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Abstract

Many filamentous ascomycete fungi reproduce primarily asexually, with only occasional sexual generations. This can lead to a departure from the 1:1 mating type ratio that is expected in obligate sexual populations. The relaxed selection on sexual traits also can lead to a decrease in the frequency of female fertile strains in field populations, while male fertility does not similarly decrease since male gametes also can serve as asexual spores. Both changes ultimately impact the strength of genetic drift in populations. The frequency of female sterility likely increases with the time since the last generation of sexual reproduction, such that it can be used to estimate the relative frequency of sexual reproduction. Here I provide additional details relevant to Leslie and Klein's (1996) model of mixed sexual reproduction and vegetative propagation as related to the frequency of female sterility. This includes new or modified equations that allow for simpler calculations of i) two estimates of relative *Ne*, ii) the expected range of hermaphrodite frequencies during the cycles of mixed reproduction, and iii) the relative frequency of sex. These equations also are included in spreadsheet templates into which researchers can directly enter frequencies computed from their population data to estimate these parameters for their own populations. These resources will make the results of Leslie and Klein (1996) more accessible and should increase the use of this model in evaluating the frequency of sexual reproduction of filamentous fungi.

Keywords

sexual reproduction, asexual reproduction, female sterility, recombination, fungal mating-type idiomorphs

Cover Page Footnote

I thank J. F. Leslie for inviting me to be an instructor at several Fusarium Laboratory Workshops and for comments on the manuscript. This work originated from my Population Genetics lectures in these workshops. I thank Ivain Martinossi and two other reviewers whose comments on an earlier version of this manuscript have greatly improved it, and Pietro Poggi-Corradini for assistance with showing that the frequency of each mutation class after sex is given by a Poisson distribution with parameter (M_b)/2. This is contribution number 22-073-J from the Kansas Agricultural Experiment Station.

On computing relative effective population size estimates and parameters from an equilibrium cycle of hermaphrodite frequency fluctuation due to mixed reproductive modes in filamentous fungi

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Abstract

Many filamentous ascomycete fungi reproduce primarily asexually, with only occasional sexual generations. This can lead to a departure from the 1:1 mating type ratio that is expected in obligate sexual populations. The relaxed selection on sexual traits also can lead to a decrease in the frequency of female fertile strains in field populations, while male fertility does not similarly decrease since male gametes also can serve as asexual spores. Both changes ultimately impact the strength of genetic drift in populations. The frequency of female sterility likely increases with the time since the last generation of sexual reproduction, such that it can be used to estimate the relative frequency of sexual reproduction. Here I provide additional details relevant to Leslie and Klein's (1996) model of mixed sexual reproduction and vegetative propagation as related to the frequency of female sterility. This includes new or modified equations that allow for simpler calculations of i) two estimates of relative N_e, ii) the expected range of hermaphrodite frequencies during the cycles of mixed reproduction, and iii) the relative frequency of sex. These equations also are included in spreadsheet templates into which researchers can directly enter frequencies computed from their population data to estimate these parameters for their own populations. These resources will make the results of Leslie and Klein (1996) more accessible and should increase the use of this model in evaluating the frequency of sexual reproduction of filamentous fungi.

Introduction

Many filamentous haploid ascomycete fungi follow a mixed mode of reproduction, with vegetative propagation by hyphal elongation and through asexual spores interspersed with occasional sexual reproduction and production of ascospores. In filamentous ascomycetes, mating compatibility is determined by the mating-type locus (MAT), where two highly dissimilar but complementary genes, termed idiomorphs, can be present. In heterothallic species, pairs of individuals coming together for sexual reproduction must carry opposite MAT idiomorphs, which prevents self-fertilization that can be called intra-haploid mating in haploid fungi (Giraud *et al*. 2008). In a purely sexual population, a 1:1 ratio of MAT idiomorphs is expected at the population level, since greater reproductive opportunity would benefit individuals with the rarer idiomorph and increase its frequency (Fisher 1930). Additionally, due to anisogamy each individual in the mating pair takes on a distinct function, with one (male parent) contributing male gametes and the other (female parent) producing the female reproductive structure, which is more complex and requires more resources. As male gametes can act as asexual spores or propagules, while the female reproductive structure cannot, female sterility is generally much more common than male sterility. Thus, in populations of heterothallic ascomycetes that follow a mixed mode of reproduction, it is not uncommon to find hermaphrodites, capable of both male and female functions, as well as female-sterile (FS) strains that can participate in sexual reproduction but can only contribute male gametes. For example, populations in the *Fusarium fujikuroi* species complex are usually composed of hermaphrodites and FS strains (*e.g*., Leslie and Klein 1996; Mohamed Nor *et al*. 2019).

The frequency of hermaphrodites varies within and between species, and may serve as an indication of the frequency of sexual reproduction in each population. Leslie and Klein (1996) developed a model for filamentous fungi with mixed reproductive modes, alternating between sexual reproduction and vegetative propagation, that uses hermaphrodite frequency to estimate the relative frequency of sexual reproduction. The publication focuses on heterothallic filamentous fungi, in which intra-haploid mating is not permitted and all sexual crosses occur between genetically distinct parents.

