# Evolutionary Dynamics of Spore Killers

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ABSTRACT

Spore killing in ascomycetes is a special form of segregation distortion. When a strain with the Killer genotype is crossed to a Sensitive type, spore killing is expressed by asci with only half the number of ascospores as usual, all surviving ascospores being of the Killer type. Using population genetic modeling, this paper explores conditions for invasion of Spore killers and for polymorphism of Killers, Sensitives and Resistants (which neither kill, nor get killed), as found in natural populations. The models show that a population with only Killers and Sensitives can never be stable. The invasion of Killers and stable polymorphism only occur if Killers have some additional advantage during the process of spore killing. This may be due to the effects of local sib competition or some kind of "heterozygous" advantage in the stage of ascospore formation or in the short diploid stage of the life cycle. This form of segregation distortion appears to be essentially different from other, well-investigated forms, and more field data are needed for a better understanding of spore killing.

IN several species belonging to the ascomycete order Sphaeriales, spore killing has been found. In some crosses between different wild strains of these species, only half of the ascospores in the ascus survive as a consequence of the action of a so-called Spore killer gene, which has the ability to kill the sensitive ascospores not carrying the killer gene.

The most extensively studied Spore killers are those observed in Neurospora (TURNER and PERKINS 1979, 1991; RAJU 1979). In three Neurospora species they found four different Spore killer genes (TURNER 1993). In *Fusarium moniliforme* (*Gibberella fujikuroi*) a Spore killer has been described by KATHARIOU and SPIETH (1982) and in *Podospora anserina* two Spore killers appeared in a crossing reported by PADIEU and BERNET (1967). In the same species we recently found another Spore killer (NAUTA *et al.* 1993). In all these cases a cross between a Killer strain and a Sensitive strain results in the production of asci with only half the normal number of viable ascospores. All surviving spores have the killer genotype, and the spores that are killed have the sensitive genotype.

Spore killing is an example of segregation distortion, just as has been found in some male animals, like for example the Segregation Distorter in *Drosophila melanogaster* (HARTL, HIRAIZUMI and CROW 1967; TEMIN *et al.* 1991) and the *t*-complex in mice (SILVER 1985): it is manifested postmeiotically, resulting in one member of a pair of heterozygous alleles being transmitted in excess of the expected Mendelian proportion of 50%.

Another frequently used term for segregation distortion is *meiotic drive*, which refers to the effect that the gene segregating in excess of the Mendelian proportion will increase in frequency (unless counterbalancing negative effects on fitness are too strong). In his comparison of different segregation distorters, LYTTLE (1991) notices an important difference between Spore killers and other mechanisms of meiotic drive: in mice and Drosophila the amount of sperm is reduced by segregation distortion, but the total number of progeny is not. Distorters are both absolutely and relatively represented in more progeny. In the Spore killer system, however, the number of progeny is affected, and the Killer only has a relative advantage, as its absolute number of offspring does not increase through killing. As will be shown below, this advantage is very small when the Spore killer is rare, and it cannot produce a "drive" of the killer gene.

A remarkable characteristic that these meiotic drive genes seem to have in common, is that they are located in a region where, in the heterozygous condition, recombination is suppressed. In Neurospora, CAMPBELL and TURNER (1987) found a recombination block of about 30–40 map units long, containing two different and independent Spore-killer genes. Most probably this block is the result of a series of small inversions (TURNER and PERKINS 1991). This can be compared to the well-studied situation for the *t*-haplotype in mice, where a recombination block of about 20 cM is found, due to a series of four inversions (HAMMER, SCHIMENTI and SILVER 1989). Segregation Distortion in Drosophila is typically associated with two inversions, though they do not seem to be absolutely required (TEMIN *et al.* 1991).

Besides Killers and Sensitives, also Resistant strains have been found for the two Spore killers in *Neurospora intermedia* (TURNER 1977) and for the one found

in *Neurospora celata* (TURNER 1993). These strains are neither sensitive to spore killing, nor able to perform it and can therefore be considered as neutral. Two loci appear to be involved in the action of the Spore killer, one for a killer gene (called *Sk* in Neurospora) and one for a sensitivity gene (*r-Sk*). CAMPBELL and TURNER (1987) demonstrated that the recombination block prevents crossing over between the two loci in a cross with one Killer. As recombination is not blocked in a cross without a Killer, the sensitivity gene could be mapped, but the killer gene could not.

