Female Fertility and Mating Type Effects on Effective Population Size and Evolution in Filamentous Fungi

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ABSTRACT

The idealized individual in many fungal species is a haploid self-sterile hermaphrodite that may be propagated by asexually produced spores or that may reproduce sexually. In field populations, polymorphism occurs for female-sterile/hermaphrodite status, and female-sterile mutants, which function only as males during sexual reproduction, may comprise >50% of the population. The effective population number may be based on the number of strains of different mating type or the relative frequency of hermaphrodites. The female-sterile mutants are at a selective disadvantage every time sexual reproduction occurs, and must have an advantage during vegetative propagation to persist at a significant frequency. When a high frequency of female-sterile strains is observed in field populations, it indicates that vegetative propagation is a significant component of the fungus' natural history. Depending on the mutation rate to female sterility and the selective advantage of the female-sterile strains during vegetative propagation, the ratio of sexual:asexual generations can range from 1:15 to 1:2300 for species in the *Gibberella fujikuroi* complex. The relative rarity of sexual reproduction may permit female-sterile strains to accumulate to a level such that local populations could completely lose sexuality and appear as asexual (imperfect) species.

FILAMENTOUS ascomycete fungi are usually found as haploid mycelia that propagate vegetatively via conidial spores and hyphal elongation. In some fungi, termed imperfect fungi, the Fungi Imperfecti or the Deuteromycetes, sexual reproduction is unknown or absent and all propagation is asexual. Recent studies (LOBUGLIO *et al.* 1993; GEISER *et al.* 1995; BURT *et al.* 1996) have indicated that many imperfect fungi have as their closest evolutionary relative a perfect species, *i.e.*, a species with a known sexual stage. This linkage between perfect and imperfect species has caused a rethinking of some basic taxonomic concepts within the fungi. Conditions that could lead to a transition from a perfect to an imperfect life cycle have not been extensively described but could provide novel insights into how these microorganisms evolve.

Within the ascomycetes, sexually reproducing species usually follow one of three basic sexual reproductive strategies—homothallic, pseudohomothallic (also termed secondary homothallic), and haploid heterothallic—with each species limited to a single reproductive strategy (FINCHAM *et al.* 1979; NELSON 1996). Individual strains of homothallic fungi and individual haploid, but heterokaryotic, strains of pseudohomothallic fungi may be self-fertile or may cross with other individual strains of their species to complete the sexual portion of the life cycle. In this communication, we focus on the heterothallic filamentous ascomycetes, which require contributions from two genetically distinct parents for a successful sexual cross. Crosses in these fungi are governed by the mating type locus and by the need for one of the two parents to provide female reproductive functions. Although our focus is on filamentous heterothallic ascomycetes, the arguments we develop can also be applied to a number of other haploid systems.

In heterothallic species, the parents of a cross must be of different, usually termed opposite, mating type. Mating type in these fungi is controlled by a single locus with two alleles termed variously "*A*"/"*a*", "*a*"/"*α*", "+"/"−", or "*mat1-1*"/"*mat1-2*" depending on the organism (YODER *et al.* 1986; NELSON 1996). All of these loci appear to function as regulators of complex genetic pathways containing numerous genes, many of which are poorly characterized. For population purposes, mating type in filamentous ascomycetes can be considered to be determined by one locus with two functional alleles.

In addition to mating type, the male/female/hermaphroditic nature of the mating participants adds a second layer of complexity to the mating interaction. An idealized member of a heterothallic population of filamentous ascomycetes is a self-sterile hermaphrodite that is capable of producing male gametes and elaborating the female reproductive structure. When the female structure is fertilized by a male gamete, then the structure matures to produce ascospores that are derived

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from the meiotic products of a diploid, biparental nucleus. The parent from which the male gamete originated is termed the male parent while the parental mycelium upon which the female reproductive structure develops is termed the female parent. The male gamete may be an asexually produced spore, a sexually produced spore, or a mycelial fragment. The female structure is usually much more elaborate, requiring a high degree of cellular specialization and the development of a highly organized structure.

The female reproductive structure usually is limited in function to sexual reproduction while the male gamete is usually not so limited. In theory, male or female functions can be lost independently to give strains that are male only, female only, or hermaphrodites in an equilibrium that is dependent upon population history and environmental stability (NAUTA and HOEKSTRA 1992). In strains of *Gibberella fujikuroi* that we have observed (LESLIE 1995), female fertility is often lost and populations are composed of hermaphrodites and female-sterile (FS) strains, although occasional male-sterile, female-fertile strains can be found. The multiple types and roles of the male gametes are probably an important factor in explaining these data, especially since these propagules may function both in vegetative propagation and sexual reproduction.

Our objectives are to assess the effects of mating type and male/female/ hermaphroditism on inbreeding and variance effective population numbers in filamentous ascomycetes, to develop theory to assess the role that the loss of female fertility plays in shifting species from sexual to asexual reproductive modes, and to use this theory to evaluate data from different biological species of the *G. fujikuroi* species complex (LESLIE 1991, 1995). A preliminary report of some of this information has been made (LESLIE and KLEIN 1995). The remainder of this manuscript is organized into three large sections on theory, data and discussion. The theory section presents background on fungi (for nonmycologists) and contains the derivation of the equations that can be used to evaluate experimental data. The experimental data are from field populations of two filamentous ascomycetes, *G. fujikuroi* and Neurospora spp., and are analyzed as examples using the theory developed in the preceding section. The discussion section includes material of a more general nature on fungal evolution.