In addition to developing their fungal mixed reproduction model, Leslie and Klein (1996) included several equations pertaining to effective population number. In population genetics, the process of stochastic allele frequency changes between generations, known as genetic drift, is more easily characterized by assuming populations simpler and more idealized than actual populations with respect to the process of reproduction and the population's breeding structure. In particular, the Wright-Fisher model is usually assumed, where gametes are randomly sampled from individuals in one generation and randomly combined to produce the next generation. The concept of effective population number (or size), N_e, was introduced by Wright (1931) as the size of such an idealized population that has the same rate of genetic drift or amount of inbreeding as the actual population being considered. In contrast, the census population size, N, is simply the number of organisms in the population being considered. Crow (1954) realized that there are multiple ways of defining N_e depending on the quantity to be matched between the idealized and actual population. He specified the variance effective number when the one-generation increase in allele frequency variance across replicate populations was to be matched. Similarly, he specified the inbreeding effective number when matching the one-generation change in the inbreeding coefficient, a quantity referring to the probability that two alleles from a locus are identical by descent from a common ancestor.

Leslie and Klein (1996) give equations for variance and inbreeding effective population number relevant to fungi, and the estimates they produce help quantify the potential effects of genetic drift in populations. A particularly important potential consequence of strong genetic drift, if the frequency of female fertility is already low and vegetative propagation far exceeds sexual reproduction, is the complete loss of female fertility from a population and the evolution of total asexuality. Most subsequent studies that have applied their results only use the two equations for inbreeding effective population number: one based on unequal frequencies of MAT idiomorphs; and the other based on the proportion of a population or sample that is FS versus female fertile (*i.e.*, hermaphroditic). I too will ignore the variance effective population number in this work, and focus on inbreeding effective population number. We can use the term relative effective population size to describe the ratio of effective population size to census population size, N_e/N. In Table 1 of Leslie and Klein (1996) and in a fillable spreadsheet (Toomajian 2021) that accompanies this paper (Figure 1), relative N_e is displayed, and papers that apply their results to field populations similarly give relative N_e, since the actual count of individuals with either mating type or hermaphrodite individuals from field populations is too large to obtain practically. To give a specific example, Figure 1 shows that the relative inbreeding N_e based on the frequency of hermaphrodites among the species included is lowest for *F. subglutinans*, at 0.23, reflecting a much higher chance that two alleles from the same locus randomly sampled from the population are identical by descent from some recent ancestor. This also translates into a lower expected level of genetic diversity in this population of *F. subglutinans*. Since N_e is usually smaller than N, relative N_e will usually be less than one, but values approaching one correspond to very low levels of inbreeding, and hence higher genetic diversity.

Estimating the frequency of sex in populations can be important. Sexual reproduction between genetically distinct individuals results in genetic recombination shuffling alleles into new combinations, which can promote genetic adaptation and allow populations to rid themselves of deleterious mutations (Milgroom 2015). When sexual reproduction occurs predominately through intra-haploid mating, the production of recombinant genotypes may be greatly limited. The current work focuses on heterothallic filamentous fungi, where intra-haploid mating is avoided. Intra-haploid mating is possible in homothallic fungi, allowing for the rapid spread through sexual reproduction of a genotype in places where no other distinct genotypes exist. The amount of outcrossing in homothallic ascomycetes is often uncertain, yet the potential for long-distance wind dispersal of microscopic spores is expected to result in more recombinant genotypes over broader geographic and temporal scales. The benefits of sexual recombination arising solely from recombination between genetically distinct haploids disappear in homothallic systems at the extreme of a complete lack of outcrossing, but other factors, such as DNA maintenance and repair or the suppression of genomic parasites through repeat-induced point mutations (RIP), may still contribute to the advantage of sex in this case.

The use of models of mixed reproduction can indicate which populations are reproducing strictly clonally and which undergo sexual reproduction frequently, providing clues about their demographic history and evolutionary potential. As such, the model and relative N_e estimates provided in Leslie and Klein (1996) have remained popular, and the paper is well-cited even 25 years after its publication. The model and relative N_e estimates also have been taught regularly through the Fusarium Laboratory Workshop (Leslie and Summerell 2006a, 2006b).

The purpose of this communication is to make the results of Leslie and Klein (1996) more accessible and to make it easier for fungal researchers to use the data they have collected on population isolates to generate estimates related to these results. I provide additional details relevant to the model and include additional equations, some modified from Leslie and Klein (1996), that allow for simpler calculations of the estimates of relative N_e and the relative frequency of sex. These equations also are included in a spreadsheet template (Toomajian 2021), allowing researchers to directly enter frequencies computed from their own population samples and obtain estimates of relative N_e and the relative frequency of sexual reproduction.

Theory and Equations

Assumptions: Unless otherwise indicated, the following assumptions from Leslie and Klein (1996) are made throughout.

- Individuals are haploid.
- Generations do not overlap.
- Male-sterile strains are absent in the population.
- Census population size, N, is large and constant.
- Hermaphrodite and FS strains are equally effective as males.
- Female-fertility is a binary trait, and all female fertile strains are equally fertile.
- Sexual reproduction and vegetative propagation do not occur simultaneously, but rather may require distinct environmental cues, and all individuals are participating in mating.
- An asexual generation is the mean time to produce a new fungal individual by asexual means, while an individual is defined relative to the median amount of vegetative propagation of colonies in the population.

