THE EVOLUTIONARY DYNAMICS OF CYTOPLASMIC MALE STERILITY

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The different forms of flowers on plants of the same species reflect the complex interactions among heredity, development, population demography, and ecological associations that shape adaptations (Darwin 1877). The case of gynodioecy, a mixture of hermaphroditic and female (male-sterile) individuals, is even more instructive about the subtleties of adaptation than Darwin could have guessed from his limited knowledge of hereditary mechanisms. In many gynodioecious populations, the male-sterile trait is transmitted matrilineally, and the genetic determinants are therefore believed to reside in the cytoplasm. These cytoplasmic determinants usually coexist in a population with autosomal genes that repress their action and cause the restoration of pollen fertility (Kheyr-Pour 1980, 1981; van Damme 1983).

The special aspect of cytoplasmic male sterility (CMS) for studying adaptation arises from the different modes of transmission by the cytoplasmic and autosomal factors. Matrilineally inherited cytoplasmic genes are passed mainly through successful ovules; by contrast, autosomal genes are passed equally through successful ovules and pollen. Cytotypes gain a fitness advantage over other asexual, haploid cytotypes only by increasing success through ovules, whereas autosomal alleles gain an edge over their competitors by increasing the sum of ovule and pollen success. There is a conflict between subsets of the genome over the allocation of resources to ovules and pollen (Hamilton 1967; Alexander and Borgia 1978; Eberhard 1980; Cosmides and Tooby 1981; Charnov 1982; Frank 1983; Gouyon and Couvet 1985). If a male-sterile cytotype begins to spread in the population because of a slight fitness advantage through ovules, then the conflict intensifies since, as pollen becomes increasingly scarce, autosomal alleles associated with enhanced pollen fertility gain an increasingly large fitness advantage over competing alleles.

Darwin's logic for natural selection still applies, but the focus shifts from the competition among individuals to the combination of, first, the struggle between genomic subsets defined by mode of heredity and, second, the common interest for these competing parts of the genome of residing in an individual that performs

well against other individuals in the population. The distribution of male-sterile individuals among hermaphrodites is an easily scored phenotypic marker in wild populations that reflects the current resolution of this genomic conflict. Interpreting the intensity of conflict and its resolution is made difficult by complex interactions of the competing hereditary factors with the usual set of problems in the study of adaptation: population demography, ecological factors such as pollination, developmental constraints able to prevent the appearance of certain phenotypes that would otherwise be favored, and historical events.

Several previous studies have presented quantitative models to explain the observed patterns of CMS or have made qualitative suggestions about the role of genomic conflict when interacting with other key variables (reviewed below). These quantitative models make an important start, but they are inadequate because they are based on an oversimplification of the genetic system and require unrealistic parameter values to approximate observed patterns. Qualitative statements about conflict and other factors are difficult to evaluate because of the numerous potentially important variables and their complex interactions.

My goals are to define clearly the many variables that may influence the evolution of CMS and then to explore quantitatively parts of the parameter space in search of plausible explanations for observed patterns. In a future paper, I will introduce quantitative methods for analyzing the intensity and resolution of the conflict inherent in this system, along with suggestions for extending the quantitative analysis of conflict and resolution to other systems.

BACKGROUND

Review of Key Observations

Cytoplasmic male sterility (CMS) was first discovered by Correns (1906). As of 1972, CMS had been reported in 140 species from 47 genera and 20 families (Laser and Lersten 1972; see also Edwardson 1970; Grun 1976). More than one-half of these cases occurred naturally, about 20% were uncovered by intraspecific crosses, and the rest were observed in interspecific crosses.

Four striking characteristics of CMS in wild populations, in somewhat idealized form, are (1) the maintenance of a number of distinct cytotypes, each capable of causing male sterility by an apparently different mechanism since each is susceptible to only a particular subset of autosomal restorer alleles; (2) the maintenance of polymorphism at several autosomal restorer loci, with particular alleles or loci specialized for restoring pollen fertility when associated with particular cytotypes; (3) the maintenance of genetic differentiation among geographically distant populations; and (4) the maintenance of phenotypic diversity among populations, measured as the percentage of male-sterile individuals. Note that phenotypic diversity does not necessarily follow from genetic diversity because, with the complex genetic interactions among nucleus and cytoplasm, there are many genetic ways in which to obtain male sterility or male fertility.

Cytotypes.—Careful studies of populations exhibiting CMS often show a number of genetically distinct cytotypes that cause male sterility. For example, direct

genetic evidence from wild species has been obtained for *Plantago lanceolata*, in which two genetically distinct cytotypes have been found among study populations in the Netherlands and Luxembourg (van Damme 1983, 1984, 1986), and for *Origanum vulgare*, in which a sample of 24 male-sterile lines from four populations were interpreted by Kheyr-Pour (1980, 1981) as having at least seven distinct cytotypes, although the evidence in this case is not conclusive. Circumstantial evidence in natural populations has been reported for *Thymus vulgaris*, in which crosses between geographically distinct populations yield a higher frequency of females than crosses within populations, suggesting the presence of different cytotypes among populations (Couvet et al. 1985). Direct genetic evidence for the existence of distinct male-sterile cytotypes is also available for several cultivated species (*Zea mays*, review in Laughnan and Gabay-Laughnan 1983; *Triticum aestivum*, Wickersham and Patterson 1980; references for other species in Edwardson 1970; Hanson and Conde 1985). Few data are available concerning the number of cytoplasmic loci involved in male sterility. Hanson and Conde (1985) suggested that the nine observable phenotypes in *Solanum* probably depend on at least two distinct cytoplasmic loci. Aviv and Galun (1986) concluded from protoplast-fusion experiments that each of two physically distinct locations on the *Nicotiana* mitochondrial chromosome can independently cause male sterility.

Different cytotypes may have distinct anther morphologies associated with different stages at which microsporogenesis fails. Van Damme and van Delden (1982) and van Damme (1983) have observed three distinct anther morphologies clearly associated with two genetically distinct male-sterile cytotypes in *Plantago lanceolata*, and Ross (1970) observed at least two distinct anther morphologies in *Plantago coronopus* (see also Laser and Lersten 1972; Gottschalk and Kaul 1974; review of *Solanum* in Hanson and Conde 1985). These distinct anther morphologies suggest that the different cytotypes have different physiological mechanisms by which they interfere with normal pollen development.

Strong correlative evidence suggests that the cytoplasmic genes causing male sterility in *Zea*, *Petunia*, and *Nicotiana* are in the mitochondria (Butow 1986; *Zea*, Levings and Pring 1976; Levings et al. 1980; *Petunia*, Boeshore et al. 1985; *Nicotiana*, Aviv and Galun 1986). A transposable element in both the nucleus and mitochondria is implicated in the evolution of male sterility in the *S* cytoplasm of maize, but the details are unknown at present (Kemble et al. 1983; Gabay-Laughnan and Laughnan 1983). In *Vicia faba*, both small circular DNA's in the mitochondria and free cytoplasmic viruses have been correlated with CMS (Hanson and Conde 1985).

Autosomal restorer alleles.—Autosomal alleles that restore pollen fertility in the presence of male-sterile cytotypes are found at a number of distinct loci in several species. These alleles usually have a dominant effect in intraspecific crosses and often appear to be specific for a particular cytotype (Edwardson 1970). The best-studied cases of autosomal pollen-fertility restoration are among crop species, in which CMS lines are often used so that only pollen from controlled sources is introduced into a field (Hanson and Conde 1985). At least six separate autosomal loci of maize restore pollen fertility of one of the four known cytotypes (Laughnan and Gabay-Laughnan 1983). For the cytoplasm *cms-T*, the

simultaneous presence of a dominant restorer allele at each of two loci on different chromosomes is required for complete restoration of pollen fertility; presence of a dominant restorer at only one of the two loci may cause partial restoration, depending on genotypic background and environment (Duvick 1965). For *cms-S* (Buchert 1961), a single autosomal locus causes restoration by a gametophytic mechanism. Only pollen grains with the restorer allele complete normal development. Heterozygotes for the restorer allele therefore produce one-half the amount of viable pollen as homozygotes. Clear evidence about the other two cytotypes is currently lacking. Dewey et al. (1986) have found a transcript in the mitochondria that is unique to *cms-T* and have shown that an autosomal restorer gene alters this transcript. Cases in which autosomal restorer genes affect mitochondrial translation products are summarized by Pring and Lonsdale (1985).

For the *Monon* cytotype of winter wheat, Wickersham and Patterson (1980) suggested that the simultaneous presence of at least one restorer allele at each of two separate autosomal loci is required for complete restoration of pollen fertility. When restorer alleles are absent at one locus, the restorer allele of the second locus is dominant for partial restoration of pollen fertility; by contrast, when restorer alleles are absent at the second locus, the restorer allele of the first locus is recessive for partial restoration. In rye, alleles of at least three autosomal loci affect the amount of pollen-fertility restoration. For example, partial restoration occurs in the simultaneous presence of dominant alleles at each of a particular pair of loci, and complete restoration occurs when the dominant allele at a third locus is present with the dominant allele in at least one of the other two loci (Scoles and Evans 1979). Several other cases are discussed by Hanson and Conde (1985).

Genetic subdivision.—Genetic differentiation among populations is implicated when crosses between groups yield a higher frequency of male-sterile individuals than do crosses within groups, suggesting locally high frequencies of particular cytotypes and their associated autosomal restorers and simultaneously low frequencies of restorers for nonresident cytotypes (Couvet et al. 1986). An increased frequency of male sterility has been found in crosses among populations of *Thymus vulgaris* (Couvet et al. 1985). Van Damme (1986) studied the genetic structure of a population of *Plantago lanceolata* with respect to one male-sterile cytotype (*R*) and its male-fertile alternative (*P*) along with a set of autosomal loci that interact with these cytotypes. The overall frequency of *R* was 5%–6%, but these cytotypes were clustered into areas covering less than 0.1% of the total 7-ha area. The frequencies of the associated autosomal restorer alleles, also quite low, appeared to be higher near the concentrations of the *R* cytotype.

Phenotypic differentiation.—The frequency of male-sterile (female) individuals varies widely among wild populations of the same species: 100 populations of *Origanum vulgare* contained 1%–62% females (Kheyr-Pour 1980; similar data in Ietswaart et al. 1984); 110 populations of *Thymus vulgaris* contained 5%–95% females, with the median greater than 50% (Gouyon and Couvet 1985); 27 populations of *Plantago lanceolata* in the Netherlands and Luxembourg contained 0.4%–29% females or partial male steriles (individuals who make a small amount of pollen relative to fully fertile hermaphrodites; van Damme and van Delden 1982); 10 populations of *P. lanceolata* in California had 2%–31% females (Krohne

et al. 1980); and up to 70% females were observed in another study of this species (Baker 1963). See Lloyd (1976) for a summary of other reports on percentages of females in gynodioecious species.