THEORETICAL CONSIDERATIONS

The evolution of fungal populations presents problems not found in bacteria, plants and animals but reminiscent of all. Fungi, and in particular most ascomycetes, are usually vegetatively haploid and can propagate vegetatively or reproduce sexually. Thus, the nature of the genetic changes that drive evolution in fungi is governed by both the asexual clonal selection processes prevalent in bacteria (MAYNARD-SMITH *et al.* 1993) and the sexual genetic exchange mechanisms of higher eukaryotes. When sexual reproduction does occur, it is possible for a single self-sterile individual to act as a male, a female or both. The haploid vegetative nature of many fungi restricts the genetic variants that can be maintained in the population, *e.g.*, no heterozygotes, and no recessive lethals except for loci that function only during sexual reproduction when dikaryosis and diploidy occur briefly (LESLIE and RAJU 1985). This unusual combination of biological constraints has probably been a factor in limiting the theoretical treatment given to the fungi.

Another difficulty with fungal populations is determining the number of individuals that they contain. Fungal individuals may be extremely large, *e.g.*, the Armillaria clones found in some forests (SMITH *et al.* 1992), or compact, *e.g.*, the multiple individuals of *G. fujikuroi* that can be recovered from a single maize stalk (KEDERA *et al.* 1994). With animals the definition of an individual is relatively straightforward: a single individual is a distinct physical entity. This definition blurs when considering plants that reproduce clonally by budding, tillering, etc., but if clonal members are considered as a unit, as with the idea of stands of a certain plant, then the concept usually still holds. The problem is more acute with fungi that have asexually produced propagules, *e.g.*, conidial spores, that can be dispersed in the same manner as the sexually produced propagules, *e.g.*, ascospores. In plants, the term genet is defined as the members of a clone, *i.e.*, a genet is composed of all of the spatially discrete entities that originated from the same meiotic product. A ramet is defined as a spatially discrete entity and may be the same as a genet or a subclone of a genet. Thus, the number of ramets must equal, and usually will exceed, the number of genets within a population. These terms have also been applied to fungi (RAYNER 1990). The inbreeding effective number is usually associated with the number of ramets, since each ramet is a possible parent for the next generation. The variance effective number is usually associated with the number of genets, since the genets represent the number and genetic diversity of the progeny that resulted from the previous round of sexual reproduction. To simplify our presentation, we will use "individual" to mean ramet and "clone" to mean genet.

Assumptions: Fungal life cycles have many different variations on a common theme. The life cycle we are modeling contains a number of assumptions that we state here:

- Discrete generations are used to simplify the analysis. In the event of overlapping generations, the long-term results would be the same.
- Sexual reproduction does not occur at the same time as vegetative propagation, although asexually produced spores that function as male gametes are syn-

thesized during both vegetative propagation and sexual reproduction. This assumption permits a set of environmental conditions and/or genotypes to be favored for one of the two reproductive modes but not the other. Under defined laboratory conditions, vegetative propagation is commonly favored by conditions of relative abundance, while sexual reproduction is often induced by starvation for an essential nutrient, frequently fixed nitrogen, and repressed under conditions that favor vegetative propagation (GRIFFIN 1994). Similar responses are expected for fungi in the field.

- An asexual generation is the mean time to the establishment of a new fungal individual (ramet) by asexually produced spores or other asexual means. At equilibrium, when the available space is fully occupied, the generation time is the mean time to the replacement of one individual by another. An individual is defined by the median amount of vegetative propagation of the colonies in the population such that a colony with twice the median would count as two individuals. An asexual generation time is analogous to a doubling time and *in extremis* the generation time would equal the cell doubling rate.
- The only sexual phenotypes present are hermaphrodites and FS strains. Few male-sterile mutants are known in well-studied model fungi, whereas many FS mutants are known. Male-sterile/female-fertile strains have not been detected in the populations we have sampled (LESLIE 1995). We expect such strains, when they do occur, to be at severe disadvantage since they have greatly reduced fitness during vegetative propagation.
- Spatially discrete individuals, whether hermaphrodites or FS strains, may vary in size, but the distribution of sizes is random with respect to genotype. FS strains may have a greater tendency toward vegetative propagation than hermaphrodites, and therefore a larger average size, but within each of the two reproductive types we assume a random distribution of differences in vegetative propagation.
- Population size is relatively constant. We assume that the ecological niche can accommodate a certain number of fungal individuals, and that over time, this number is relatively constant. This assumption is not meant to preclude fluctuations in population size on an annual basis.
- Female fertility. For simplicity we have assumed that all female fertile strains are equally fertile. In practice, however, the degree of female fertility can vary widely. Further development of our theory may require the consideration of a quantitative (additive) component to the basis for FS. Such data are difficult to obtain, however, and we do not anticipate any major changes in the trends described here when such a component is introduced.

Sexual reproduction: In the production of sexual progeny, hermaphrodites and FS strains do not contribute equally to the next generation; the hermaphrodites always contribute more. If both FS strains and hermaphrodites contribute male gametes with equal efficiency, then the hermaphrodites will contribute all of the female gametes, and a fraction of the male gametes (h) to the next generation. The FS strains contribute only a fraction of the male gametes ($1 - h$), where h and $1 - h$ are the frequencies of the two strain types in the population. Consequently, any gene that causes FS should be selected against every time sexual reproduction occurs, since a strain carrying a FS allele can contribute only to the male gamete pool. Let h_0 equal the fraction of the genetic material in the population from the hermaphroditic strains and let h_1 equal the fraction of the genetic material in the succeeding generation that was derived from the hermaphrodites. Then the fraction of genetic material contributed by the hermaphrodites to the next generation is,

$$h_1 = \frac{(1 + h_0)}{2}. \qquad (1)$$

If the contribution of the hermaphrodites to the pool of male gametes is less efficient than the FS strains (a lower "maleness"), then the rate at which genetic material from the FS strains is lost is reduced, but the net result is the same: sexual reproduction favors the hermaphrodites. If m is the relative maleness of the hermaphrodites to the FS strains, then Equation 1 becomes,

$$h_1 = \frac{(1 + mh_0)}{2.} \qquad (2)$$

When $m = 1$, *i.e.*, FS strains and hermaphrodites have the same relative maleness, then Equation 2 simplifies to Equation 1. At the other limit, when $m = 0$, *i.e.*, the hermaphrodites have lost the ability to produce male gametes, then the population is composed of *de facto* males and females, and the frequency of both classes will tend toward one-half. For all intermediate cases ($0 < m < 1$), there is net loss of genetic material from the FS strains during sexual reproduction.