- Within hermaphrodite and FS classes, differences in vegetative propagation are randomly distributed with respect to genotypes.
- Mutations at a large number of loci can result in female sterility and these loci are unlinked. During vegetative propagation, mutations can accumulate at multiple loci, and assuming the number of female-sterility mutations per individual is randomly distributed, then the frequency of strains with different numbers of mutations is given by a Poisson distribution.
- The average number of progeny per individual is assumed to be binomially distributed, and there is no differential male sexual reproductive success of hermaphrodite and FS individuals.
- The population has reached a stable equilibrium such that the frequency of hermaphrodites reliably fluctuates between its maximum value (after sex), its minimum value (before sex), and back again to its maximum value after the next sexual generation.
- Within a species, the rate of female sterility mutations and the selective disadvantage of hermaphrodites during asexual generations are constant.

Estimates of the ratio of inbreeding effective population size to census population size (N_e/N): Isolates sampled from a field population of fungi can provide information about the strength of genetic drift in the population. But a sample should not be confused with a whole population. Since researchers generally do not know the total number of breeding individuals with either MAT idiomorph or the total numbers of hermaphrodites and FS strains in a field population, they use the frequency of MAT idiomorphs or hermaphrodites from their sample to calculate relative N_e rather than an absolute value for N_e. To derive an equation for relative N_e based on MAT idiomorph frequencies, we can start with the result from Wright (1931), shown as Equation 4 in Leslie and Klein (1996), for the inbreeding effective population size when there are two discrete sexes and self-fertilization is not allowed:

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

Here, N_m and N_f represent the number of breeding males and females, respectively. In this equation, f represented females, but for the rest of this work f will be used only to signify frequency. Replacing N_m and N_f with N_+ and N_-, the number of strains carrying one or the other of the MAT idiomorphs, gives

$$N_e = \frac{4N_+ \times N_-}{(N_+ + N_-)} = \frac{4N_+ \times N_-}{N}$$

Dividing both sides by N, the census population size, gives

$$\frac{N_e}{N} = \frac{4N_+ \times N_-}{N \times N} = 4\frac{N_+}{N} \times \frac{N_-}{N} = 4f_+f_-$$

where f_+ and f_- are just the frequencies of each MAT idiomorph in the population (*e.g.*, N_+/N).

Similarly, we can write an equation for relative N_e by starting with Equation 6 in Leslie and Klein (1996) for the inbreeding effective population size based on the number of hermaphrodites and FS isolates:

$$N_e = \frac{4N^2 N_h}{(N + N_h)^2}$$

Here, N is again census population size, which is assumed to be large, and N_h is the number of hermaphrodites in the population. Dividing both sides by N and further simplification gives,

$$\frac{N_e}{N} = \frac{4NN_h}{(N+N_h)^2} = \frac{4\frac{N_h}{N}}{\frac{(N+N_h)^2}{N^2}} = \frac{4f_h}{\left(\frac{N+N_h}{N}\right)^2} = \frac{4f_h}{(1+f_h)^2}$$

where f_h is the frequency of hermaphrodites (N_h/N).

These two ratios each represent the proportional reduction of the inbreeding effective population size compared to the census population size, due to either a skew in the expected 1:1 ratio of mating type idiomorphs or the presence of FS individuals in the population. Written in terms of the frequency of the mating type idiomorphs or the hermaphrodites, frequencies that researchers can directly compute from their population sample(s), they are convenient forms of these equations.

The range of hermaphrodite frequencies during asexual/sexual cycles: When assuming the equilibrium model of a cycle of one sexual generation followed by multiple asexual generations as detailed in Leslie and Klein (1996), the observed frequency of hermaphrodites can provide information about g, the number of asexual generations per sexual generation. For clarity, I will repeat much of the logic first spelled out in Leslie and Klein (1996), though in places I have changed some of the mathematical notations.

We assume a model where the frequency of hermaphrodites is at a fluctuating equilibrium. The frequency is at a maximum immediately after sexual reproduction, after which the frequency steadily decreases due to new female sterility mutations and a relative fitness benefit of FS strains over hermaphrodites during vegetative propagation. After g asexual generations and just before sexual reproduction, hermaphrodite frequency is at its minimum, but right after each sexual generation, the frequency of hermaphrodites increases again to its maximum. This occurs because hermaphrodites can contribute to sexual progeny through both gamete types, while FS strains can only contribute through male gametes, hence contributing less. Assuming hermaphrodites and FS strains contribute male gametes with equal efficiency, then hermaphrodites contribute all female gametes plus a fraction of the male gametes equal to their frequency in the population, while FS strains contribute the balance of the male gametes. Due to this lower contribution from FS strains, we expect mutations to female sterility have a disadvantage in being inherited by sexual progeny. This results in a predicted increase in hermaphrodites due to matings in a sexual generation. We assume a stable, but fluctuating, equilibrium of hermaphrodite frequency since we assume that the increase in the frequency of hermaphrodites that is expected in each sexual generation exactly balances the decrease in their frequency across g asexual generations due to the joint action of the accumulation of female sterility mutations and the relative fitness advantage of FS strains. Because hermaphrodite frequency is expected to be at a maximum right after sexual reproduction and at a minimum right before sexual reproduction, we can use these two values as boundaries for the frequency, and subsequently deduce the relative frequency of sex by making assumptions on the rate of female sterility mutations and the relative cost of hermaphroditism during asexual phases.