Review of Key Ideas and Related Evidence

Ovule-fitness advantage for females; autosomal-cytoplasmic interactions.—Theoretical analyses of male sterility that consider cytoplasmic factors and autosomal-cytoplasmic interactions begin with Lewis (1941). He noticed that if male-sterile individuals have a higher fitness through ovules than do hermaphrodites, a cytoplasmic gene causing male sterility will spread in the population. The reason is simply that matrilineal genes make up an asexual gene pool with fitnesses determined by the rate of transmission through ovules. At least a slight advantage in ovule fertility or seed viability of male steriles seems reasonable since some energy may be saved by not producing pollen.

Data on ovule fitness in male steriles relative to hermaphrodites ranges from rare cases in which male steriles and hermaphrodites have nearly equal ovule fitness (Vaarema and Jääskeläinen 1967) to cases in which the seed output per male-sterile flower is more than twice that per hermaphrodite flower (Assouad et al. [1978], who explicitly factored out the potentially confounding effect of inbreeding depression). These data were reviewed by Lloyd (1976), van Damme (1984), and van Damme and van Delden (1984).

Lewis (1941) pointed out that a male-sterile cytoplasmic gene associated with increased ovule fitness continues to spread until checked by some opposing force. A number of later theoretical studies have analyzed a subset of autosomal-cytoplasmic interactions, population subdivision, pollen limitation, frequency of selfing, inbreeding depression, and pleiotropic fitness effects of both cytoplasmic male-sterile genes and autosomal restorer genes. I limit the review here to models that consider a cytoplasmic component, since the empirical evidence suggests that, in populations containing a nontrivial proportion of male steriles, the trait usually depends in part on cytoplasmic genes (Edwardson 1970; Ross 1978; van Damme 1984; Couvet et al. 1986). These models study the effects of either a single cytoplasmic locus or the interaction between a single cytoplasmic locus and a single autosomal locus.

Lloyd (1974) studied a model with cytoplasmic determinants of male sterility. He derived the equilibrium frequency of females as a function of pollinator limitation and relative seed fecundity and viability of hermaphrodites and females. Three studies have assumed that (1) autosomal restorer genes and cytoplasmic male-sterility genes are present, (2) self-fertilized and outcrossed ovules are of equal viability, and (3) the ovule production is the same for both male-sterile and hermaphroditic plants. These three studies all concluded that male sterility cannot be maintained, even when there is considerable mutation pressure or pollen flow (Watson and Caspari 1960; Caspari et al. 1966; Costantino 1971).

Pleiotropy and inbreeding effects.—Lloyd (1975, 1976) extended his earlier model of cytoplasmic control by also considering selfing and inbreeding depression. D. Charlesworth and Ganders (1979) extended the models with cytoplasmic male sterility and autosomal restorer genes by allowing the viabilities of self-

fertilized and outcrossed ovules to differ and by assuming that male-sterile plants may have an increased ovule output (see above for references on relevant empirical work). They showed that under these assumptions either the population approaches fixation for male sterility or the autosomal restorer gene becomes fixed and there are no male-sterile individuals.

D. Charlesworth (1981) and Delannay et al. (1981) extended the model by showing that polymorphism can be maintained when the cytoplasmic male-sterility or autosomal restorer genes have additional (pleiotropic) effects on ovule fertility or viability. Charlesworth's model shows that the evolutionary trajectory toward equilibrium is easily perturbed and has long, damped oscillatory cycles of gene and phenotype frequencies. This would explain the observed genetic and phenotypic variation among populations. Although some pleiotropic effects associated with these sorts of genes are quite possible (see data in van Damme 1984, 1986), the model must invoke either large pleiotropic effects or both a high selfing rate and a highly deleterious effect of selfing in order to explain the observed patterns of variation. Gregorius and Ross (1984; Ross and Gregorius 1985) have extended Charlesworth's model by providing general conditions for the maintenance of polymorphism for two cytotypes and two alleles at one autosomal locus. In their model, the cytotypes and autosomal alleles interact in a variety of ways and affect ovule and pollen fertilities and ovule selfing rate. These conditions for the maintenance of polymorphism are compared in the Discussion with the results of the simulation model presented below.

Conflict of interest and evolutionary dynamics.—An aspect of all these theoretical studies is that selection of cytoplasmic variants with any ovule-fertility or viability advantage tends to favor an increase in the frequency of male-sterile individuals, whereas selection of autosomal variants tends to favor the elimination of male steriles (D. Charlesworth 1981; Delannay et al. 1981). Put another way, the direction of selection differs between the autosomal and cytoplasmic points of view—there is a conflict of interest. Although the conflict is inherent in the studies that consider autosomal and cytoplasmic genes, it is never discussed explicitly. Cosmides and Tooby (1981), Charnov (1982), Frank (1983), Gouyon and Couvet (1985), and Couvet et al. (1986) have stressed the conflict aspect of this phenomenon but have not presented any new quantitative analyses. Kheyr-Pour (1981), Gouyon and Couvet (1985), and Couvet et al. (1986) have pointed out that, on the basis of reasonable assumptions, the above models cannot explain three of the most striking features observed in wild populations of male-sterile plants: (1) the large number of cytoplasmic and autosomal loci that affect male sterility, (2) the large variation in the frequency of male steriles among populations of the same species, and (3) the genetic diversity among populations.

THE SIMULATION MODEL

Description of the Life Cycle and Parameter Space

Models must focus on the dynamics of the system in order to be consistent with both the complex patterns of cytoplasmic male sterility (CMS) observed in the

TABLE 1

SUMMARY OF SOME FACTORS AFFECTING CYTOPLASMIC MALE STERILITY AND THE
ASSUMPTIONS USED FOR COMPUTER-SIMULATION EXPERIMENTS

Factor	Assumption
Life cycle	annual
Population subdivision and inbreeding	single breeding group with mixture of random mating and selfing
Determinants of breeding-group size	N, population size fixed at 250
Availability of pollen determines probability of outcrossed fertilization $= Cm^x$; m is frequency of hermaphroditic plants	C, probability of outcrossed fertility with abundant pollen; C is set at one
	x, rate at which fertilization decreases with shortage of pollen; x is set at one
Number of cytoplasmic loci	assumed to be two linked loci with no recombination
Number of alleles per cytoplasmic locus	three male-sterile alleles and one male-fertile allele
Number of autosomal loci	six unlinked loci
Number of alleles per autosomal locus	one restorer and one non-restorer
Autosomal-cytoplasmic interactions	each autosomal locus can restore a particular cytoplasmic allele; restorer alleles are dominant
Phenotype space: relative amounts of resources for reproduction allocated to pollen and ovules	male steriles allocate only to ovules; each hermaphrodite allocates a fixed proportion to pollen and ovules
Increase in progeny viability of male steriles	assumed to be zero
Pleiotropy by cytotypes	assumed to be zero

wild and the inherent conflict between the autosomal and cytoplasmic genes. I have chosen to use a computer-simulation model to capture the many biological details in a manner that is realistic and allows these dynamics to be explored directly. This approach provides a formalization of assumptions and quantitative conclusions based on the dynamics caused by the conflict of interest between cytoplasmic and autosomal genes.

The simulation model has an annual life cycle and fixed population size, with a mixture of selfing and random mating in the local breeding group. The two phenotypic classes are females and hermaphrodites. The ovule-to-pollen ratio of hermaphrodites may vary according to genotype because of factors affecting the efficiency of pollen production (see below). There are two linked, non-recombining cytoplasmic loci, each with three different alleles that can cause male sterility plus a single male-fertility allele, yielding 16 possible cytoplasmic haplotypes (cytotypes). Cytoplasms are assumed to be homoplasmic (genetically monomorphic within an individual) and therefore essentially haploid (see Birky 1978, 1983a,b). Six unlinked autosomal loci each have two alleles: a "non-restorer" allele, and an allele that restores male fertility in the presence of a particular one of the six cytoplasmic alleles causing male sterility. The physiological mechanisms involved in pollen sterility and restoration depend on a "gene-for-gene" interaction between the six cytoplasmic sterility alleles and six autosomal restorer loci. A cytoplasmic male-sterility allele in the absence of its associated autosomal restorer allele has complete penetrance. (See tables 1 and 2 for a listing of assump-

TABLE 2

Summary of Some Factors Affecting Cytoplasmic Male Sterility and the
Parameters Used in Five Computer-Simulation Experiments

		EXPERIMENT NUMBER				
FACTOR	PARAMETER	1	2	3	4	5
Selfing rate for her-maphrodites	s (%)	5–15	5–10	1–4	2	10–85
Ovule- and pollen-fertility reduction of progeny produced by selfing	d (%)	0–10	0	0	0	0–45
Increase in ovule fer-tility of male steriles	f (%)	25–75	5–25	2–10	15	75
Pleiotropy per auto-somal restorer al-lele independent of cytotype	reduction in male fer-tility and additive gene action, p (%)	2–8	4	3–4	4	2
Introduction of new alleles through mu-tation or migration	$1/N\mu_c$, generations per introduction per (haploid) cyto-plasmic locus	20	20	20	20–100	20
	$1/2N\mu_a$, generations per introduction per (diploid) auto-somal locus	10	10	10	10–50	10

tions and parameters.) There are $3^6 \times 4^2 = 11,664$ possible genotypes. This is an evolutionary potential: over any short period only one or a few cytotypes will be present in a simulated population of 250 individuals, and some autosomal loci will be close to fixation or actually fixed (see the Results). Different genetic systems are studied briefly in experiment 7 of the Results.

There are a number of genetic and frequency-dependent effects on the fitnesses of the two phenotypic classes. Females have an ovule-fertility advantage of f over hermaphrodites. Each autosomal restorer allele causes a pleiotropic loss in pollen fertility of p, where the effects are additive across the 12 autosomal alleles and independent of cytotype. The pleiotropic-fitness effect on an individual depends on the frequency of restorer alleles among hermaphrodites. If a hermaphroditic individual has a_i restorer alleles and the average number of restorer alleles per hermaphrodite is $\bar{a}$, then the relative pollen fertility of the ith individual is $(1 - Q_i)/(1 - Q)$, where $Q_i = a_i p$ and $Q = \bar{a} p$. (See the Discussion for a comparison of possible assumptions about types of pleiotropic effects.) Individuals that are the product of a selfed mating suffer a reduction d in both pollen and ovule fertility. The probability that an ovule from a hermaphrodite is selfed is s, and the probability that a non-selfed female or hermaphrodite ovule is fertilized is Cm^x, where C and x are parameters and m is the frequency of hermaphrodites. The proportion of selfed ovules among the fertilized ovules of a hermaphrodite is, therefore, $r = s/[s + (1 - s)Cm^x]$ (see table 3).