In terms of the relative contributions by the FS strains and the hermaphrodites to the next generation, the genetic basis of FS is irrelevant; however, in terms of the total number of progeny and the relative frequency of FS strains and hermaphrodites within the progeny the genetic basis of the FS is of critical importance. If there is only a single FS gene segregating, then h and $1 - h$ are the frequencies of the female-fertile and FS alleles, respectively, and $1 - h$ is reduced to half of its previous value in each round of sexual reproduction. FS is a phenotype that can result from mutations at far more than one locus, however, and under this scenario, the frequency of any individual mutant could be rela-

tively low while the frequency of FS strains remained relatively high. The accumulation of mutations during vegetative propagation should result in some FS strains having mutations that block female fertility at more than one genetic locus. If we assume a random distribution for the number of mutations per individual, then the frequency of strains with any given number of mutations is given by the Poisson distribution, $e^{-M}(M^i/i!)$, where M = the mean number of mutations per strain, and i = the number of mutations in a given class (fs_i) of strains. The hermaphrodites are the only members of the class $i = 0$ (fs_0), and their frequency can be used to estimate the frequency of all of the other classes.

All matings in a sexual generation must have a hermaphrodite as one parent, so all crosses are of the form $fs_0 \times fs_i$ and crosses between two FS strains, *e.g.*, $fs_1 \times fs_1$, cannot occur. Thus, the relative frequency of mating for any given mutational class is given by its frequency in the overall population. The frequency of FS progeny resulting from each class of matings is not the same. Matings of class $fs_0 \times$ class fs_0 (two hermaphrodites) have no FS offspring, matings of class $fs_1 \times$ class fs_0 have 50% FS offspring, matings of class $fs_2 \times$ class fs_0 have 75% FS offspring (less if the loci are linked) and so on. Thus, all matings of class fs_2 and higher FS strains result in more FS strains than hermaphrodites among the progeny. These relationships can be summarized as:

$$h_a = \Sigma fs_i\left(\frac{1}{2}\right)^i, \quad \text{for } i = 0, 1, 2, \ldots, \qquad (3)$$

where h_a and $1 - h_a$ are the relative frequencies of hermaphrodites and FS strains after sexual reproduction and fs_i is the relative frequency of the class with i female sterility mutations before sexual reproduction. As fs_0 is the frequency of hermaphrodites before sexual reproduction, it is clear from Equation 3 that the relative frequency of hermaphrodites increases following each round of sexual reproduction. If the hermaphrodites are not as efficient in male functions as are their FS counterparts ($0 < m < 1$), however, then it is possible for the number of hermaphrodites to decrease following sexual reproduction. In essence the contribution of the fs_0 class in Equation 3 is reduced to $m \cdot fs_0$ and h_a is reduced accordingly. This difference in maleness between hermaphrodites and FS strains becomes less important as the proportion of hermaphrodites in the population decreases since the fs_0 contribution to the male gamete pool for the next generation is reduced under these conditions.

If m is sufficiently small, then h_h (the frequency of hermaphrodites before sexual reproduction) will be larger than h_a. The key relationship is that between fs_0 and fs_h (the proportion of hermaphrodites resulting from crosses between the FS strains and the hermaphrodites) where $fs_h = \Sigma fs_i\ (1/2)^i$, for $i = 1, 2, 3, \ldots$ Whenever $m \cdot fs_0 + fs_h > fs_0$ the number of hermaphrodites must increase following every sexual generation until the inequality is reversed. The value on the left of this relationship must be corrected by multiplying it by

$$\frac{1}{1 - 1 - mfs_0}$$

to reflect the relatively larger contributions made by each class to the next generation. When $m = 1$, then this correction factor is 1. If m is set at 0, the lower limit for male fertility of the hermaphrodite, then if $fs_h > fs_0 - fs_0^2$, the number of hermaphrodites must always increase. If the distribution of the FS mutations follow a Poisson distribution, then if $fs_0 < 0.39$, the number of hermaphrodites must always increase following sexual reproduction regardless of the value of m. This is easily seen by noting that when $m = 0$, the extreme case, the inequality reduces to $\Sigma(M^i/i!2^i) < 1 - e^{-M}$, where $e^{-M} = fs_0$, which can be easily solved for M since fs_0 is known. Greater than 0.39, the number of hermaphrodites in a succeeding sexual generation may increase or decrease depending on the values of fs_0 and m.

Asexual (vegetative) propagation: Spores resulting from sexual reproduction are often produced under one set of environmental conditions and spores for vegetative propagation under another. The ability to asexually produce dispersive spores to colonize substrate could be an important advantage in some contexts. Vegetative propagation is also important because the production of sexual structures, and hence offspring, is often directly related to the size of the organism. Clones, which consist of many individuals, are essentially a single "superorganism" with respect to selection of this kind. Conditions that favor vegetative propagation favor increased production of male gametes, even among hermaphrodites, since male gametes and asexually produced spores frequently are the same thing. In a population that contains FS strains at a level greater than predicted by the mutation rate to FS, a selective advantage for the FS strains during vegetative propagation is required to balance the disadvantage they suffer as male-only contributors to sexual reproduction. This reasoning implies that there is a "cost" to female reproduction by the hermaphrodites. This cost might be incurred, for example, if the FS strains use resources for propagation via asexually produced spores that the hermaphrodites must use for the elaboration of the female reproductive structures.