To specify the probability that mutations to female sterility are inherited by progeny from male parents during the sexual generation, we first assume that female sterility can arise from mutations at many presumably unlinked loci, and that the number of female sterility mutations per individual is randomly distributed. In this case, the frequency of strains with i female sterility mutations just

before sexual reproduction follows the Poisson distribution, $e^{-M_b}\frac{M_b^i}{i!}$, where M_b = the mean number of mutations per strain and the subscript denotes *before* sexual reproduction. Hermaphrodites are the only strains with no female sterility mutations (i=0), and so before sexual reproduction, their frequency is given by $f_{0(b)} = e^{-M_b}$. In this notation, the subscript 0 reflects the value of the number of female sterility mutations in this strain class (i=0 in hermaphrodites), and the b again indicates the frequency *before* sexual reproduction. Since it is much simpler to estimate the frequency of hermaphrodites than the mean number of female sterility mutations per strain in a population, rearranging the preceding equation as M_b in terms of $f_{0(b)}$ gives the following more practical form below:

$$M_b = -\ln\left(f_{0(b)}\right)$$

The fertile female parent is always a hermaphrodite, while we can assume that the relative frequency of each female-sterility mutation class (including hermaphrodites) acting as the male parent is given by its frequency in the population, $f_{i(b)}$. In other words, random mating is assumed once we condition on a fertile female parent. The proportion of FS progeny produced differs for matings in which the male parents have different numbers of mutations. Due to the independent assortment of unlinked female-sterility mutations in meiosis, the expected proportion of hermaphrodites produced from males with i mutations is $(\frac{1}{2})^i$. Combining the relative frequency of each mutation class as male parent with the expected proportion of hermaphrodite progeny produced by each class of male parent and summing over all classes of male parent, the total frequency of hermaphrodites immediately after sexual reproduction, $f_{0(a)}$, is:

$$f_{0(a)} = \sum_{i=0}^{\infty} f_{i(b)} \left(\frac{1}{2}\right)^i$$

where $f_{i(b)}$ represents the frequency of each mutation class **before sex** and is given by the Poisson distribution mentioned previously (repeated below):

$$f_{i(b)} = e^{-M_b}\frac{M_b^i}{i!}$$

Since $f_{i(b)}$ here represents the frequency of each mutation class **before sex**, then the mean number of mutations per strain, M_b in the equation, must be the value of M before sex (M is expected to differ before and after sex). Substituting the expression for $f_{i(b)}$ into the equation for hermaphrodite frequency after sex gives,

$$f_{0(a)} = \sum_{i=0}^{\infty} e^{-M_b}\frac{M_b^i}{i!}\left(\frac{1}{2}\right)^i = e^{-M_b}\sum_{i=0}^{\infty}\frac{\left(M_b/2\right)^i}{i!}$$

As the exponential function e^x can be written as the Taylor series below:

$$e^x = \sum_{i=0}^{\infty}\frac{x^i}{i!}$$

then we can substitute the exponential function $e^{M_b/2}$ for the infinite sum above to get,

$$f_{0(a)} = e^{-M_b} e^{M_b/2} = e^{(M_b/2 - M_b)} = e^{-M_b/2}$$

The equation above relates hermaphrodite frequency after sex with the mean number of mutations per strain before sex.

To proceed, we need to determine the frequency distribution of progeny in all of the different mutation classes, and the mean number of mutations per strain after sex. We can use the random variable X_a for the number of mutations in the progeny produced by the matings and the random variable X_b for the number of mutations in the male parents (just before sex). Since the male parent can never have less mutations than their resulting progeny, we can define j as the number of mutations in the male parent of a progeny minus the number of mutations in that progeny. The probability that a progeny carries i mutations can be expressed as a summation of conditional probabilities shown below:

$$\mathrm{P}(X_a = i) = \sum_{j=0}^{\infty} \mathrm{P}(X_a = i | X_b = i + j) \mathrm{P}(X_b = i + j)$$

The first term in this summation follows a binomial distribution with parameters $n=i+j$ and $p=1/2$, since each of the $i+j$ unlinked mutations carried by the male parent has a ½ chance of being inherited by a progeny. In other words,

$$P(X_a = i | X_b = i + j) = \binom{i+j}{i} \frac{1}{2^{i+j}}$$

And the second term in this summation follows the Poisson distribution introduced above, but now with slightly different notation since we are describing a strain with $i+j$ mutations.

$$P(X_b = i + j) = e^{-M_b} \frac{M_b^{i+j}}{(i+j)!}$$

Putting all of this together gives:

$$P(X_a = i) = \sum_{j=0}^{\infty} \binom{i+j}{i} \frac{1}{2^{i+j}} e^{-M_b} \frac{M_b^{i+j}}{(i+j)!}$$

Pulling out terms independent of j, expanding the binomial coefficient, and rearranging gives:

$$= e^{-M_b} \sum_{j=0}^{\infty} \frac{(i+j)!}{i!\, j!} \frac{1}{(i+j)!} \left(\frac{M_b}{2}\right)^{i+j}$$

Cancelling the $i+j$ factorials, pulling out further terms independent of j, and separating the fraction into separate terms with i and j exponents gives:

$$= \frac{e^{-M_b}}{i!} \sum_{j=0}^{\infty} \frac{1}{j!} \left(\frac{M_b}{2}\right)^{i} \left(\frac{M_b}{2}\right)^{j}$$

Pulling out further terms independent of j gives:

$$= e^{-M_b} \frac{\left(\frac{M_b}{2}\right)^i}{i!} \sum_{j=0}^{\infty} \frac{1}{j!} \left(\frac{M_b}{2}\right)^j$$