Population genetic models of nonfungal forms of meiotic drive (*e.g.*, FELDMAN and OTTO 1991) show that polymorphism in drivers and nondrivers can be stable, depending on the fitnesses of the diploid genotypes and the strength of meiotic drive. Linkage between a segregation distorter and its target locus is necessary for the drive system to become established (PROUT, BUNDGAARD and BRYANT 1973; LYTTLE 1991) and a tightly linked modifier reducing recombination between the two loci can invade a population (THOMSON and FELDMAN 1974; FELDMAN and OTTO 1991). The latter result can offer an explanation for the occurrence of the inversions in the chromosome at the region of distortion.

All these models have in common that they are dealing with diploid organisms. The fungi where Spore killers have been found are haploids for the major part of their life cycle. The population genetic consequences of this aspect, and the special characteristics of Killers are studied in the models below. Attention is focused on the conditions that allow the invasion of a Spore killer in the population and on conditions for the existence of polymorphism in Killer, Resistant and Sensitive types, as is found in nature.

## THE MODEL

Consider a population of a haploid ascomycete, with a life cycle like *Neurospora crassa* (PERKINS and BARRY 1977; RAJU 1992). It is assumed to reproduce sexually each generation. Fertilization results in a short diploid stage, immediately followed by meiosis, ascus formation and possibly spore killing.

Now suppose the Spore killer-complex consists of two loci, a killer-locus with two alleles, killer ($K$) and non-killer ($k$), and a sensitivity-locus with two alleles sensitive ($S$) and resistant ($s$). Then a Killer has genotype $Ks$, a Sensitive $kS$ and a Resistant $ks$. The genotype $KS$ will kill itself and is therefore assumed to be inviable.

Fitness differences can be assumed in different parts of the life cycle. In a general model, a fitness scheme can be used as given in Table 1. Spore killing results in the death of Sensitives after a cross with Killers (fitness zero). The fitness of ascospores resulting from a crossing of two Sensitives is fixed at unity; the other fitnesses are expressed relative to this standard.

TABLE 1

**The fitnesses of the different crosses in a general model**

| Phenotype | Killer | Sensitive | Resistant |
|---|---|---|---|
| Killer | $w_{11}$ | $w_{12}$ | $w_{13}$ |
| Sensitive | 0 | 1 | $w_{23}$ |
| Resistant | $w_{31}$ | $w_{32}$ | $w_{33}$ |

Fitness values apply to the types at the left, when crossed to the types above.
All $w_{ij} > 0$.

Two special, biologically relevant cases have been studied. First, fitness differences in the major part of the life cycle, the haploid vegetative stage, are considered. Second, fitness differences in the stage of ascospore formation, where spore killing occurs, are studied. In this stage, shortly after meiosis, the genes from the two parents share the same cytoplasm and side effects of their interaction may influence fitness. A third possibility, fitness differences in the diploid stage of the life cycle, will not be modeled in this paper. Although this stage may be important, because many genes are expressed (LESLIE and RAJU 1985), its duration is very short and there are no indications that it is relevant for spore killing. Therefore, only a few comments on this case are given in the Appendix. It is, however, easy to see that such a model would resemble other models on Segregation Distorters in diploid organisms (PROUT, BUNDGAARD and BRYANT 1973; FELDMAN and OTTO 1991), where stable polymorphisms can be explained.

In the models below, it is assumed that recombination between the two loci occurs with frequency $r$. Moreover, a Killer may have an additional advantage when it mates with a Sensitive: because half of the spores in the ascus are killed, the Killer will suffer less from local sib competition and presumably have access to more nutrients. This advantage is represented by a factor $c$ in the model ($1 \leq c \leq 2$). Relative frequencies of Killers, Sensitives and Resistants are given by $x_1$, $x_2$ and $x_3$, respectively. ($x_1 + x_2 + x_3 = 1$)