The relationship can be expressed by letting:

x_{fs} = asexual fitness of FS strains, *i.e.*, the relative vegetative propagation rate of the FS strains, and x_h = asexual fitness of hermaphrodites, *i.e.*, the relative vegetative propagation rate of the hermaphrodites.

Define a parameter: $\Theta = x_h/x_{fs}$, $0 \leq \Theta \leq 1$. For any $\Theta \neq 1$, the frequency of hermaphrodites will decrease in every asexual "generation". The extremes present interesting cases. When $\Theta = 1$, there is no asexual selec-

tive advantage for the FS strains and all of the strains should be hermaphrodites, due to the hermaphrodite's advantage in sexual reproduction. When $\Theta = 0$, the hermaphrodites are not propagated vegetatively and the FS strains will increase as long as sex is not required. If x_{fs} is always greater than x_h, then x_{fs} can be set equal to 1, and $\Theta = x_h$. Selection either for hermaphrodites during sexual reproduction or for FS strains during vegetative propagation can drive the population to an extreme (all hermaphrodites or all FS strains).

Mutation: If the FS strains do not have a selective advantage during vegetative propagation, it is still possible for a significant portion of the population to be FS as a result of mutation. For this to happen, mutation from hermaphrodite to FS must be relatively common (mutations at a large number of loci can result in the phenotype) or there must be a relatively large number of asexual generations between sexual generations, or some combination of both. The mutations are generated somatically, since somatic cells in these organisms all are potentially germ line cells. To have an effect, however, the mutation must be present in the cell(s) that differentiates to form the female reproductive structure. If the mutation rate from hermaphrodite to FS is μ, then the proportion of hermaphrodites remaining is $h \cdot (1 - \mu)^i$ where i is the number of asexual generations.

The probability of the occurrence of mutations conferring FS is a significant consideration. The number of loci involved in the development and differentiation of the female structures is undoubtedly large, resulting in a correspondingly high probability for the FS phenotype. Suppose that the mutation rate per locus is 10^{-5} and mutations at any of 10–1000 loci can confer the FS phenotype, then $10^{-4} \leq \mu \leq 10^{-2}$. Thus mutation can play a significant role in this system because of the relatively large number of loci that can be altered to give the FS phenotype.

Equilibrium considerations for mixed modes of reproduction: Selection either for hermaphrodites during sexual reproduction or for FS strains during vegetative propagation can drive the population close to an extreme (all hermaphrodites or all FS strains). If, however, the environment is such that alternating selection favoring first the hermaphrodites and then the FS strains occurs, then a sort of equilibrium should ensue. We conceive of this equilibrium as a cycle (Figure 1). Sexual reproduction increases the relative number of hermaphrodites (Equation 3), while the number of hermaphrodites declines during vegetative propagation due to mutation and the selective advantage of the FS strains. Hermaphrodite losses due to mutation or selection during the asexual portion of the life cycle need not be distinguished from one another since the two values are multiplied to give a cumulative loss of hermaphrodites per asexual generation. Thus, the variables (μ, Θ, and g = number of asexual generations per sexual generation) affecting this cycle are all primarily associated with the asexual portion of the cycle.

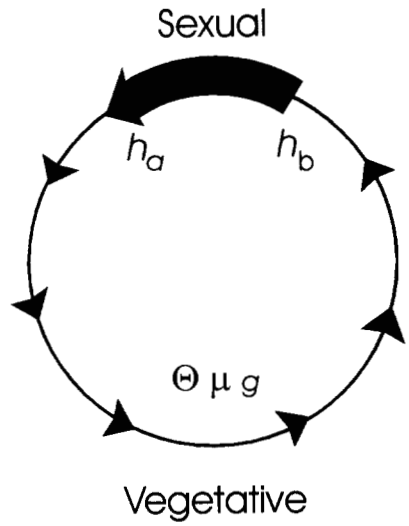

FIGURE 1.—Equilibrium cycle. A single sexual generation alternates with multiple asexual generations to affect the frequencies of hermaphrodites in a population with a mixed reproductive cycle. μ, mutation rate from female fertility to female sterility; Θ, selective disadvantage of hermaphrodites during vegetative propagation; g, number of generations of vegetative propagation per sexual generation; h_a, frequency of hermaphrodites immediately following the sexual generation; h_b, frequency of hermaphrodites immediately preceding the sexual generation.

$h = 1$ and $h = 0$ are limits for the hermaphrodite frequency, but the processes in the cycle only allow h to approach these limits, and an extrinsic factor, *e.g.*, drift, is required to actually reach them. $h = 1$ is not stable since hermaphrodites will always be lost due to mutation. Should h drift to 0, however, there will be a biologically stable equilibrium in the form of a vegetatively propagating population in which the possibility for sexual reproduction is lost. If a hermaphrodite were to migrate into this population, then sexual reproduction could commence again. A vegetatively propagating population also could evolve to become reproductively isolated from its progenitors, thereby giving rise to a new species.

An infinite number of equilibrium cycles are possible, each one representing a different set of reproductive and environmental factors. For a cycle to be stable over the long term, the loss of hermaphrodites during vegetative propagation must be balanced by their replacement during sexual reproduction. h_a is the highest value for hermaphrodite frequency during the cycle and h_b is the lowest. If h_b becomes sufficiently small, then it is possible for all of the hermaphrodites to be lost from the population by chance, rendering the population asexual. In general, the greater the frequency of hermaphrodites the more frequently (or recently) sexual recombination occurs. Within a species, μ and Θ may be assumed to be constants allowing the comparisons

of different populations for the relative frequency (g) with which sexual recombination has occurred.