Alleles may be introduced into the population by immigration or mutation. The

TABLE 3

Summary of Relative Numbers of Successfully Pollinated Ovules and Numbers of Successfully Outcrossing Pollen Grains

	Outcrossed	Selfed
	FEMALES	
Relative number of fertilized ovules	$(1 + f)Cm^x$	$(1 - d)(1 + f)Cm^x$
Relative number of successfully outcrossing pollen grains	0	0
	HERMAPHRODITES	
Relative number of fertilized ovules	$s + (1 - s)Cm^x$	$(1 - d)[s + (1 - s)Cm^x]$
Relative number of successfully outcrossing pollen grains	$(1 - Q_i)/(1 - Q)$	$(1 - d)(1 - Q_i)/(1 - Q)$

Note.—An individual is *outcrossed* if it is the product of an outcrossed mating; it is *selfed* if the pollen and the ovule that formed it came from the same hermaphroditic parent. Realized frequency of selfing among hermaphrodites, $r = s/[s + (1 - s)Cm^x]$.

combined migration-mutation process is simulated by specifying a probability that an allele changes state in (or between) generations. These combined migration-mutation rates are μ_c for a cytoplasmic locus and μ_a for an autosomal locus. At each haploid cytoplasmic locus, the expected number of generations until the next change in the population is $1/N\mu_c$, and at each diploid autosomal locus, $1/2N\mu_a$, where N is the size of the population. Two allelic states occur at each autosomal locus; therefore, the expected waiting time before the first occurrence of a particular mutant (migrant) is also $1/2N\mu_a$. Because of the four allelic states at each cytoplasmic locus, the expected waiting time for the first occurrence of an absent allele is $3/N\mu_c$. Each migration-mutation event at a cytoplasmic locus is equally likely to produce any of the three alternative alleles different from the current state of the allele in the randomly chosen individual. Each migration-mutation event at an autosomal locus causes one of the two alleles to switch from its present form to the alternative state (for additional information about assumptions, see tables 1–3).

Choice of Parameter Values and Genetic System

The parameter space is huge, particularly when one considers the range of possible genetic systems. It is difficult to decide where to focus attention. Previous theory has shown that male sterility can occur under what seem unrealistically large fitness effects of inbreeding depression, ovule-fertility advantage of females relative to hermaphrodites, or negative pleiotropic effects of autosomal restorer alleles or male-sterile cytotypes (references above). I therefore chose which parameters to vary and over what range by setting the goal of finding the smallest set of fitness effects and the simplest genetic assumptions that yield results consistent with observed genetic and phenotypic patterns.

The genetic system of gene-for-gene interactions between six cytoplasmic male-

sterile alleles and six associated autosomal restorer loci is a simple assumption about the interactions between cytoplasmic alleles and autosomal loci. I chose six cytoplasmic-autosomal pairs and two separate cytoplasmic loci, each with three of the male-sterile alleles. Under this arrangement one will fairly often infer, by classical crossing experiments, that the simultaneous presence of restorer alleles at two autosomal loci is necessary to restore pollen fertility for a single cytotype, a genetic system that could not occur with only one cytoplasmic locus and gene-for-gene interactions. Two or more autosomal loci interacting with one cytotype is a common observation (see the review above under ''Background''), and experiment 6 (below) presents the manner by which such a system would be inferred according to the simulation model. The results presented below in experiment 7 briefly summarize the simulations of a variety of genetic systems.

I chose a population size of 250 because this number is sufficient to make the effects of drift negligible when compared with the magnitude of selection coefficients that occur in the model. The major features of the dynamics are therefore insensitive to the stochastic effects of simulation. The quantitative effects of drift could be measured by studying a range of population sizes, which has not been done in this study, although small pilot studies with population sizes of 500 and 1000 did not yield results noticeably different from a population of 250. Results such as those reported below in experiments 3 and 7 suggest that even minor stochastic perturbations are negligible when compared with the effects of small changes in parameter values.

Simulation Algorithm

The simulation program, written in Microsoft Pascal, runs on an IBM PC-AT computer. For a particular combination of parameters, each of the six unlinked autosomal diploid loci are first initialized with ''non-restorer'' alleles, and the two linked, non-recombining cytoplasmic loci are initialized with male-fertility alleles for all $N = 250$ hermaphroditic individuals in the population. The program is then run for 1000 generations to complete initialization, and during each of the following 2000 generations frequency statistics are collected for phenotypes, cytotypes, and autosomal alleles. For all procedures with more than one possible outcome, the outcomes are chosen by a 32-bit random number with a cycle of 2^{32}, or greater than four billion. The model is therefore fully stochastic.

Each generation begins with the potential for migration or mutation to occur, followed by mating. Ovule and pollen donors are chosen according to the success weights in table 3. First, an ovule donor is chosen. If it is a male-sterile plant, then a pollen donor is chosen. For hermaphrodites, the ovule is selfed according to the realized probability of selfing (table 3); otherwise, a pollen donor is chosen from the population. The process is repeated once for each new individual of the next generation, up to the fixed population size.

Statistics on cytotype and autosomal-allele frequencies give information about the *true* polymorphism in the population, which contrasts with the *detectable* polymorphism that one could actually infer by crosses in classical genetic experiments. Information on detectable polymorphism in the simulated population is important because consistency of the model with extant data depends on compar-

ing simulation results with the results of inferential genetics based on crosses. If one had available only the simulated population without knowing the genetic basis of male sterility for that population, the genetic system inferred by crosses would differ considerably over time. The inferred system would reveal few of the details of the genetic system built into the simulation model (see the Results). The model assumptions represent a long-term evolutionary potential rather than the state of a population over any short period of time.

In order to collect information on detectable polymorphism and the genetic system that would be inferred by crosses, an algorithm was constructed to simulate the inference process. The algorithm extracts the maximum amount of information possible from crosses, given a set of individuals. In practice, one never obtains all the information available. To show the trends that occur with diminishing information, the algorithm was applied in each generation to the entire population and to a sample of 20 composed of a random selection of females up to 10 individuals, if available, with the remainder chosen randomly from among the hermaphroditic individuals. Information from actual crossing experiments will probably tend to be less than the maximum information available from a sample of 20.

RESULTS

The three main attributes of interest are the distribution of the percentage of male-sterile individuals (females) over the 2000 generations of a simulation, the true levels of genetic polymorphism, and the polymorphism and genetic system that would be inferred by performing genetic crosses on a stock derived from a sample of the population. All parameters and results are reported as percentages.

Summaries of the evolutionary dynamics of the percentage of females are presented in figure 1 for three parameter combinations. Each peak in the time-series graphs in this figure represents the spread of a newly introduced sterility cytotype when one of the associated autosomal restorer alleles is rare or absent, followed by the spread of the restorer allele. If peaks are infrequent relative to the rate at which new cytotypes are introduced by migration or mutation, then the frequency of the restorer alleles at each autosomal locus is high, and each of the two linked, non-recombining cytoplasmic loci is nearly fixed for the alleles that spread in the most recent outburst of females. Increasingly frequent cycles of spreading females and autosomal restoration of pollen fertility, relative to the introduction of new alleles, are associated with increasing polymorphism of both autosomal and cytoplasmic loci (e.g., see fig. 1, legend; experiment 6, below).

Statistics

The results for each generation are the percentage of females, the percentage of the restorer allele at each autosomal locus, the percentage of each cytotype among all cytotypes, and the presence or absence of a particular inference about the genetic system by simulated crossing experiments. These percentages, or the presence or absence of an attribute, must then be summarized over the 2000
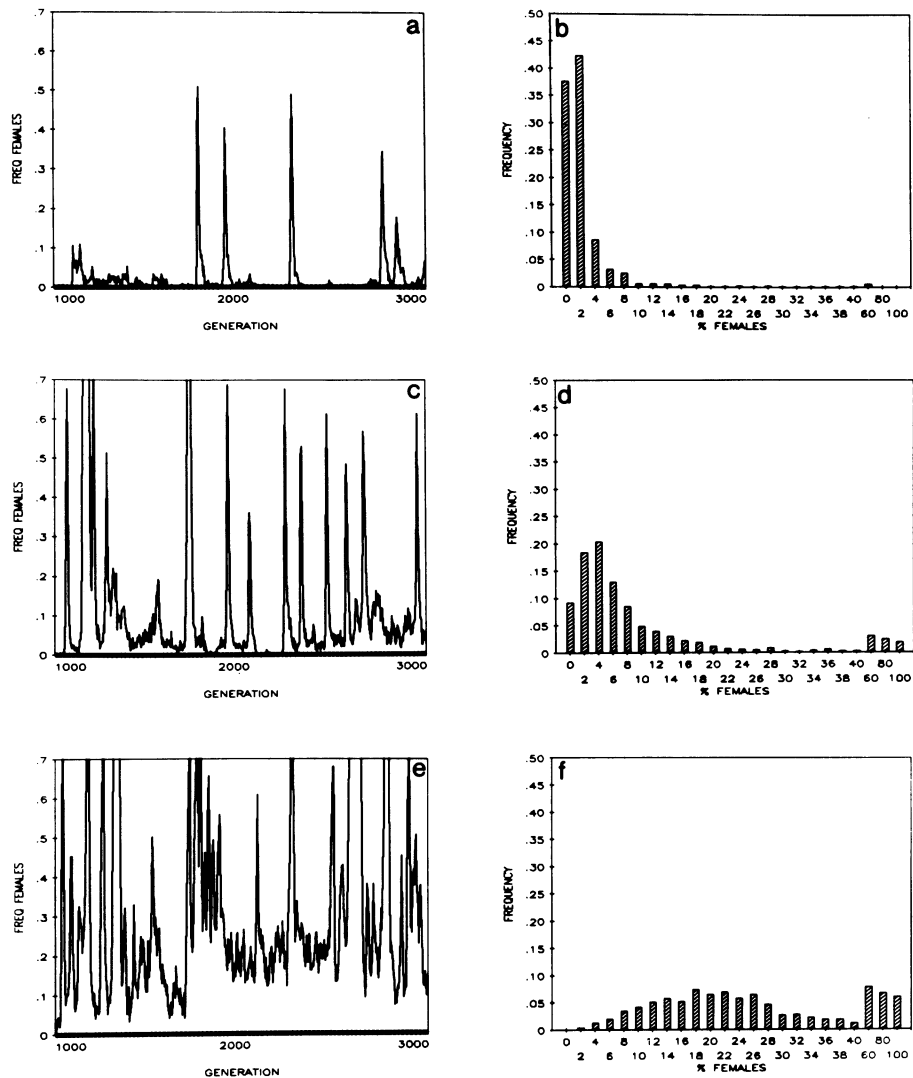
Fig. 1.—Time series and histograms of the percentage of females for three combinations of parameters. For all three runs, $s = 10$, $d = 5$, $f = 50$, $N = 250$, and $\mu_a = \mu_c = 2 \times 10^{-4}$ (for definition of parameters, see table 2). Only autosomal pleiotropy varies among the three runs: a and b, $p = 2$; c and d, $p = 4$; e and f, $p = 8$. When $p = 2$, the average range of allele frequencies per generation of the autosomal restorer allele was 68%–96%; $p = 4$, 41%–82%; $p = 8$, 9%–54%. For the cytoplasmic loci, the male-fertility alleles were nearly absent, as expected, since male-sterile cytotypes were assumed to have no negative pleiotropic effects, and the average frequency per generation of the primary, secondary, and tertiary alleles were, for $p = 2$, 93%, 6%, and 1%; for $p = 4$, 91%, 8%, and 1%; and for $p = 8$, 76%, 22%, and 2%. In panels d and f, high percentages of females indicate that a cytotype has spread and there is a long wait until the introduction of an autosomal restorer allele.

generations of each run. The results over all generations can be described as a frequency distribution of percentages or as the percentage of occurrence of an attribute. For example, the right column of figure 1 shows the frequency distribution of the percentage of females over 2000 generations. In order to compare results among runs that take the form of frequency distributions, percentiles of the distribution from each run are used, especially the lower quartile (25th percentile), median (50th percentile), and upper quartile (75th percentile). The results from several runs are often summarized by the mean value of a particular percentile taken over all runs in the set.

