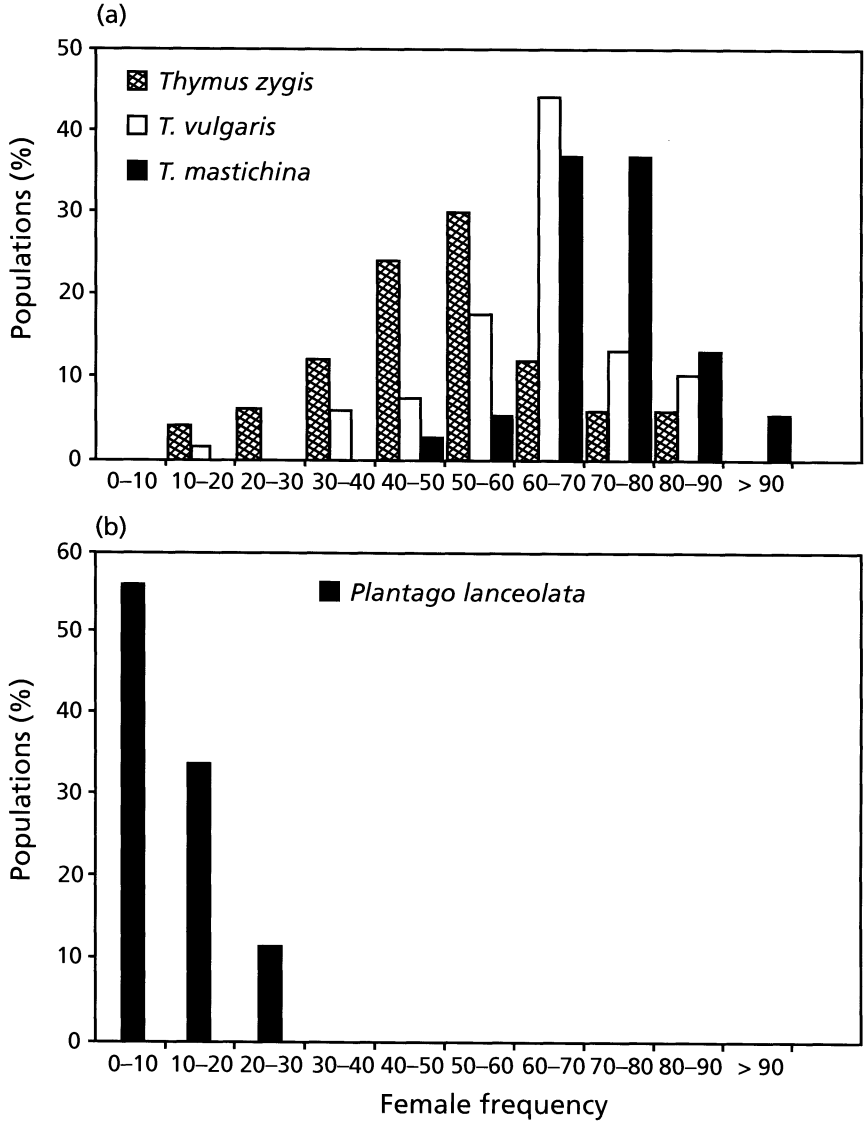

Figure 11.3 Frequency distribution of females among populations in four species. (a) The sex type of 100 individuals in each of 50 *Thymus zygis* populations, 68 *T. vulgaris* populations, and 38 *T. mastichina* populations. (From Manicacci *et al.* 1998.) (b) van Damme and van Delden (1982) measured the sex type in 27 populations of *Plantago lanceolata*, with sample sizes per population ranging from 111 to 6902.

ment of hermaphrodites, and female frequency. The three *Thymus* species show these trends.

Demographic extinctions and colonizations of patches may be more common in T. vulgaris *than in* P. lanceolata

Studies of *T. vulgaris* emphasize that this species has a patch-structured metapopulation in which patches proceed through cycles of colonization and extinction. For *T. vulgaris*, Belhassen *et al.* (1989) note that fire often destroys patches in southern France. *Thymus vulgaris* rapidly colonizes disturbed patches and is often characterized as an early successional species. By contrast, no studies of *P. lanceolata* discuss patch extinctions or colonizations.

Belhassen *et al.* (1989) report data for 15 sites for which the date of a fire had been recorded. The sites varied in age since burn from 1 to 23 years. The data clustered into three groups. The three sites less than 5 years since burn had a median frequency of females of 59%, the six sites between 5 and 11 years had a median frequency of females of 76%, and the six sites between 14 and 23 years had a median frequency of 58% females.

Belhassen and colleagues interpret these data in support of the Gouyon and Couvet (1985) model. Unrestored cytotypes may be relatively common upon colonization of empty patches (founder events). Cytoplasmic control pushes up the frequency of females to high levels until matching restorers arrive. Thus, the frequency of females tends to rise and then fall with the age since colonization. The data match the predicted trend. Given the small sample size and lack of information about the genetics, this study encourages further work.

Manicacci *et al.* (1996) analysed the role of founder events with a molecular study of cytotype diversity and spatial patch structure in *T. vulgaris*. They used RFLP to characterize cytotype in four distantly separated populations. They divided each population into patches with spatially circumscribed groups of plants and separated by regions that lacked *T. vulgaris*.

Almost all of the cytotype diversity was among patches, demonstrating a very strong effect of founder events. None of the 13 patches among the four populations shared a cytotype. Diversity within patches was low. In four patches that contained only female plants, samples of three, five, five and seven plants for each patch revealed only one cytotype per patch.