Effective population number (N_e): The reduction in the frequency of hermaphrodites by either mutation or selection during vegetative propagation, may be further accelerated by genetic drift during sexual reproduction. To calculate the effects of drift, it is necessary to know the effective population number (CROW 1954; CABALLERO 1994). There are two commonly used effective population numbers (CROW 1954; CROW and DENNISTON 1988): the inbreeding effective population number [$N_{e(f)}$], which is based on the probability of identity due to common ancestry, and the variance effective population number [$N_{e(v)}$], which is based on the amount of allele frequency drift per generation as measured by its variance. Derivations of effective population number generally follow the arguments presented by CROW and KIMURA (1970), which are based on earlier work by WRIGHT (1931) and CROW (1954). $N_{e(f)}$ is a concept that applies to sexually reproducing populations and relies on parameters related to sexual reproduction. The effective population number is a critical parameter used to estimate the effects of drift and inbreeding, and to compare field populations to an idealized population.

For differences based on mating type (or for any case where there are two discrete sexes or mating types and selfing is not allowed) the population effective number can be calculated from the equation first derived by WRIGHT (1931):

$$N_e = \frac{(4N_mN_f)}{(N_m + N_f)}. \qquad (4)$$

For ascomycete populations the number of strains with one mating type allele is substituted for N_m and the number of strains with the other mating type allele is substituted for N_f.

Effective population numbers for the FS/hermaphrodite polymorphism require special derivation. For $N_{e(f)}$, the derivation is based on equations 7.6.2.17 and 7.6.2.18 from CROW and KIMURA (1970). For a constant haploid population, $\bar{k}$, the average number of sexual progeny per strain is one. The proportion of males in the population is one (since all strains can serve as males), so the average number of gametes contributed per male strain, $\bar{k}_m$, is 1/2. The hermaphrodites serve as the females, so the average number of gametes contributed per female strain, $\bar{k}_f$, is dependent upon the relative frequency of the hermaphrodites in the population, and $\bar{k}_f \cdot h = 1/2$, so $\bar{k}_f = N/2N_h$, where N is the total number of individuals in the population and N_h is the number of hermaphrodites in the population. If the number of gametes contributed per individual follows a binomial distribution, then:

$$N_{e(f)} = \frac{8N^2N_h}{(N + N_h)^2}, \text{ whenever } N \text{ is large.} \qquad (5)$$

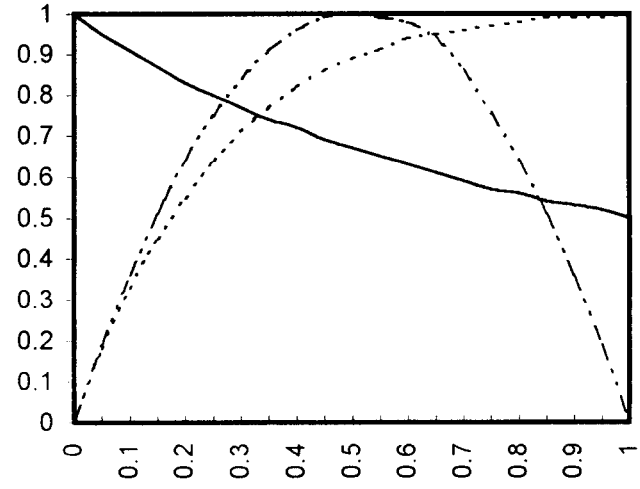

FIGURE 2.—N_e as a proportion of the population count. Inbreeding effective population number (Equation 6; $N_{e(f)}$; · · ·), variance effective population number (Equation 7; $N_{e(v)}$; ——), and mating type effective population number (Equation 4; - · · -). The y axis is the proportion of the count; the x axis is the proportion of hermaphrodites for the inbreeding and variance effective population numbers and is the proportion of strains of one mating type for the mating type effective population number.

Since the individuals are haploid, however, this number must be divided by two to account for the fewer number of genomes that are present in the population, so

$$N_{e(f)} = \frac{4N^2N_h}{(N + N_h)^2}, \text{ whenever } N \text{ is large.} \qquad (6)$$

At the limit of all FS strains, $N_{e(f)} = 0$, while if $N = N_h$, then $N_{e(f)} = N$. For a diploid population, $\bar{k} = 2$, $\bar{k}_m = 1$, $\bar{k}_f = N/N_h$, and $N_{e(f)}$ is the same as Equation 6.

The variance effective number, $N_{e(v)}$, derivation is based on equations 7.6.3.30 and 7.6.3.31 from CROW and KIMURA (1970). Again, the total number of individuals is used as the number of males, the number of hermaphrodites is used as the number of females, and the average number of offspring per individual is assumed to be binomially distributed. As before, $\bar{k} = 1$, $\bar{k}_m = 1/2$, $\bar{k}_f = N/2N_h$, when the population is haploid, and

$$N_{e(v)} = \frac{N^2}{(N + N_h)}. \qquad (7)$$

At the limit of all males ($N_h = 0$), $N_{e(v)} = N$, while at $N = N_h$, $N_{e(v)} = N/2$. For a diploid population, $\bar{k} = 2$, $\bar{k}_m = 1$, $\bar{k}_f = N/N_h$, and

$$N_{e(v)} = \frac{2N^2}{(N + N_h)}. \qquad (8)$$

At the limit of all males, $N_{e(v)} = 2N$, while at $N = N_h$, $N_{e(v)} = N$, as has been shown previously (CROW and KIMURA 1970).