Most experiments consist of a single replicate of a factorial design. An analysis of variance (ANOVA) was conducted by assuming that third- and higher-order interactions are negligible or by assuming that particular second-order interactions are negligible on the basis of results from previous experiments. This is a common approach for maximizing the information per experiment for large parameter spaces while maintaining the advantages of replication (Cochran and Cox 1957). The small standard errors for estimates obtained under this procedure suggest that little would be gained by replication. In three of seven experiments, it was necessary to collect data from additional replicates in order to increase precision.

Experiment 1: Core of the Parameter Space

The first experiment consists of four parameters at three levels each (for parameter ranges, see table 2). The design is therefore a 3^4 factorial experiment with a single observation for each treatment combination. The purpose of the experiment is to uncover general trends in what seemed a priori to be a biologically realistic part of the parameter space and to suggest what future experiments might be fruitful (see "Choice of Parameter Values and Genetic System," above).

Separate ANOVA's were calculated at the lower, middle, and upper quartiles of the distribution of the percentage of females over the 2000 generations of each run. They all suggest that the only factor with a significant influence in the context of the ranges of parameters chosen is the main effect of autosomal pleiotropy, p (see fig. 2). Pleiotropy, with two degrees of freedom, explains 67.7%–81.8% of the total variation in the three ANOVA's, each of which has 80 total degrees of freedom. As would be expected from figure 1, a high percentage of females occurs occasionally. The mean percentage of females at the 90th, 95th, and 100th percentiles for $p = 2$ are 9.4, 25.9, and 77.4; for $p = 4$, 37.1, 65.5, and 87.9; and for $p = 8$, 73.9, 80.9, and 90.0.

Experiment 2: Small Ovule-Fertility Advantage for Females

This experiment explores the effect on evolutionary dynamics and the distribution of the percentage of females when the ovule-fertility advantage for females relative to that for hermaphrodites is small. Data are presented for a single observation for each of 10 combinations with selfing, s, at 5% or 10%, the ovule-fertility advantage for females, f, ranging from 5% to 25% in increments of 5, and the negative pleiotropy of autosomal restorer alleles, p, set at 4% (table 2). Assuming that the interaction between s and f is negligible (following the results of
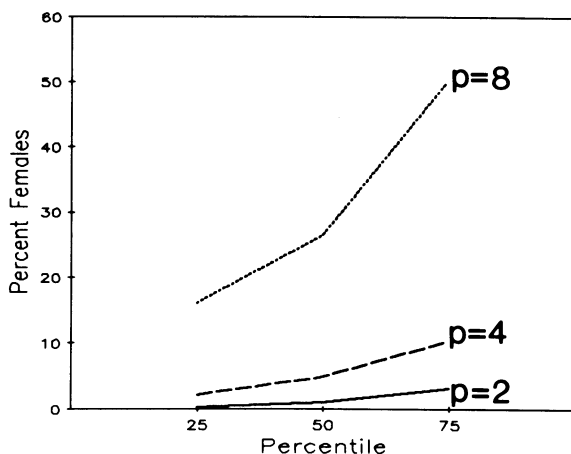
FIG. 2.—The average percentage of females at the lower, middle, and upper quartiles for experiment 1 (see table 2), which explores the core of the parameter space. Each line represents a particular level of autosomal pleiotropy, p. The quartiles are from the distribution of the percentage of females over 2000 generations of a parameter combination (see, e.g., fig. 1). There are only nine points on the graph, and the lines are intended to serve as visual aids. Each point represents the average of 27 observations, or one-third of 3^4. Standard errors of the means are 5.1%–14.8% of the means, except for the lower quartile for $p = 2$, which has a standard error of the mean (0.178) of 38.6% of the mean. Standard deviations are the standard errors multiplied by $(27)^{1/2}$.

experiment 1), then neither main effect (neither ovule fertility nor selfing rate) has a significant influence on the observed variation in the quartiles of the distribution of the percentage of females across the 2000 generations of each run. The means and standard errors of the means are, for the lower quartile, 0.60 ± 0.11; for the median, 2.44 ± 0.23; and for the upper quartile, 8.32 ± 1.54. From experiment 1, with $p = 4$, the means of the lower, middle, and upper quartiles and the standard errors of the means are 2.13 ± 0.30, 4.74 ± 0.43, and 10.28 ± 0.53, respectively.

The lower- and middle-quartile means for small ovule-fertility advantage of females are significantly lower than in the core experiment (95% confidence intervals do not overlap), whereas the upper quartiles do not differ significantly. However, even with a small ovule-fertility advantage, the average median percentage of females is 2.4.

Once again, populations occasionally have a high percentage of females. The 90th-, 95th-, and 100th-percentile means and standard errors are 27.16 ± 4.27, 39.88 ± 4.87, and 66.28 ± 4.75.

Experiment 3: Very Small Ovule-Fertility Advantage and Autosomal Pleiotropy

This experiment is designed to explore a part of the parameter space that assumes only a very small ovule-fertility gain for females relative to hermaphrodites (five levels), a small amount of negative pleiotropy by autosomal restorer alleles (two levels), low amounts of selfing (three levels), and no inbreeding
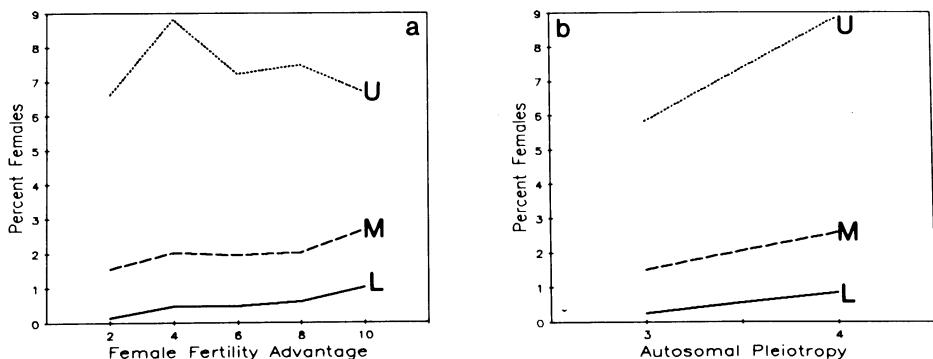
Fig. 3.—The average percentage of females at the lower (L), middle (M), and upper (U) quartiles for experiment 3 (see table 2), which considers a small ovule-fertility advantage for females relative to hermaphrodites and small levels of negative pleiotropy for autosomal restorer alleles. Since there are a total of 30 parameter combinations, there are six observations for each point in *a* and 15 for each point in *b*. The means and standard errors of the means of the 90th, 95th, and 100th percentiles for all 30 combinations are 20.23 ± 1.67, 32.17 ± 2.23, and 67.16 ± 12.26.

depression (see table 2). Data are presented for a single observation for each of the 30 combinations. ANOVA's on the lower, middle, and upper quartiles suggest that only the main effects of pleiotropy and ovule-fertility advantage of females significantly influence the observations, with the exception that the main effect of female ovule-fertility advantage is insignificant for the upper quartile. The quartile means are shown in figure 3. Although the trends are clear, the magnitude of the changes across this particular range of parameters is small.

Some generations have a high percentage of females, as before, even with these small parameter values. Since the total changes in the magnitudes of the observations are small across these ranges of parameters, the means for all 30 parameter combinations are reasonable summary statistics for the upper percentiles. The means and standard errors of the means for the 90th, 95th, and 100th percentiles are 20.23 ± 1.67, 32.17 ± 2.23, and 67.16 ± 12.26.

Experiment 4: Lower Migration-Mutation Rates

The rate at which new alleles are introduced is varied in this experiment (for parameter values, see table 2). Three levels are used for cytoplasmic and for autosomal rates, yielding nine combinations. Eight replicates of each combination are studied, for a total of 72 runs. The migration-mutation rates are presented as the expected number of generations per introduction in the population per locus, which is $1/N\mu_c$ for a haploid cytoplasmic locus and $1/2N\mu_a$ for a diploid autosomal locus. Since the population size, N, is set at 250 in all experiments, varying the waiting times until introductions means varying μ, the generations per introduction per allele for each individual in the population. In this experiment, μ_a and μ_c each varies from 2×10^{-4} to 4×10^{-5}. The results (fig. 4) suggest that even for lower cytoplasmic and higher autosomal rates of introduction, which are the most
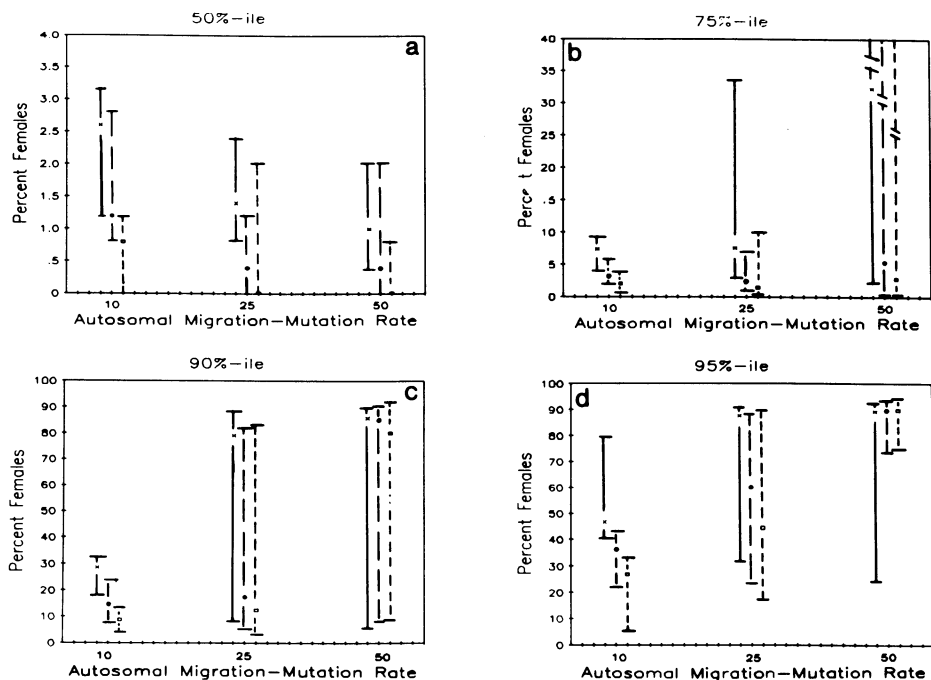
Fig. 4.—The median and range for the different percentiles of the distribution of the percentage of females per generation over 2000 generations, for eight replicates of each parameter combination (experiment 4; see table 2). Combined migration-mutation rates are given as the expected number of generations until one change in the allelic state at a particular locus for one member of the population. Cytoplasmic rates: × and *solid line*, 20; *dots and long-dashed lines*, 50; *open squares and short-dashed lines*, 100. All lines represent ranges. The 100th-percentile medians are greater than 92% females for all combinations except at an autosomal migration-mutation rate of 10 and at cytoplasmic rates of 50 (89.0% females) and 100 (76.8% females). The medians at the 25th percentile are all below 1% females. The autosomal rates are 10, 25, or 50 for each observation: the data for each level are off-set to make the ranges easier to see. Note the increase in variation with increasing time between migration or mutation events (see the text). *b*, Upper range boundaries for an autosomal rate of 50: *solid line*, 82.4% females; *long-dashed line*, 45.2%; *short-dashed line*, 86.8%.