Noting that the infinite sum is a Taylor series that can be expressed as an exponential function, we get:

$$= e^{-M_b} \frac{\left(\frac{M_b}{2}\right)^i}{i!} e^{M_b/2}$$

And combining the exponential terms finally gives:

$$= e^{-M_b/2} \frac{\left(\frac{M_b}{2}\right)^i}{i!}$$

This expression is just a Poisson distribution with mean $M_b/2$. This mean can be expressed as M_a, the mean number of mutations per strain immediately after sex. So, the frequency of hermaphrodites immediately after sex (the i=0 case) is,

$$f_{0(a)} = e^{-M_b/2}$$

which matches the result obtained before this section investigating the progeny frequency distribution. And we can write an expression for the hermaphrodite frequency after sex in terms of the frequency before sex by substituting the M_b in the exponent in the last equation above with a previous expression in terms of $f_{0(b)}$ to get:

$$f_{0(a)} = e^{-(-\ln(f_{0(b)}))/2}$$

$$= \left(e^{ln(f_{0(b)})}\right)^{1/2} = \sqrt{f_{0(b)}}$$

And thus, one also can write the expression for the frequency before in terms of the frequency after:

$$f_{0(b)} = f_{0(a)}^2$$

In summary, the frequency distribution of each mutation class follows a Poisson distribution both before and after sex, but the mean number of mutations per strain immediately after sex is exactly one half of that immediately before sex, indicated here:

$$M_a = \frac{M_b}{2}$$

This makes intuitive sense, as we expect half of the genetic material in the progeny to come from female parents that carry no mutations, while the other half of the genetic material should reflect the amount of mutations that have accumulated before sex.

Using all of the above, we can now compute the hermaphrodite frequency ranges given the sampled frequency. Assuming the sampled hermaphrodite frequency is that of the population immediately before sex, we can compute the expected hermaphrodite frequency after sex (the maximum frequency in the cycle). As a specific example from a fillable spreadsheet that

accompanies this paper (Figure 2, example 1), assume the sampled hermaphrodite frequency is 0.75. Setting $f_{0(b)}$ to 0.75, we can find the frequency after sex, which is the maximum boundary, as follows:

$$f_{0(a)} = \sqrt{f_{0(b)}} = \sqrt{0.75} = 0.866$$

In this case, the mean number of mutations per strain before sex is:

$$M_b = -\ln\left(f_{0(b)}\right) = -\ln(0.75) = 0.29$$

and the mean number of mutations per strain after sex is:

$$M_a = \frac{M_b}{2} = \frac{0.29}{2} = 0.14$$

Or, assuming the sampled frequency is that of the population immediately after sex, we can compute the expected frequency before sex (the minimum frequency in the cycle). Using the same example of 0.75 for the sample hermaphrodite frequency, we can set $f_{0(a)}$ to 0.75 and then solve for the frequency before sex, which is the minimum boundary:

$$f_{0(b)} = f_{0(a)}^2 = 0.75^2 = 0.562$$

Here, the mean number of mutations per strain after sex matches M_b from the case above, 0.29.

$$M_a = -\ln\left(f_{0(a)}\right) = -\ln(0.75) = 0.29$$

And the mean number of mutations per strain doubles by the time strains reach the next sexual generation, increasing to 0.575.

Estimating the number of asexual generations per sexual generation: To produce an estimate of g, the expected number of asexual generations between each sexual generation in the equilibrium cycle, one must assume values of the mutation rate to female sterility, μ, and Θ, the relative selective disadvantage of hermaphrodites compared to FS strains during the asexual generations (vegetative propagation). One also must assume that the population has reached its stable equilibrium of fluctuating hermaphrodite frequencies, so that each sexual generation brings this frequency to its predicted maximum in the cycle and the frequency drops to its predicted minimum in the cycle just before the next sexual generation. Using either of the assumptions above about the sampled hermaphrodite frequency, and assuming one or more plausible values for $(1-\mu)\Theta$, we can now compute g. After sex, when the frequency of hermaphrodites is at a maximum, it decreases by a factor $(1-\mu)\Theta$ each asexual generation, as a fraction μ of the hermaphrodites become FS due to mutation, and then the remaining hermaphrodites that did not experience a mutation (the proportion 1-μ) will decrease further by the next asexual generation due to their lower relative asexual fitness Θ compared with the FS strains. The frequency of hermaphrodites immediately before sex (when it is at its minimum, after g asexual generations) should equal the frequency after sex times this factor raised to the power g, the number of asexual generations.

$$f_{0(a)}((1-\mu)\Theta)^g = f_{0(b)}$$

Rearranging, substituting the hermaphrodite frequency before sex for the expression in terms of the frequency after sex, and then further simplifying gives:

$$((1-\mu)\Theta)^g = \frac{f_{0(b)}}{f_{0(a)}} = \frac{f_{0(a)}^2}{f_{0(a)}} = f_{0(a)}$$

Then, taking the natural logarithm of each side of the equation gives:

$$g \times ln\big((1-\mu)\Theta\big) = \ln\left(f_{0(a)}\right)$$

$$g = \frac{ln\big(f_{0(a)}\big)}{ln((1-\mu)\Theta)}$$

The inverse of *g* is also useful, as it can be interpreted as the proportion of the total population that is undergoing sexual reproduction during any generation, assuming that individuals in the population initiate sexual reproduction at random rather than synchronously.