The inbreeding and variance effective numbers be-

TABLE 1

Experimental data and effective population size parameters for three biological species in the *Gibberella fujikuroi* species complex

Biological species	Mating type[a]	$N_{fs}:N_h$	N_e			M
			M.T.[b]	$N_{e(f)}$[c]	$N_{e(v)}$[d]	
A	237:446	342:341	90.6	88.8	66.7	0.7
D	72:63	94:41	99.5	71.5	76.7	1.2
F	53:40	84:9	98.1	32.1	91.2	2.3

N_{fs}, number of female-sterile male-fertile strains; N_h, number of hermaphroditic strains; N_e, effective population number; and M, mean number of female sterility mutations per strain. Biological species for this species complex are defined in LESLIE (1991). Mating type and fertility data are from LESLIE (1995).

[a] Mating types are +:−.

[b] Effective population number based on mating type and expressed as a percent of the actual count (Equation 4).

[c] Inbreeding effective number based on numbers of males and hermaphrodites and expressed as a percentage of the actual count (Equation 6).

[d] Variance effective number based on numbers of males and hermaphrodites and expressed as a percent of the actual count (Equation 7).

have in opposite manners as the relative numbers of FS strains and hermaphrodites change (Figure 2). As the number of hermaphrodite strains increases, the inbreeding effective number also increases, but the variance effective number decreases. At the limit of 100% FS strains, $N_{e(f)} = 0$, reflecting the inability of the population to go through the sexual portion of the life cycle. The variance effective number is at its maximum under these conditions. The values for N and N_h will also differ for the effective numbers since the number of individuals should be used for $N_{e(f)}$ and the number of clones for $N_{e(v)}$. The number of individuals will always equal or exceed the number of clones and is usually the number most readily obtained from field data. Thus, we expect that $N_{e(f)}$ will be more generally useful than will $N_{e(v)}$.

EXPERIMENTAL DATA

***G. fujikuroi*:** The filamentous ascomycete *G. fujikuroi* (anamorphs in *Fusarium* section *Liseola*) consists of at least six distinct biological species, often referred to as mating populations (LESLIE 1991). The frequency of FS strains in field samples of these fungi ranges from 50 to 90% (Table 1). LESLIE (1995) noted that the percentage of FS strains varies considerably and might be related to the environmental or ecological niche in which each of these biological species thrives. The biological species designated A (LESLIE 1991) was collected primarily as a pathogen on maize from temperate (*e.g.*, North American) regions. Species D is found on maize, rice, sorghum, and many other hosts in both temperate and tropical regions. Species F is primarily a pathogen of sorghum and is found with global distribution. Genetic evidence from these species (*e.g.*, vegetative compatibility group analysis) is consistent with the hypothesis that there is significant genetic variation within at least some of these species.

Vegetative propagation produces individuals that can be grouped into an equal or smaller number of clones. The number of clones in field samples of *G. fujikuroi* may be inferred from data that categorizes each isolate based on vegetative compatibility group or VCG (LESLIE 1993). Vegetative compatibility is controlled by a large number of loci and/or alleles (probably both) such that members of a single VCG often have very similar, if not identical, genotypes. In field isolates taken from the same population, members of a VCG usually share other genetically determined characteristics such as mating type, spore-killer phenotype and female fertility. In the populations discussed here, there is a higher degree of clonality among the F species than among the D or A species; this had previously led us to speculate that vegetative propagation was relatively more important in species F than in the other two species. This conclusion is similar to that reached by GEISER *et al.* (1994) using data on linkage disequilibrium and vegetative compatibility group variation in *Aspergillus nidulans*.

The effective population numbers in the D and the F species are not reduced significantly by the minor discrepancies observed in the relative frequencies of the two different mating types (Table 1). The difference observed in the A species is significantly different from 1:1 ($P < 0.001$) and reduces the effective population number to ~90% of the count. Thus, even relatively large discrepancies in mating type frequency do not reduce the effective population number to a degree such that drift can play a large role.

All three species have FS strains in frequencies (>50%) well above the mutation rate (Table 1). These data are presumptive evidence for significant levels of vegetative propagation for extended time periods. For species A, N_e derived from either the mating type or the FS/hermaphrodite dimorphism is similar (90.6% compared with 88.8%). For species D and F, however,

TABLE 2

Length and range in hermaphrodite frequencies for equilibrium cycles based on observed data from three biological species in the *Gibberella fujikuroi* species complex

Biological species	Time[a]			Hermaphrodites			Time[b]		
	0.98[c]	0.99[c]	0.999[c]	Maximum[d]	Observed	Minimum[e]	0.98[c]	0.99[c]	0.999[c]
A	17.2	34.4	347	0.706	**0.499**	0.249	34.4	68.8	695
D	29.5	59.0	595	0.551	**0.304**	0.092	59.3	119	1200
F	57.0	114	1150	0.316	**0.100**	0.010	114	228	2300
—	74.2	148	1500	0.224	**0.050**	0.003	148	297	3000
—	114	228	2300	0.100	**0.010**	<0.001	—	—	—
—	130	261	2630	0.070	**0.005**	<0.001	—	—	—
—	172	343	3470	0.032	**0.001**	<0.001	—	—	—

Biological species for this species complex are defined in LESLIE (1991). Observed frequency of hermaphrodites is from LESLIE (1995).

[a] Time, in asexual generations, to cycle from h_a to the observed frequency given the value of $(1 - \mu)\Theta$.

[b] Time, in asexual generations, to cycle from the observed frequency to h_b given the value of $(1 - \mu)\Theta$.

[c] $(1 - \mu)\Theta$.

[d] Value for h_a if the observed hermaphrodite frequency is used as fs_0 in Equation 3.