unfavorable combination for male sterility, a low percentage of females occurs during about 40% of the generations in each run of 2000 generations, and females make up 10% or more of the population during at least 10% of the generations. Experiment 3 and other simulation data suggest that increasing the female plants' advantage in ovule fertility (from the 15% studied in this experiment up to the 30%–50% range) uniformly increases the percentage of females by a small amount (about 1%–3% in the 25th–50th-percentile range, and slightly more at the higher percentiles) and that increasing the autosomal pleiotropy slightly (from 4% to 5% or 6%) increases the percentage of females at the 25th percentile from near zero to a 1%–2% range, increases the 50th percentile to a 2%–8% range (cf. fig. 4a), and has less relative effect at the higher percentiles.
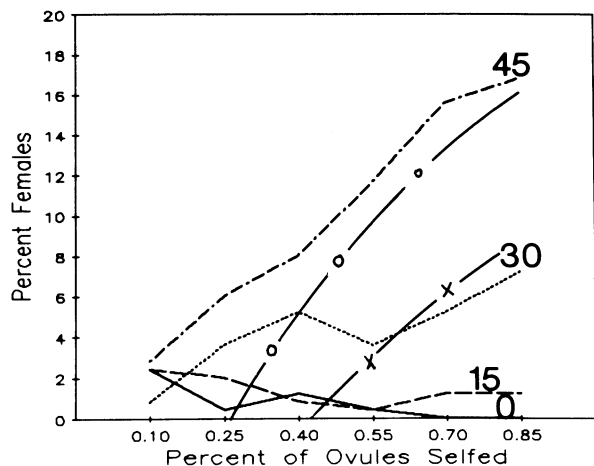
FIG. 5.—The median percentage of females for experiment 5 (see table 2), which examined the interaction between the proportion of selfed ovules in hermaphrodites and inbreeding depression. The number above each line is the amount of inbreeding depression, d. The trends are similar for the lower and upper quartiles. The line with the open circles is the prediction for $d = 45$, and the line with the x's is the prediction for $d = 30$, from equation (2).

Experiment 5: High Selfing Rate and Inbreeding Depression

The fifth experiment examines the effects of selfing, inbreeding depression, and their interaction across a wide range of values, with a large (75%) ovule-fertility advantage of females relative to hermaphrodites (table 2). The purpose is to compare results with a body of theory that focuses on this range of parameters (see the Discussion). A single observation for each of six levels of selfing and four levels of inbreeding depression is analyzed. There is an interaction between the selfing rate and the amount of inbreeding depression (fig. 5), which is expected since in the present model the effect of inbreeding depression occurs only in individuals that are the product of a selfed mating. The data shown in figure 5 are the medians of the distribution of the percentage of females over 2000 generations of each parameter combination. The trends are very similar for the lower and upper quartiles.

Experiment 6: True and Detectable Polymorphism; Inferred Genetic System

In this experiment the core of the parameter space (see experiment 1) is reexamined with the focus on the genetics of male sterility rather than on the frequency of phenotypes. Here, the edges of the core (table 2) are studied for ovule-fertility advantage ($f = 25, 75$), selfing rate ($s = 5, 15$), and inbreeding depression ($d = 0, 10$), along with all three levels of autosomal pleiotropy ($p = 2, 4, 8$), forming a $3 \cdot 2^3$ factorial design. Data are analyzed for three replicates of this design.

True polymorphism.—The *true* cytoplasmic polymorphism is calculated by ranking the frequency (percentage) of the cytoplasmic haplotypes (cytotypes) in
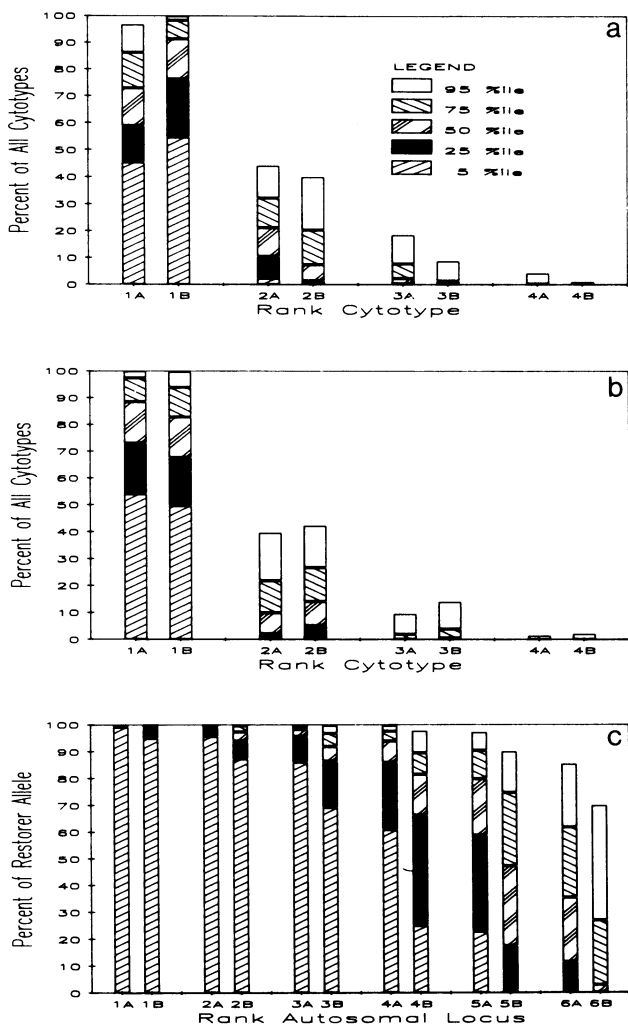
FIG. 6.—True polymorphism of cytotypes and autosomal loci (explained in the text). *a*: *A*, *f* = 25; *B*, *f* = 75. *b* and *c*: *A*, *p* = 2; *B*, *p* = 4.

each generation and then calculating the percentiles of these frequencies for each rank category over the distribution from the 2000 generations of each run. This yields information on the temporal distribution of the frequency of the most common cytotype, the second most common cytotype, and so on. For example, in figure 6*a*, the top of the 50th-percentile bar above 1*A* implies that the most common cytotype is more than 73% of all cytotypes in 50% of the generations and is less than 73% in the remaining 50% of the generations. The top of the 5th-percentile bar to the top of the 95th-percentile bar is the 90% confidence interval for each rank category.

The most important factor for cytotype polymorphism across the range of parameters studied is the ovule-fertility advantage, f. In figure 6a, the A and B categories represent $f = 25$ and $f = 75$, respectively, averaged across all other parameter combinations. Note that higher values of f lead to a decline in polymorphism, shown by the increased frequency of the most common type in 1B compared with 1A, the decline in 2B compared with 2A, etc. This effect is probably caused by the more rapid spread and the higher attained frequency of unrestored cytotypes causing male sterility and the generally increased selection intensity among cytoplasmic variants.

The second most important factor is pleiotropy of autosomal restorer alleles, p. In figure 6b, categories A and B represent $p = 2$ and $p = 4$, respectively, averaged over all other parameter combinations. Increasing pleiotropy causes an increase in polymorphism, but the effect is small for a shift from $p = 2$ to $p = 4$. A slightly greater increase in polymorphism occurs for a shift from $p = 4$ to $p = 8$ (not shown).

Autosomal polymorphism is summarized by ranking the frequency (percentage) of the restorer allele at each of the six loci and then calculating percentiles of the temporal distributions of ranked percentages as above. The factor that explains most of the variation is, not surprisingly, the pleiotropic effect of the restorer allele, p. In figure 6c, the A and B categories represent $p = 2$ and $p = 4$, respectively. For example, category 6B shows that with $p = 4$, the restorer allele is absent from the population at one or more loci in 45% of the generations, opening up the possibility for the introduction and spread of the associated cytoplasmic male-sterility allele(s). With $p = 2$, the absence of a restorer allele at one or more loci occurs only 10%–15% of the time. This explains why pleiotropy has a strong effect on phenotypic polymorphism and dynamics.

Detectable polymorphism; inferred genetic system.—The cytotype polymorphisms described in figure 6 are the true frequencies in the simulated population, which can be easily tabulated by the computer. By contrast, polymorphisms for naturally occurring populations or agricultural varieties have been detected by classical genetic crossing experiments, and the genetic control of the phenotype has also traditionally been inferred from crosses. The number of cytotypes and the inferred genetic control *detectable* by crossing experiments are shown in figures 7 and 8. Each category is described in the following paragraphs.

The middle bar above the number of cytotypes (#Cyt) category shows the percentage of all generations in which a given number of cytotypes are detectable by crossing experiments. For example, in figure 7a one cytotype is detectable in 12% of the generations, two cytotypes in 40% of the generations, three in 31%, and so on.

The left, middle, and right bars over each category are the statistics for generations with 0%–2% females, all generations, and 30%–50% females, respectively. In most categories, the percentage of females explains a large part of the variation.