In two patches with very rare hermaphrodites, all females tested within a patch had one cytotype and a single hermaphrodite from each patch had a different cytotype. This pattern suggests an unrestored cytotype and a restored cytotype, with cytoplasmic control dominating within those patches. Finally, a patch with an intermediate frequency of females had one cytotype in two females and two hermaphrodites, and a second cytotype in two other hermaphrodites, consistent with a joint nuclear–cytoplasmic polymorphism in that patch.

The study by Manicacci *et al.* (1996) has small sample sizes and lacks segregation analyses to correlate RFLP type to genetic control of male sterility. Nevertheless, this study suggests that founder events play an important role in the CMS dynamics of

T. vulgaris. Perhaps more importantly, this work demonstrates the great potential of molecular tools to test hypotheses about spatial process.

Conclusions

We have argued that differing nuclear equilibria strongly influence the broad contrasts between *P. lanceolata* and the three *Thymus* species. The value of the seed productivity of females versus hermaphrodites, F, predicts the equilibrium frequency of females under nuclear control, $z^{\star}_{\text{nuc}} = (F-2)/2(F-1)$. Using the values of F in Figure 11.2(a), the predicted nuclear equilibria of female frequency follows the trend *P. lanceolata* < *T. zygis* < *T. vulgaris* < *T. mastichina*. The lower bounds for female frequency in Figure 11.3 match the trend predicted by the nuclear equilibria.

The value of F is confounded with pollen compensation by hermaphrodites. Suppose, for example, that hermaphrodites reduced their seed production by one-half and doubled their pollen production. The outcome would be an apparent doubling of seed productivity of females relative to hermaphrodites. This would increase the apparent female fitness advantage, F, which would raise the nuclear equilibrium.

Self-compatible species such as *Thymus* may be particularly prone to pollen compensation and thus to large values of F. Selfing may initially cause a nuclear equilibrium frequency of females greater than zero. The higher the nuclear equilibrium, the lower the frequencies of restorers, and the more often control shifts to the cytoplasm. Cytoplasmic control raises the frequency of females and favours additional pollen compensation and an increase in F, which in turn further raises the nuclear equilibrium for the frequency of females.

We mentioned the contrast between *P. lanceolata*, which is self-incompatible and has a relatively low frequency of females, and the *Thymus* species, which are self-compatible and have relatively high frequencies of females. Self-compatibility may facilitate high frequencies of females by raising the nuclear equilibrium. However, selfing is certainly not the whole story. *Plantago coronopus*, a congener of *P. lanceolata*, is self-compatible, yet has a relatively low frequency of females when compared with the *Thymus* species (Koelewijn 1993). Clearly, self-compatibility by itself does not explain differences among species, although it may contribute in some way. Broader comparative studies would greatly help in identifying important patterns.

Other differences between *P. lanceolata* and *T. vulgaris* include demography and spatial scaling. *Thymus vulgaris* appears to have more frequent extinctions and colonizations of patches than *P. lanceolata*, probably contributing to relatively greater spatial variation in CMS genetics and frequency of females. Within patches, the spatial scale of pollen and seed dispersal is very short for *P. lanceolata*, allowing significant spatial variation within populations (Figure 11.1).

Manicacci *et al.*'s (1996) molecular study of *T. vulgaris* suggests that most seed dispersal occurs over short distances in that species. They did not mention pollen movement, but isolated females may sometimes set seed, suggesting that pollen can

move over relatively longer distances. The relative movement of seeds and pollen plays a crucial role in CMS dynamics—these processes deserve further attention (McCauley 1998; Taylor *et al.* 1999).

The analyses of *P. lanceolata* and the *Thymus* species show the potential for comparative work; almost all CMS species remain unstudied in the wild. The preliminary molecular analyses also hint at the widespread polymorphisms and the powerful interaction between spatial processes and natural selection. Further molecular work tracking the movement of genes will be the key to unraveling this complex and fascinating problem.

Summary

Mitochondria sometimes cause male sterility in hermaphroditic plants by interfering with pollen development. Male-sterile plants usually produce more seeds than do hermaphrodites, probably because male sterility allows resources to be reallocated from pollen to seeds. Enhanced seed production benefits cytoplasmically inherited mitochondrial genes, which are transmitted through seeds but not through pollen. However, reduced pollen success lowers the fitness of nuclear genes, which are transmitted through both pollen and seeds. The different fitness consequences of male sterility for cytoplasmic and nuclear genes create a conflict of interest between these different subsets of the genome. Consistent with this idea of conflict, nuclear restorers occur that counteract cytoplasmic effects and restore pollen fertility.

Wild populations of cytoplasmically male-sterile plants typically have widespread polymorphisms of male-sterile mitochondria and nuclear restorer genes. These polymorphic genes appear to fluctuate over time and space, driven by the genomic conflict between the mitochondrial advantage of male sterility and the nuclear advantage of hermaphroditism.

We review various theories that explain the dynamics of cytoplasmic male sterility. We also propose a new theory, the runaway allocation model, which predicts a positive association across species between the frequency of male-sterile plants and the seed productivity of male-sterile plants relative to hermaphrodites.

Finally, we review observations from wild populations of *Plantago* and *Thymus*. The data suggest that the movement of mitochondrial and nuclear genes in spatially subdivided metapopulations controls the spatiotemporal dynamics of male sterility. The data also show a positive association across species between the frequency of male sterility and the relative seed productivity of male steriles compared with hermaphrodites, supporting the main prediction of our runaway allocation model. Recent studies with molecular tools show great promise for tracking the movement of genes over space and unravelling the processes that drive cytoplasmic male sterility.

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