[e] Value for fs_0 if the observed hermaphrodite frequency is used as h_a in Equation 3.

the inbreeding effective population numbers for the FS/hermaphrodite polymorphism, 71.5 and 32.1%, respectively, are much smaller than the corresponding values for the mating type polymorphism.

The observed hermaphrodite frequencies can be used to estimate length, in asexual generations, and the range of hermaphrodite frequencies that might occur in an equilibrium cycle (Table 2). The length of the cycle depends on N_h, Θ and μ with cycle length increasing as N_h or μ decreases or Θ increases. The range and the cycle length also depend on where in the cycle the population is when N_h is measured. For example, if biological species A in Table 2 has just completed the sexual portion of the cycle, then N_h would be expected to fall from ~50 to 25% before the next round of sexual reproduction. On the other hand, if this population was now to go through a sexual cycle, then N_h would increase from ~50 to 70% after which the asexual decay in N_h would begin once again. The values in Table 2 were calculated based on the assumption that the FS strains and hermaphrodites are equal in their maleness. If the hermaphrodites are not as effective as males as their FS counterparts, then the relative change in the frequencies of the hermaphrodites following sexual reproduction will be reduced and cycle times correspondingly shortened. As noted above, the significance of this reduction becomes less important as the proportion of hermaphrodites in the population decreases.

Based on these data, sexual reproduction in species A, with the lowest percentage of FS strains, is nearly twice as frequent as in species D and nearly three times as frequent as in species F. Both the D and the F species could cycle to <10% hermaphrodites (Table 2). Species D averages more than one FS mutation per genome, and for species F, the average is greater than two. In crosses made to develop female-fertile tester strains of species F, KLITTICH and LESLIE (1992) obtained sterility: fertility ratios that were consistent with the segregation of FS alleles at multiple loci. To further exacerbate the situation in this species, the number of individuals is much larger than the number of clones (KLITTICH and LESLIE 1988) as measured by diversity in the number of VCGs. The severely reduced inbreeding effective population number (32% of the actual count) increases the tendency for genetic drift. The data are consistent with the hypothesis that the F species is moving toward imperfect status and that local populations exist in which hermaphrodites are completely absent. Thus two processes are at work in this species: a reduction in genetic diversity due to clonality, as exemplified by the reduced VCG diversity and the preponderance of FS strains over hermaphrodites, and a reduction in effective population number as a consequence of the high proportion of the FS strains. There is a kind of synergy in the reduction of population number in these processes where the effects of drift are felt during both vegetative propagation and sexual reproduction.

Neurospora spp.: Data from organisms in the genus Neurospora also provide insight into the utility of our theory. *Neurospora crassa* and *N. intermedia* populations recovered from burned substrates usually contain very few FS strains. These strains are thought to flourish vegetatively in the brief ecological "window" immediately after a fire and then to sexually produce long-lived ascospores, which can persist until the next burn occurs and activates the ascospores (PERKINS and TURNER 1988). In effect, the sexual stage is essential for these organisms since, although the ascospores can survive long-term, there is no evidence that either the hyphae or the asexually formed spores can. Our theory predicts that when the sexual stage is essential, then the population should be predominantly hermaphrodites with rel-

atively few, if any, FS strains. The *N. intermedia* yellow ecotype is recovered primarily from nonburned substrate, *e.g.*, the Javanese native food *ontjom*, but is cross-fertile with the orange ecotype that is found on burned substrate (TURNER 1987). The fertility of the yellow ecotype strains is significantly reduced when compared with their orange ecotype counterparts. Since the yellow ecotype can be maintained through serial subculturing as inoculum for this native food, the selection against FS would be relaxed and the populations of the yellow ecotype expected to contain a substantial number of FS strains.

DISCUSSION

The theory we have developed is based on the relative sexual fertility of the strains in the population. A unique feature of the organisms we are modeling is the ability of asexually produced spores to function as male gametes and for vegetative propagation. This dual nature is essential for our theory and provides these organisms with the ability to pursue a sexual strategy in a diverse environment and a vegetative strategy in a uniform environment. In our analysis, all strains are capable of vegetative propagation and of producing male gametes but not all are female fertile. The resulting equations provide hypotheses that are relatively easy to test. These hypotheses are relevant both to fundamental questions in model organisms and to applied problems in economically important species.

As a first step in the analysis of a fungal population, the determination of FS level provides extensive information. If sexual reproduction is always required as part of the life cycle, FS strains would not be expected at a rate above a few percent because the FS alleles are selected against during sexual reproduction (see above). If sexual reproduction is never necessary, then the population eventually should become all FS. If both FS strains and hermaphrodites are present at meaningful levels, then both vegetative propagation and sexual reproduction must be significant long-term modes of increase for the species. Both the relative proportions of the FS and hermaphrodite classes and the genetic basis of the FS phenotype are important characters of the population.

In many fungi, of which Neurospora spp. are a good example, the sexual structure may be necessary to survive a period of unfavorable growth conditions. We would expect FS strains to be relatively rare in these fungi. Other fungi can asexually produce long-term survival structures, *e.g.*, sclerotia and chlamydospores, or can propagate themselves vegetatively on an alternative host. Fungi with asexual alternatives for surviving unfavorable growth conditions or that are found in constant environments are the fungi in which FS strains could most easily increase to a high frequency.

Our expectations are very different from those of NAUTA and HOEKSTRA (1992), who studied this problem from the perspective of relative viability of the different propagule types. In their analyses, selection for or against sexually and asexually produced spore types was considered, and they showed that a stable polymorphism for FS strains and hermaphrodites can occur. Our selection model, in which there is an advantage for hermaphrodites during sexual reproduction and for FS strains during vegetative propagation, is equivalent to their model with the simplification of the alternation of reproductive schemes between sexual and asexual rather than have the two occurring concurrently. In our approach, we examined female fertility as the critical character. This character is relatively easy to score and certainly is much easier to score than are selective differences between propagule types. Female fertility also is probably of greater biological significance since different propagule types are not usually produced under conditions in which they would be competing with one another.