The ability to detect a cytotype depends on the autosomal polymorphisms available for crosses. Different cytotypes are not distinguishable by crosses when they differ only in their response to autosomal variation not present in the current population. Often the number of detectable cytotypes is less than the number in
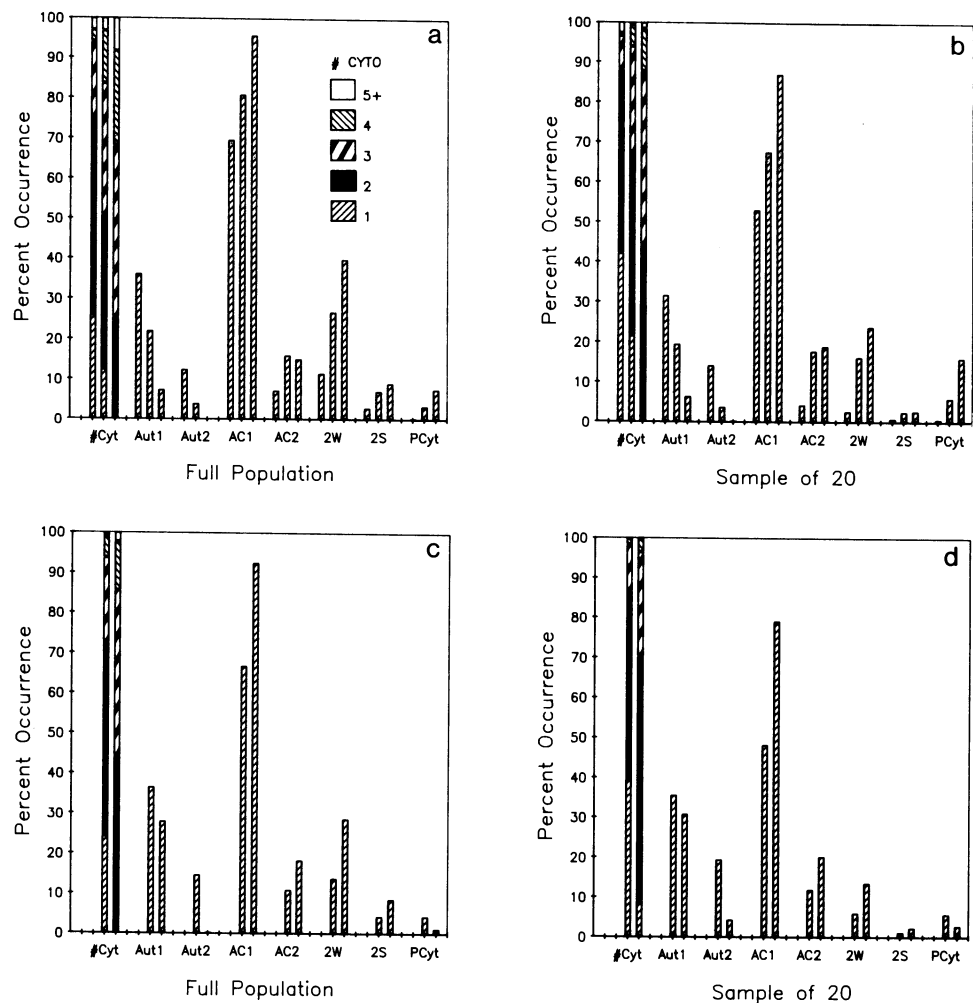
FIG. 7.—The number of cytotypes and the genetic systems detectable by crossing experiments (explained in the text). *a* and *b, f* = 25; *c* and *d, f* = 75.

the population. For example, in figure 6*a*, the actual occurrence of only a single cytotype in the population happens less than 5% of the time (bar 1*A*), whereas for the same set of runs one would infer, by crossing experiments on the entire population, a single cytotype 13% of the time (fig. 7*a*, middle bar over #Cyt).

The left pair of graphs in figures 7 and 8 summarizes the information in the entire population. The right pair corresponds to the information in a sample of 20 individuals: a random selection of all the females, up to 10, plus a random selection of enough hermaphrodites to fill out the sample of 20. An example of the diminished information in the sample of 20 can be seen by comparing the middle bar over #Cyt in panels 7*a* and *b*. One would infer only a single cytotype 12% of the time when there is full information (fig. 7*a*), whereas with less information one
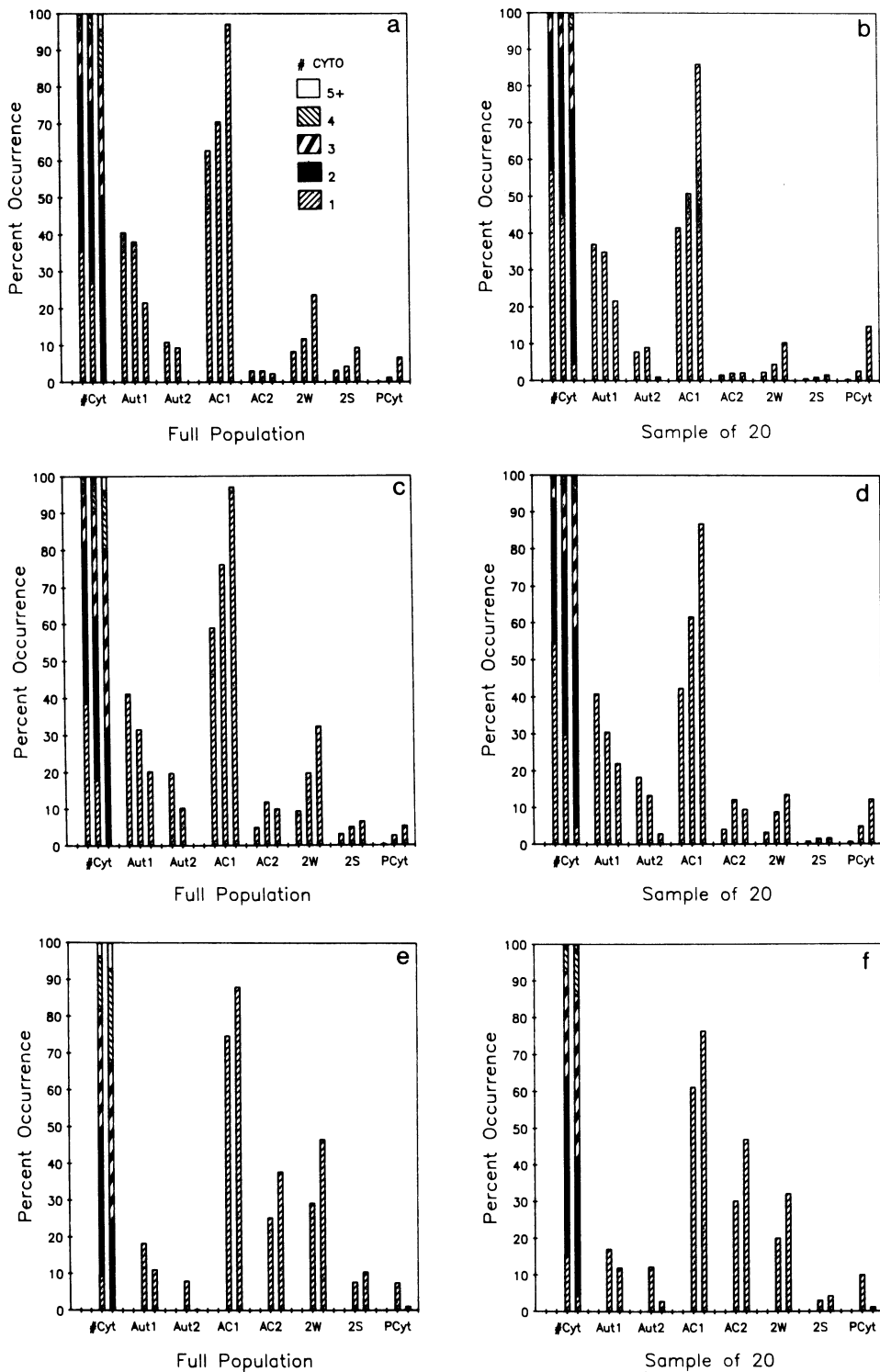
FIG. 8.—The number of cytotypes and the genetic systems detectable by crossing experiments (explained in the text). *a* and *b*, $p = 2$; *c* and *d*, $p = 4$; *e* and *f*, $p = 8$.

would infer a single cytotype 22% of the time (fig. 7b). When studying only a sample of the population, two factors determine the reduction in the number of detectable cytotypes: a loss in the number of cytotypes present in the sample, and a loss of autosomal polymorphism needed to identify each cytotype uniquely.

Ovule-fertility advantage, f, and autosomal-restorer pleiotropy, p, explain most of the variation in inferred genetics over the range of parameters studied. The top and bottom rows of figure 7 are for $f = 25$ and $f = 75$, respectively, and the top, middle, and bottom rows of figure 8 are for $p = 2$, $p = 4$, and $p = 8$, respectively. In the bottom rows of figures 7 and 8 only two bars are over each category, the left bar for all generations and the right bar for generations with 30%–50% females. Generations with 0%–2% females occur too rarely in these cases.

The categories Aut1 and Aut2 are cases in which one would infer that a particular allele at a particular autosomal locus or pair of loci, respectively, must be present in all cytoplasmic backgrounds for the plant to be a hermaphrodite. There is a slight increase in Aut2 with a loss of information; Aut1 and Aut2 decrease slightly when f increases; both Aut1 and Aut2 drop noticeably at $p = 8$; and both are much higher when the percentage of females is low. Overall, the most striking result is that, when the percentage of females is low, one would infer Aut1 20%–40% of the time and Aut2 10%–20% of the time (figs. 7, 8).

AC1 and AC2 are interactions between autosomal and cytoplasmic polymorphisms. Either each single autosomal locus interacts with the cytoplasmic polymorphism in a unique way (AC1), or a pair of autosomal loci interacts epistatically with the cytoplasmic polymorphism (AC2). Necessary and sufficient conditions for AC2 are that the simultaneous presence of a pair of autosomal restorer alleles at different polymorphic loci is required to repress the male-sterility effect of a cytotype and that in the presence of all other cytotypes, of which there must be at least one, the phenotype is not affected by either restorer allele at these two autosomal loci. AC1 decreases with a loss of information, and AC2 increases with increasing pleiotropy. Both increase sharply with an increasing frequency of females.

The categories 2W and 2S are the detection of two distinct cytoplasmic loci affecting male sterility. Necessary and sufficient conditions for weak evidence for two cytoplasmic loci, 2W, are the existence of three cytotypes and two polymorphic autosomal loci such that (1) both loci have an effect in the presence of one cytotype, (2) only one has an effect in the presence of a second cytotype, (3) neither has an effect in the presence of a third cytotype, and (4) a one-to-one relationship is assumed between separable cytoplasmic effects and physically distinct factors in the cytoplasm. For strong evidence, 2S, the third condition is that, of the two autosomal loci specified, only the alternative autosomal locus from that in condition 2 has an effect in the presence of a third cytotype. Alternatively, one may drop condition 4 and, instead of attempting to infer two distinct factors (loci) within non-recombining cytotypes, search for autosomal loci that affect more than one cytotype. The categories 2W and 2S are then cases in which at least one (2W) or two (2S) autosomal loci have an effect on different cytotypes but not on all cytotypes present.