$$\frac{1}{g} = \frac{ln((1-\mu)\Theta)}{ln\big(f_{0(a)}\big)}$$

Spreadsheet file for computing both N_e/N ratios, range of hermaphrodite frequencies, and g: To aid in the computation of both N_e/N ratios, the expected range of hermaphrodite frequencies, and the number of asexual generations per sexual generation, g, I have created spreadsheet templates with the relevant equations encoded (Toomajian 2021). The first template presents, as an example, the two relative N_e estimates and corresponding strain counts from a few previous publications (Leslie and Klein 1996; Mohamed Nor *et al*. 2019; Fumero *et al*. 2021) as well as several hypothetical samples. At the bottom, color-coded cells indicate where strain counts should be entered in order for the template to compute each estimate of relative N_e (Figure 1). An equal frequency of mating type idiomorphs or 100% frequency of hermaphrodites each results in a relative N_e of 100%, while a skew in the MAT idiomorph ratio or a lower frequency of hermaphrodites causes values below 100%. In particular, the greater the skew, or the lower the hermaphrodite frequency, the lower the value. It can be assumed that census population sizes are often extremely large, meaning the resulting effective population sizes are still large. Yet lower relative N_e values emphasize how only a subset of the population is likely to contribute to future generations. Additional factors can contribute to greater-than-expected drift in populations besides female sterility and a skew in the mating type ratio, such as population bottlenecks, and these factors would further decrease the overall relative N_e value.

The second template takes the observed proportion of hermaphrodites from a sample and computes the expected minimum and maximum frequencies of hermaphrodites in the equilibrium cycle, the expected number of asexual generations in each cycle under various assumptions, and this last quantity's inverse, which can be interpreted as the proportion of individuals participating in sexual reproduction in any given generation. The template also calculates the mean number of mutations per strain parameters M_a and M_b under the dual assumptions that the sample had just completed a sexual generation and that it was just about to start one. Again, sample calculations and results are given for the same populations used in the first template. Towards the bottom, a color-coded cell indicates where the observed proportion of hermaphrodites in the sample population should be entered, and in another color-coded cell the user can optionally specify a new value of the combined parameter $(1-\mu)\Theta$ to use in the calculation (Figure 2). Few data exist to inform the choice of μ and Θ, though they are likely very similar for closely related species, so that given a set of assumed values the relative differences in g, or $1/g$, between species or populations within a species can be informative about the relative frequency of sex.

Discussion

Here I have provided additional details relevant to Leslie and Klein's (1996) model of mixed sexual reproduction and vegetative propagation as related to the frequency of female sterility, including new or modified equations, that allow for simpler calculations of the estimates of relative N_e, the expected range of hermaphrodite frequencies during the cycles of mixed reproduction, and the relative frequency of sex. I make available spreadsheet templates in which researchers can directly enter frequencies computed from their population samples to calculate these various parameter estimates (Toomajian 2021). These resources will make the results of Leslie and Klein (1996) more accessible and should increase the use of this model in evaluating the relative frequency of sexual reproduction in populations of filamentous fungi.

Estimating the frequency of sex in fungal populations can be important, since sexual reproduction coupled with outcrossing results in the recombination of genetic variants found in populations, which helps to maintain genotypic diversity and allows populations to purge deleterious mutations (Milgroom 2015). Recombination also may play a role in fungal pathogen adaptation, particularly in unstable, rapidly changing environments, even though recombination may not be required for adaptation, given that mutation alone can at times result in relatively fast adaptation in strictly asexual populations (Milgroom 2015). This work focuses on heterothallic species, where mating occurs between genetically distinct individuals and intra-haploid mating is avoided, meaning that sexual reproduction can efficiently recombine genetic variants as long as most matings are not between closely related individuals. For some homothallic ascomycete species where nearly all mating may be intra-haploid, the population-level benefit of sexual recombination is not as obvious, and therefore the utility of the Leslie and Klein (1996) model for estimating the frequency of sexual reproduction is questionable and merits further study. The presence of mating type loci and other machinery needed during meiosis in fungal genomes provides strong evidence for at least occasional sexual reproduction (Nieuwenhuis and James 2016). Strong deviations from equal frequencies of mating types is consistent with rare sexual reproduction. Such deviations may be much more common in small populations, where drift is stronger, and need not be representative of a species as a whole. Researchers can measure the relative frequency of FS and hermaphrodite strains in samples from populations and adopt the model and assumptions of Leslie and Klein (1996) easily without needing access to whole-genome resequencing or even minimal DNA analysis. As such, it offers a useful and relatively low-tech tool, complementary to more genomics-intensive methods as listed in Nieuwenhuis and James (2016), for estimating the relative frequency of sexual reproduction.

Other approaches are available for detecting meiotic recombination and estimating the frequency of sex, and effort should be devoted to comparing these approaches with estimates from the Leslie and Klein (1996) model. A recent abundance of population-level, and often genome-wide, data have provided clearer evidence for meiotic recombination (*e.g*., Paoletti *et al*. 2005; Carbone *et al*. 2007; Ropars *et al*. 2016). Relevant molecular population genetic tests of recombination include the four-gamete test (Hudson and Kaplan 1985), the index of association (Brown *et al*. 1980), and investigations of the decay of linkage disequilibrium (LD) with high density markers (*e.g*., Tsai *et al*. 2008; Taylor *et al*. 2015). The four-gamete test (Hudson and Kaplan 1985) is particularly sensitive to detecting even rare recombination events. The index of association uses as the null hypothesis the free recombination (independent assortment) between all loci included in the test, so that the rejection of this null model clearly indicates the presence of some LD, but can still be consistent with recombination (Nieuwenhuis and James 2016). Better estimates of the frequency

of recombination are required, and LD decay statistics may fill this need. Patterns of LD decay for markers at increasing distances apart can be used both as a test for recombination as well as to provide relative information regarding the frequency of sex (*e.g*., Fumero *et al*. 2021). Nieuwenhuis and James (2016) demonstrate that patterns of LD decay in several fungal species appear to match the generally accepted levels of sexual recombination in these species. Additionally, patterns of overrepresentation of few multilocus genotypes (*i.e*., clonal lineages) in individual samples, or when resampled across generations (*e.g*., Ali *et al*. 2016), also can be used to estimate both the rate of sexual reproduction and effective population size.