The genetic complexity associated with the elaboration of female reproductive structures provides a large number of loci that can be mutated to confer FS. This large number of loci together with a relatively long time in which the mutations can accumulate permits drift and mutation to play a much larger role in determining the frequency of FS than is found in single locus processes. The origin of the FS strains may not be known, and their high frequency may be due to selection, mutation, drift or a combination of effects with the same end results. The critical difference in these processes is the time required by each to achieve the observed number of FS strains. In the case of selection, a high proportion of FS strains may be achieved relatively quickly, dependent only upon the intensity of selection in favor of the FS strains during vegetative propagation. Under natural conditions, we think that mutation and drift, rather than selection, play the dominant role in determining the frequency of FS and in the evolution of asexual species from sexual ones. Under more artificial, *e.g.*, agricultural, conditions, however, selection of a particular pathogen genotype(s) also could lead to the loss of female fertility. Such selection, or drift, could lead to lineages that evolve into asexual taxa. Phylogenetic analysis of several haploid fungal species supports this hypothesis, as the nearest relatives of those species not known to have a sexual stage are invariably sexual species (LOBUGLIO *et al.* 1993). The effects of extinction and recolonization in this setting could be significant and need to be further explored in a fungal context that parallels the work that has been done in higher organisms, *e.g.*, MCCAULEY (1991) and MOYA *et al.* (1995).

If selection is the primary force leading to a loss of sexuality, then there must be an advantage to losing female fertility or a cost, in terms of asexual fitness, to the production of the female fruiting structures and/

or sexually produced spores. If there is such a cost, it should be quantifiable as the parameter Θ. As long as the population does not accumulate too many FS strains, it can fluctuate between states with more and fewer FS strains by alternating vegetative propagation with an occasional sexual generation. The number of FS strains in the population depends on whether vegetative propagation or sexual reproduction was favored recently. For example, some plant pathogenic fungi multiply asexually on the host crop plant during the growing season and are maintained as sexually produced spores or fruiting bodies during the off season. Such a scenario can lead to a quasi-equilibrium state that is dependent upon Θ. If Θ is near 1, then the asexual fitness of the FS strains and the hermaphrodites is similar, hermaphrodites would be lost relatively slowly during vegetative propagation, and sexual generations need not be frequent to maintain the hermaphrodites in the population. As Θ decreases, the frequency of sexual generations needs to increase to counterbalance the decreasing asexual fitness of the hermaphrodites.

Female sterility also limits genetic exchange in field populations and reduces the inbreeding effective population size. Inbreeding effective population size is a property of both the proportions of the population in the different classes and the size of the population. We considered two factors that impact the inbreeding effective population size: mating type and FS. Mating types are expected at a 1:1 ratio in heterothallic ascomycetes since the trait is known to be under the control of a single Mendelian locus. In the Gibberella species for which we have data, female fertility is far more important in determining the effective population size than is the relative numbers of strains of different mating type. Even in the A species, in which the "−" mating type is nearly twice as common as the "+," the effective population size is still >90% of the count. The FS:hermaphrodite ratio of 50:50 reduces the effective population number slightly more than the 2:1 ratio of one mating type to the other. As the proportion of FS strains approaches 73% (M = 1.3), the inbreeding effective population size begins to drop rapidly and the inbreeding effective population size becomes substantially less than the count.

The decrease in inbreeding effective number increases the effect of drift in these populations. With respect to fertility, effective population size is much more a function of the number of hermaphrodites than it is of the number of FS strains. This result is similar to that of WRIGHT (1931) for dioecious species with different numbers of males and females, in which it is the average contribution of each of the two sexes that determines their weight in the analysis of population size.

Several additional points need to be made. First, inbreeding effective population size is a concept that is relevant only to sexually reproducing populations, where it is used to estimate the effects of drift and inbreeding. Drift in asexual populations is a function of the extinction of lineages, rather than the loss of alleles. Selection in asexual populations also effects lineage persistence and the ability of lineages to accumulate deleterious mutations (HIGGS and WOODCOCK 1995). Next, as the maleness (m) of the hermaphrodites declines, the effective population number approaches the harmonic mean of the counts of FS strains and hermaphrodites. At a relative maleness of 0, there are no hermaphrodites (only males and females), and the effective number is calculated the same as for any dioecious species.

This theory provides a number of hypotheses that are relatively easy to test since many filamentous ascomycetes can form the sexual stage under controlled laboratory conditions. Some of these hypotheses include: (1) there should be significant differences in female fertility between populations of the same species that are found in native (diverse) *vs.* agricultural (more uniform) environments. This hypothesis could be easily checked in Gibberella by an examination of local D species populations, which are diverse in terms of host species colonized. (2) Within the same species, the frequency of hermaphrodites in local populations could vary significantly. The differences could be pronounced if there are significant environmental differences that favor either sexual reproduction or vegetative propagation prevail at different locations. (3) The number of loci in which FS mutations can occur should be large. This prediction can be examined in both the A and the F species. The prediction for the F species is that the average number of FS mutations per genome is more than 2 and for the A species less than one. In both cases, the mutations should be found at multiple loci, rather than at a single locus. This hypothesis is testable by rapid mapping techniques (XU and LESLIE 1996). Testing these hypotheses will offer insight into the evolution of asexual fungal species and how fungi maintain mixed modes of vegetative and sexual reproduction.

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