Loss of information causes a sharp decline in the detection of 2W and 2S in all cases. Increasing f causes a moderate decline for both weak and strong evidence, and increasing p causes a sharp rise in 2W but has little effect on 2S. Both increase sharply with an increasing frequency of females.

PCyt is the inference of purely cytoplasmic control of polymorphism in male sterility without any interaction with autosomal loci. Loss of information causes an increase in PCyt. An increasing frequency of females causes an increase in PCyt (this is not obvious from the figures).

Experiment 7: Varying the Genetic System

The numbers of cytoplasmic loci and alleles per locus vary in this experiment, while maintaining a gene-for-gene interaction between male-sterility alleles in the cytoplasm and restorer alleles at distinct autosomal loci. The previous experiments assume two cytoplasmic loci, each with three male-sterile alleles and six associated autosomal loci. Here, the genetic system ranges from one cytoplasmic locus, with one to six male-sterile alleles, to two cytoplasmic loci, with one to three sterility alleles at each locus. At each cytoplasmic locus there is also a male-fertility allele. In each of the nine cases, the number of autosomal loci equals the total number of cytoplasmic male-sterility alleles. For example, there are four autosomal loci for one cytoplasmic locus with four sterility alleles or for two cytoplasmic loci with two sterility alleles each. Parameter values are the center point of the space for experiment 1: inbreeding, $s = 10$; inbreeding depression, $d = 5$; ovule-fertility advantage for females, $f = 50$; with pleiotropy over the range $p = 2, 4, 8$. Data are reported for four replicates of each of the nine genetic systems by three pleiotropic levels.

Table 4 summarizes a selection of the results. For each genetic system, described by cytoplasmic loci (CytLoci) and sterility alleles per cytoplasmic locus (CytAll), the table shows the mean over the four replicates of the percentiles of the distribution of females per run. For example, with one cytoplasmic locus and one sterility allele $(1, 1)$ or with two loci and one sterility allele per locus $(2, 1)$, the cytoplasm becomes fixed for the male-sterility allele at all loci; and, consequently, all autosomal restorer loci approach fixation for the restorer allele. For $1, 1$ and $2, 1$ at $p = 4$, during 95% of the generations there are less than 1.4% and 2.6% females in the population. The variations in the percentage of females for these two genetic systems represent stochastic perturbations about the autosomal equilibrium, which is near zero.

A second notable pattern appears when comparing $1, 4$ and $2, 2$, each with four male-sterile alleles, and $1, 6$ and $2, 3$, each with six alleles. At the higher percentiles and for all levels of pleiotropy, $1, 4$ has more females than $2, 2$, and $1, 6$ has more females than $2, 3$. For a given number of alleles, spreading cytotypes with absent restorer alleles occur more frequently with a single cytoplasmic locus than with two loci. The number of cytoplasmic loci has an effect because each cytotype with two factors maintains restorer alleles at two autosomal loci, whereas a cytotype with only one factor maintains alleles at only one autosomal locus. Since the rate of spreading cytotypes depends on the frequency at which autosomal

TABLE 4

MEAN PERCENTILES OF THE PERCENTAGES OF FEMALES FOR DIFFERENT GENETIC SYSTEMS

GENETIC SYSTEM	PERCENTILE					
	5	25	50	75	90	95
PLEIOTROPY = 2						
1,1	0.0	0.0	0.0	0.0	0.5	1.2
1,2	0.0	0.0	0.0	0.5	1.7	3.0
1,4	0.0	0.0	0.1	1.0	4.5	11.3
1,6	0.0	0.0	0.7	3.9	18.6	49.4
2,1	0.0	0.0	0.0	0.3	0.9	1.7
2,2	0.0	0.0	0.0	0.9	3.0	5.0
2,3	0.0	0.1	1.0	2.9	8.2	21.8
PLEIOTROPY = 4						
1,1	0.0	0.0	0.0	0.1	0.6	1.4
1,2	0.0	0.0	0.2	1.4	3.1	4.9
1,4	0.0	0.5	1.8	4.8	18.6	57.0
1,6	0.0	0.7	2.8	8.1	35.8	73.0
2,1	0.0	0.0	0.1	0.6	1.7	2.6
2,2	0.0	0.3	1.5	3.7	8.9	32.4
2,3	0.1	1.1	3.2	8.4	30.5	61.4
PLEIOTROPY = 8						
1,1	0.0	0.0	0.3	1.3	2.6	3.6
1,2	1.2	3.4	5.4	8.2	13.2	29.0
1,4	0.6	3.3	7.9	15.4	47.0	74.0
1,6	1.1	4.6	11.1	30.3	75.8	83.7
2,1	0.0	1.0	2.2	3.9	5.5	6.6
2,2	9.0	12.6	15.8	20.9	37.7	39.9
2,3	5.5	14.2	22.1	36.4	69.6	81.4

NOTE.—Genetic system described in left column by CytLoci, CytAll.

restorer alleles are lost from the population (see below), sequential sets of cytotypes that maintain fewer restorer alleles are associated with the higher rates at which male-sterile cytotypes sweep through the population.

DISCUSSION

Phenotypic Dynamics

The switching of genetic control between the autosomes and the cytoplasm is the major causal factor, over the range of parameters studied, that underlies the evolutionary dynamics of the percentage of females in a population.

When a male-sterile cytotype is introduced into the population by migration or mutation and the associated autosomal restorer allele is absent, the cytotype spreads because of its ovule-fertility advantage, f, over hermaphrodites. The percentage of females first increases toward the equilibrium under cytoplasmic control and then fluctuates about this equilibrium value. The cytoplasmic equilibrium occurs at approximately the frequency of females for which the ovule fitnesses of females and hermaphrodites are equal. When the product of the selfing rate, s, and the inbreeding-depression parameter, d, is small, as in each experi-

ment except experiment 5 (table 2), ovule fitnesses of females and hermaphrodites are nearly equal when (table 3)

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

where C and x are parameters describing pollination efficiency (table 1). Letting $z = 1 - m$, where m is the frequency of hermaphrodites and z is the frequency of females, the equilibrium frequency of females under cytoplasmic control is approximately (for a similar model, see Lloyd 1974, 1975)

$$z^*_{cyt} \approx 1 - [s(1 - d)/C(f + s)]^{1/x}, \qquad (1)$$

where as x approaches zero, pollen is not limiting for outcrossed ovules, and the frequency of females z approaches one if there is any ovule-fertility advantage for females ($f > 0$; Lewis 1941). In all the experiments reported above, $C = 1$ and $x = 1$, which means that pollen is not limiting when the frequency of females is zero, and the probability of successful outcrossed fertilization declines linearly as the frequency of hermaphroditic (male-fertile) plants declines. In experiment 1, when the pleiotropic disadvantage of a restorer allele to pollen fertility is $p = 8$, such that the population is often moving toward the cytoplasmic optimum (see the next paragraph), the prediction of equation (1) lies between the 90th and 100th percentiles of the observed distribution of the frequency of females over 2000 generations in 25 of 27 runs; and in the two other cases, the 85th and 100th percentiles bound the prediction.

When an autosomal restorer allele for a cytotype is introduced by migration or mutation and all other cytotypes present have associated autosomal restorers, then the frequency of females moves toward the autosomal equilibrium and eventually fluctuates about this equilibrium. An approximation of this equilibrium can be obtained as the pleiotropy, p, approaches zero. By the same reasoning as for the cytoplasmic case—the equilibration of fitnesses for the two phenotypes (Slatkin 1978)—when $p = 0$, the autosomal equilibrium frequency of females, z^*_{aut}, is given by

$$2Cm^x(f + s) - Cm^{x-1}(1 + f) - 2s(1 - d) = 0,$$

where $m = 1 - z$. When the rate of change in pollination efficiency with changing frequency of females, x, is set to zero, and $C = 1$, the result for z^*_{aut} agrees with the model of B. Charlesworth and Charlesworth (1978). When $C = 1$ and $x = 1$, as in all simulations presented here,

$$z^*_{aut} = (f - 1 + 2sd)/2(f + s). \qquad (2)$$

For all $f < 1 - 2sd$, z^*_{aut} is zero. This is the same condition as when pollen is not limited ($C = 1$, $x = 0$), which is expected since, when $C = 1$ and z approaches zero, pollen is not limited for either $x = 0$ or $x = 1$. This condition on f is always satisfied in the first four experiments, and the observed frequency of females is very near zero when restorers are present for all cytotypes. In experiment 5, the condition is not always satisfied, and for higher values of s and d, the percentage of females under autosomal control fluctuates widely over time about the observed median of the distribution shown in figure 5. The observable medians

match fairly well the equilibrium prediction for autosomal control in equation (2) when s and d are large; and since a low level of pleiotropy ($p = 2$) is used in these runs, the autosomes are most often in control of the phenotypic frequency. The wide fluctuations about the median are consistent with D. Charlesworth's (1981) observations on the easily perturbed dynamics of this region when the autosomal and cytoplasmic genes interact. The fit for small s is not as good because fluctuations near zero lead to a median greater than zero. Since it seems likely that $f > 1 - 2sd$ only rarely in natural populations, further analysis of these dynamics seems unwarranted at present. This condition on f may not be necessary for gynodioecy because several gynodioecious species are self-incompatible ($s = 0$, requiring an unrealistically strong condition on f under the purely autosomal-control model), most notably *Plantago lanceolata*, one of the best-studied cases of cytoplasmic male sterility in wild populations (van Damme 1983, 1984, 1986).

The phenotypic dynamics are driven by an interaction between changing phenotypic and genotypic frequencies. When a cytoplasmic allele is absent, the associated autosomal restorer tends to be lost because of its negative pleiotropic effect on pollen fertility, p, at a rate depending on the magnitude of p. When the autosomal restorer allele is lost, the associated cytoplasmic allele may invade and spread, and the waiting time depends on the migration and mutation rates. When this cytotype arrives and begins to spread, increasing the frequency of females, it drives out other alleles at both cytoplasmic loci since these loci are inherited as a non-recombining unit. The increase in females is coupled with the increase in frequency of a new cytotype and the decline of the other cytotypes. The frequency of females increases toward the cytoplasmic equilibrium until the associated autosomal restorer allele is reintroduced locally by migration or mutation, and then the local frequency of females again declines toward zero. After the spread of a cytotype and the loss of some of the other cytotypes, the slow process by which autosomal restorer alleles are lost begins again, which eventually opens up the possibility for the local reintroduction of a cytotype and its spread.