While the model and results of Leslie and Klein (1996) have remained popular and important, they are not without their limitations. For example, the equations related to N_e do not provide absolute estimates of N_e, but rather predict by how much N_e is expected to decrease due to a skewed mating type ratio or the presence of female sterility. They do not consider other factors that also can work in parallel to decrease the predicted size of N_e further. Another drawback is the paucity of data available to inform the choice of μ, the mutation rate to female sterility, and Θ, the relative selective disadvantage of hermaphrodites compared to FS strains during the asexual generations. At least for closely related species, these parameter values should be similar, so that comparing the relative frequency of sexual reproduction between populations and species is warranted, but putting too much faith in absolute values of this frequency is discouraged. Researchers are encouraged to consider a range of plausible values for these parameters, and are advised to summarize relative values of sexual reproduction across different populations. Additional uncertainty in model parameters, especially from estimates of MAT idiomorph and hermaphrodite frequencies from samples drawn from field populations, are not considered here, and only point estimates are generated in the spreadsheet templates for g, the range of hermaphrodite frequencies, and the relative N_e values (Toomajian 2021). Researchers should use this resource with caution and healthy skepticism, understanding that the estimates generated may deviate from the actual values, and that the estimates are primarily intended to inspire further research rather than provide definitive answers. Finally, a large number of assumptions underlie the equations outlined here, and significant violations of the assumptions in field populations could call the parameter estimates produced by these equations into question.

Moving forward, the availability of population genomics data makes at least rough estimates of absolute N_e possible, and these estimates can be paired with the relative estimates from Leslie and Klein (1996) to generate population size estimates adjusted for the female sterility and mating locus skew (Fumero *et al*. 2021). Increasingly, the relative population size and frequency of sex estimates produced using the methods of Leslie and Klein (1996) will be coupled with population genomics data to provide more complete biological pictures of filamentous ascomycete fungi, such as those in the genus *Fusarium*.

Data and Resource Availability

The spreadsheet file with templates for computing relative effective population sizes, the range of hermaphrodite frequencies, and g is freely available in the online repository figshare (https://doi.org/10.6084/m9.figshare.17049572, Toomajian 2021).

Competing Interests

The author declares no conflicts of interest.

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INSTRUCTIONS

To calculate relative inbreeding effective population size due to unequal frequencies of mating types (answers in column H), edit counts in both D16 and E16, OR directly input the FREQUENCY (<1) of MAT-1 in F16

To calculate relative inbreeding effective population size due to the presence of female sterile individuals (answers in column M), edit counts in both J16 and K16, OR directly input the FREQUENCY (<1) of hermaphrodites in L16

Heterothallic species	reference	size of MAT sample (n)	count of MAT-1	count of MAT-2	frequency MAT-1	frequency MAT-2	Ne/N (%) based on mating type frequencies	size of female fertility sample	female sterile count	hermaphrodite count	frequency hermaph	Ne/N (%) based on hermaphrodite frequency
F. verticillioides	Leslie & Klein 1996	683	237	446	0.35	0.65	**90.64**	683	342	341	0.50	**88.85**
F. proliferatum	Leslie & Klein 1996	135	72	63	0.53	0.47	**99.56**	135	94	41	0.30	**71.47**
F. thapsinum	Leslie & Klein 1996	93	53	40	0.57	0.43	**98.05**	93	84	9	0.10	**32.18**
F. verticillioides	Mohamed Nor et al. 2019	21	9	12	0.43	0.57	**97.96**	21	8	13	0.62	**94.46**
F. proliferatum	Mohamed Nor et al. 2019	24	10	14	0.42	0.58	**97.22**	24	20	4	0.17	**48.98**
F. thapsinum	Mohamed Nor et al. 2019	20	15	5	0.75	0.25	**75.00**	20	17	3	0.15	**45.37**
F. subglutinans	Fumero et al. 2021	46	26	20	0.57	0.43	**98.30**	46	43	3	0.07	**22.99**
F. temperatum	Fumero et al. 2021	27	4	23	0.15	0.85	**50.48**	27	22	5	0.19	**52.73**
example 1		683	342	341	0.50	0.50	**100.00**	683	171	512	0.75	**97.95**
example 2		135	94	41	0.70	0.30	**84.59**	135	108	27	0.20	**55.56**
example 3		93	84	9	0.90	0.10	**34.96**	93	88	5	0.05	**19.37**
Your values		0			0.00	0.00	**0.00**	0			0.00	**0.00**

Figure 1. Screenshot of spreadsheet template for the calculation of relative effective population sizes (Toomajian 2021).