The dynamics are therefore driven by the mechanisms that generate local novelty: the consistent loss in the local breeding group of male-sterile cytotypes and autosomal restorer alleles and their subsequent reintroduction. This general pattern of loss and reintroduction has also been stressed by Gouyon and Couvet (1985).

If this view of the dynamics is accepted, what are the expected observations? The results of the simulation models studied here are all presented as distributions over time of the percentage of females in a single interbreeding population. Therefore, given a particular set of assumptions and parameter values, we ask what the probability is that we will observe at least a given percentage of females at one time. For example, in figure 3a, when females have only an 8% ovule-fertility advantage (f) over hermaphrodites, negative pleiotropy (p) of autosomal restorers on pollen fertility is 3%–4%, the selfing rate (s) is low, and there is no inbreeding depression (d) (table 2), then the observations suggest that at least 1% of the population will be females 75% of the time, at least 2% females 50% of the time, at least 7% females 25% of the time, and 20%–67% females 10% of the time. Fairly mild assumptions about fitness effects can therefore lead to observable levels of male sterility (see above for a review of the observations).

Geographically distant populations of the same species that do not exchange genes will evolve independently. The spatial distribution of the percentage of females among independent populations is the same as the temporal distribution within a single population, since each spatially distant population can be viewed as an independent sample from a temporal distribution. The simulation results therefore also provide predictions about the spatial distribution of females across widely scattered locations. In general, the observed percentage of females within a particular species appears to vary considerably over space (see the review of observations, above), but empirical information is not sufficient to allow a comparison between predictions and observations.

A second type of prediction concerns the rapidity of evolutionary change over observable time scales. The time-series depictions in figures 1*a,c* show that, with only mild negative pleiotropy of autosomal restorer alleles, a population with a low percentage of females might not be expected to change much over short periods of time, but when the frequency of females rises above 5% or 10%, changes over relatively short time periods are expected. Krohne et al. (1980) found that in eight populations for which they had data on the percentage of females in successive years, the five populations with more than 13.5% females in the first year had all dropped to less than 9% in the following year, whereas the frequency of females increased in two and decreased in one of the other three populations.

Genetic Control and Polymorphism

Relating the model to empirical evidence.—The simulation model presented here is based on a single set of genetic assumptions, including the separate one-to-one interaction between cytoplasmic alleles and autosomal loci, the potential for a large number (16) of different cytotypes with two loci per cytotype, and many (729) autosomal genotypes. By contrast with the single type of genetic control of male sterility built into the model, many different types of genetic control have been claimed on the basis of genetic crossing experiments (see the review under "Background," above): (1) a *small* number of different cytotypes interacting with autosomal loci, with one, two, or more autosomal loci specific for each cytotype, and no clear evidence for two distinct cytoplasmic loci; (2) autosomal genes that affect male sterility without interacting with cytoplasmic genes (references in Lloyd 1974); (3) cytoplasmic genes that affect male sterility without interacting with autosomal genes (references in Lewis 1941); and (4) subsets of the preceding three categories occurring simultaneously in the same population (e.g., 1 and 2 in *Plantago lanceolata*; van Damme 1983).

There is an apparent difference between the assumed genetics of the model, on the one hand, and the genetics inferred from crosses in natural populations and agricultural varieties, on the other. Much of this difference can potentially be explained by contrasting the mechanisms that underlie male sterility and restoration (in other words, the genetic interactions in the sense of a long-term evolutionary potential) versus the genetic control and extent of polymorphism that can be inferred at any single point in time by performing crosses between individuals from a sample of the population. The purpose of experiment 6 is to demonstrate the difference between these two interpretations of genetic control and the extent

of polymorphism. This experiment reaches six main conclusions. (1) In any particular generation, the number of cytotypes is small and the autosomal polymorphism is limited in the simulated population (fig. 6). (2) The number of cytotypes detectable by crossing experiments is smaller (most often 1–3) than the actual number present, and the number detected declines as the information in the sample declines (figs. 7, 8). (3) It is difficult to infer either that a single autosomal locus has an effect on more than one but not all cytotypes or, with additional assumptions, that there are two separate cytoplasmic loci (2W and 2S in figs. 7, 8; for assumptions, see the Results for experiment 6). (4) Quite frequently one would infer purely autosomal control (Aut1 and Aut2 in figs. 7, 8) or infer autosomal-cytoplasmic interactions with two autosomal loci required to restore male fertility when a particular cytotype is present (AC2). (5) Occasionally there is an inference of purely cytoplasmic control (PCyt), and the frequency of this inference increases when the amount of information in the sample and subsequent crossing experiment declines. (6) Occasionally one would infer both purely autosomal control and autosomal-cytoplasmic interactions occurring simultaneously in the same population.

Relating the model to previous theory.—Two points are relevant here: the conservative nature of the simulation assumptions and parameters, which generally tend to favor hermaphrodites over females; and the conditions for the maintenance of polymorphism.

If there were no negative pleiotropic fitness effects of autosomal restorer alleles or cytotypes, then the frequencies of the restorer alleles would be driven to fixation by selection, and cytoplasmic male sterility would be extremely rare (D. Charlesworth and Ganders 1979; D. Charlesworth 1981; Delannay et al. 1981). In the simulation model, I assumed that a restorer allele in a hermaphrodite caused a reduction in the probability that the plant would have successfully outcrossing pollen grains, that is, a reduction in pollen fertility. This pleiotropic effect on pollen fertility is assumed to be independent of cytotype. Other sorts of pleiotropic effects are possible but were not included in the model. The restorer alleles might have an effect on ovule fitness, and cytotypes might cause a reduction in ovule or pollen fertility relative to other autosomal-cytoplasmic genotypic combinations. Because restorer-allele pleiotropy acting only on pollen fertilities has no effect in females, these restorer alleles are lost more slowly than would be restorers with pleiotropic effects on ovule fitness or both ovule and pollen fitness. Slower loss of restorer alleles leads to an increased frequency of hermaphrodites. Negative pleiotropic effects of cytotypes increase their rate of loss when they are restored to pollen fertility by autosomal alleles and are therefore not spreading through the population. An increased rate of loss of cytotypes would lead to an increased rate of loss of restorer alleles, which would lead to a higher frequency of females. Since cytoplasmic pleiotropy was not included in the simulation model, the model favors a higher frequency of hermaphrodites relative to most other plausible sets of assumptions about pleiotropy.

Previous models examining the effects of autosomal and cytoplasmic pleiotropy have assumed dominant or recessive rather than additive (intermediate) gene action and an effect on ovule rather than pollen fitness (D. Charlesworth 1981; Delannay et al. 1981; Gouyon and Couvet 1985) or an effect on both ovule and

pollen fitness (Gregorius and Ross 1984; Ross and Gregorius 1985). Ross and Gregorius (1985) showed that, under the range of assumptions for my simulation models, a male-sterile cytotype would become fixed when competing with a cytotype that cannot cause male sterility. From previous models it is easy to see that, with the low levels of pleiotropy assumed in most of the simulations ($p \leq 4$), restorer alleles associated with a frequent cytotype would also move rapidly toward fixation. Evolution at single autosomal-cytoplasmic pairs embedded within the simulations confirms this tendency for monomorphic hermaphroditism by fixation of restorers when only a single cytotype is present (see also experiment 7).

Population Subdivision

Population subdivision is undoubtedly an important aspect of cytoplasmic male sterility (Gouyon and Couvet 1985; van Damme 1986) because the generation of genetic novelty by migration or mutation, and its subsequent loss by selection and drift, drives the evolutionary dynamics. The simulation model presented here does not consider the concerted evolution of neighborhoods with occasional migration, but the combined migration-mutation rate that I use for the single interbreeding population is an adjustable parameter and therefore can suggest possible trends associated with given immigration rates from genetically different groups.

The role of immigration rates is examined in experiment 4 (table 2). Because the rates of gene flow through pollen and ovules are likely to differ, each rate is described by a separate parameter. As the cytoplasmic rate (μ_c) declines, the time between episodes of spreading male-sterile cytotypes increases. The probability of observing a percentage of females above a given level will decrease, and the variation in the distribution of the percentage of females will increase, since the force driving the dynamics becomes an increasingly rare event (fig. 4). As the autosomal rate (μ_a) declines, the time from the introduction of a cytotype and the spread of females until the introduction of the associated autosomal restorer increases. This increases both the variation in the percentage of females and the probability of observing a high percentage, since the restoration of pollen fertility when there is a spreading cytotype depends on an increasingly rare event (fig. 4). There is also an important interaction among the cytoplasmic and autosomal migration and mutation rates and the rate at which autosomal restorer alleles are lost. If the waiting time until the loss of autosomal alleles is long (p is small) relative to the introduction of associated cytotypes, then changing cytoplasmic migration and mutation rates will not have much influence on the pattern of dynamics.

SUMMARY

Cytoplasmic male sterility (CMS) is the maternal transmission of failed pollen production in hermaphroditic plants leading to a mixture of male-sterile and hermaphroditic individuals in the population (gynodioecy). Autosomal genes that can restore pollen fertility in the presence of male-sterile cytotypes are commonly observed. CMS in wild populations tends to be associated with (1) the mainte-

nance of distinct cytotypes, each capable of causing male sterility by an apparently different mechanism since each is susceptible to only a particular subset of autosomal restorer alleles; (2) the maintenance of polymorphism at several autosomal restorer loci, with particular alleles or loci specialized for restoring pollen fertility when associated with particular cytotypes; (3) the maintenance of genetic differentiation among geographically distant populations; and (4) the maintenance of phenotypic diversity among populations, measured as the percentage of male-sterile individuals.

Observations and previous theoretical explanations were reviewed. A simulation model was then constructed, and the results of the simulations appear to be consistent with the evolutionary dynamics and patterns of genetic polymorphism inferred from wild populations.

In the simulation models, when cytoplasmic male-sterility alleles are present and their associated autosomal pollen-restorer alleles are absent (cytoplasmic control), the frequency of females increases because of the ovule-fitness advantage usually associated with male sterility. When autosomal restorer alleles are present (autosomal control), the frequency of females tends to decline. The opposite directions of evolution favored by the cytoplasm and autosomes reflect the inherent conflict of interest between genomic subsets over the sex-allocation ratio. The evolutionary dynamics depend on an interaction between the phenotypic and genotypic frequencies and on the continual loss and reintroduction of genetic novelty over evolutionary time.

A striking difference was observed between the potential genetic control of male sterility built into the simulation model, which reflects assumptions about the underlying physiological mechanisms of normal and aberrant pollen production, and the types of genetic control that would be inferred by performing classical genetic crossing experiments on a sample of the simulated population. This contrast between potential and inferred control is possibly an important general attribute of traits that are the resolution of a continual evolutionary conflict.

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