INSTRUCTIONS

To calculate g, the number of asexual cycles per sexual generation, and the minimum and maximum of hermaphrodite frequency in the equilibrium cycle, simply edit the value in C21 (frequency, <1) and optionally Y5 (value between 0 and 1) and all values in row 21 (green cells) will autofill

			Case 1: when set f_0(b) equal to observed frequency				Case 2: when set f_0(a) equal to observed frequency				g, number of asexual cycles per sexual generation										1/g, the percer		
											(1-mu)*theta									Your value for (1-mu)*theta				
											0.9		0.95		0.98		0.99		0.999				0.9	
Heterothallic species	reference observed frequency of hermaphrodites		f_0(b)	M_b	f_0(a) maximum	M_a	f_0(b) minimum	M_b	f_0(a)	M_a	case 1	case 2	case 1	case 2	case 1	case 2	case 1	case 2	case 1	case 2	case 1	case 2	case 1	case 2	c
F. verticillioides	Leslie & Klein 1996	0.499	0.499	0.7	**0.707**	0.3	**0.249**	1.4	0.499	0.7	3.3	**6.6**	6.8	**13.5**	17.2	**34.4**	34.6	**69.1**	347.1	**694.3**			30.34	15.17	
F. proliferatum	Leslie & Klein 1996	0.304	0.304	1.2	**0.551**	0.6	**0.092**	2.4	0.304	1.2	5.7	**11.3**	11.6	**23.2**	29.5	**59.0**	59.3	**118.6**	595.6	**####**			17.68	8.84	
F. thapsinum	Leslie & Klein 1996	0.097	0.097	2.3	**0.311**	1.2	**0.009**	4.7	0.097	2.3	11.1	**22.2**	22.8	**45.5**	57.8	**115.6**	116.2	**232.4**	1167.1	**####**			9.02	4.51	
F. verticillioides	Mohamed Nor et al. 2019	0.619	0.619	0.5	**0.787**	0.2	**0.383**	1.0	0.619	0.5	2.3	**4.6**	4.7	**9.3**	11.9	**23.7**	23.9	**47.7**	239.7	**479.3**			43.94	21.97	
F. proliferatum	Mohamed Nor et al. 2019	0.167	0.167	1.8	**0.408**	0.9	**0.028**	3.6	0.167	1.8	8.5	**17.0**	17.5	**34.9**	44.3	**88.7**	89.1	**178.3**	895.4	**####**			11.76	5.88	
F. thapsinum	Mohamed Nor et al. 2019	0.150	0.150	1.9	**0.387**	0.9	**0.023**	3.8	0.150	1.9	9.0	**18.0**	18.5	**37.0**	47.0	**93.9**	94.4	**188.8**	948.1	**####**			11.11	5.55	
F. subglutinans	Fumero et al. 2021	0.065	0.065	2.7	**0.255**	1.4	**0.004**	5.5	0.065	2.7	13.0	**25.9**	26.6	**53.2**	67.6	**135.1**	135.8	**271.6**	1364.3	**####**			7.72	3.86	
F. temperatum	Fumero et al. 2021	0.185	0.185	1.7	**0.430**	0.8	**0.034**	3.4	0.185	1.7	8.0	**16.0**	16.4	**32.9**	41.7	**83.5**	83.9	**167.8**	842.8	**####**			12.50	6.25	
example 1		0.750	0.750	0.3	**0.866**	0.1	**0.562**	0.6	0.750	0.3	1.4	**2.7**	2.8	**5.6**	7.1	**14.3**	14.3	**28.7**	144.0	**288.0**			73.12	36.56	
example 2		0.200	0.200	1.6	**0.447**	0.8	**0.040**	3.2	0.200	1.6	7.6	**15.3**	15.7	**31.4**	39.8	**79.7**	80.1	**160.1**	804.3	**####**			13.09	6.55	
example 3		0.054	0.054	2.9	**0.232**	1.5	**0.003**	5.8	0.054	2.9	13.9	**27.7**	28.5	**57.0**	72.3	**144.7**	145.4	**290.9**	1460.8	**####**			7.21	3.60	
example 4		0.010	0.010	4.6	**0.100**	2.3	**0.0001**	9.2	0.010	4.6	21.9	**43.7**	44.9	**89.8**	114.0	**227.9**	229.1	**458.2**	2301.4	**####**			4.58	2.29	
example 5		0.005	0.005	5.3	**0.071**	2.6	**0.00003**	10.6	0.005	5.3	25.1	**50.3**	51.6	**103.3**	131.1	**262.3**	263.6	**527.2**	2647.8	**####**			3.98	1.99	
example 6		0.001	0.001	6.9	**0.032**	3.5	**0.000001**	13.8	0.001	6.9	32.8	**65.6**	67.3	**134.7**	171.0	**341.9**	343.7	**687.3**	3452.2	**####**			3.05	1.53	
Your value			0.000	###	**0.000**	##	**0.000000**	###	0.000	##	9999.0	**####**	9999.0	**####**	9999.0	**####**	9999.0	**####**	9999.0	**####**	9999.0	9999.0	0.00	0.00	

f_0(b) frequency of hermaphrodites before sex

M_b mean number of mutations/strain before sex

f_0(a) frequency of hermaphrodites after sex

M_a mean number of mutations/progeny after sex

mu = mutation rate to female sterility

theta = hermaphrodite disadvantage during vegetative cycle (asexual fertility of hermaphrodite divided by asexual fertility of female sterile)

for case 1, g represents number of asexual generations to cycle from f_0(a) to observed proportion of hermaphrodites

for case 2, g represents number of asexual generations to cycle from observed proportion of hermaphrodites to f_0(b)

Figure 2. Screenshot of a portion of the template for the calculation of the expected range of hermaphrodite frequencies and the number of asexual generations per sexual generation (Toomajian 2021).

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