**Fitness differences in the vegetative stage of the life cycle:** In this model a Sensitive strain is assumed to have fitness 1, a Killer strain has a relative fitness $w_1$ and a Resistant strain fitness $w_3$. In the general scheme of Table 1 this means that $w_{11} = w_{13} = w_1$, $w_{12} = cw_1(1 - r)$, $w_{23} = 1$ and $w_{31} = w_{32} = w_{33} = w_3$. Assuming random mating, the following system of recurrence relations can be deduced, $x_i'$ denoting the frequency in the next generation:

$$\begin{aligned} Wx_1' &= w_1x_1(x_1 + (1 - r)cx_2 + x_3) \\ Wx_2' &= x_2(x_2 + x_3) \\ Wx_3' &= w_3(x_3 + rcx_1x_2) \end{aligned} \tag{1}$$

with

$$W = w_1x_1 + x_2 + w_3x_3 + x_1x_2(cr(w_3 - w_1) + (1 - c)w_1 - 1) \quad (2)$$

In the simplest case, where $c = 1$, $r = 0$ and $x_3 = 0$, it is easy to see that there is an unstable equilibrium at

$$\hat{x}_1 = 1 - w_1 \quad (3)$$

In this case a Killer can only invade a population of Sensitives if $w_1 > 1$. Sensitives can never invade a population of Killers. A stable polymorphism with only Killers and Sensitives is impossible.

If $r > 0$, Resistants will always be created by recombination in a population where both Killers and Sensitives occur. As elaborated in the Appendix, Killers will invade if

$$w_1 > w_3 > 1 \quad \text{or} \quad \left(w_3 < 1 \quad \text{and} \quad w_1 > \frac{1}{c(1-r)}\right) \quad (4)$$

For $1 \leq c \leq 2$ several monomorphic stable states can exist: a Killer population is stable if $w_1 > w_3$, a Sensitive population is stable if $w_1c(1 - r) < 1$ and $w_3 < 1$, and a Resistant population is stable if $w_3 > 1$ and $w_3 > w_1$. As a polymorphism of two types is always unstable, there must be a stable polymorphism of the three types or no stable state at all if

$$1 > w_3 > w_1 > \frac{1}{c(1 - r)} \quad (5)$$

In this case a polymorphism of the three types will exist, either as a stable state, or as a quasi periodic orbit (the discrete analogue of a limit cycle).

Numerical computations of system (1) showed that such polymorphisms are not only found if (5) is true, but can also be found in addition to the stable state $x_2 = 1$, if $w_1c(1 - r) < 1$. Some examples are shown in Figure 1. It is found that the dynamics of the system can lead to the approximate disappearance of one of the types. In (small) natural populations this disappearance will frequently occur. However, reintroduction (by mutation, migration or recombination) of the type that disappeared will enable it to invade again.

The stability of the steady states in the system can be studied analytically (see Appendix), but not the parameter values for which a quasi periodic solution is found. Therefore, the parameter space has been examined numerically by studying the course of events using system (1) with 500 × 500 different values of $w_1$ and $w_3$ up to 10,000 generations. This led to results as exemplified for $c = 2$, $r = 0.01$ and $r = 0.1$ in Figure 2.

It can be concluded that Killers can only invade a population if (4) is true. A polymorphism will evolve if (but not only if) (5) is true, that is (roughly) if the fitness of the Killer in the vegetative stage is lower then the fitness of the Resistants and the Sensitives,

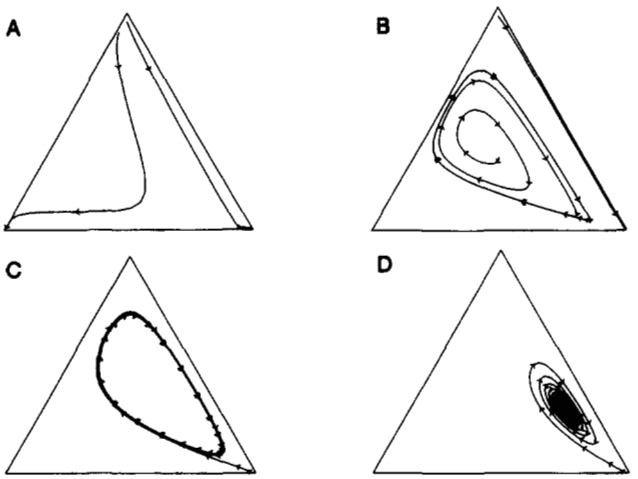


FIGURE 1.—Some examples of the dynamics in the first model. The frequencies of Killers (left), Resistants (above) and Sensitives (right) are given in a de Finetti diagram. Successive arrows mark 10-generation intervals. In all diagrams $r = 0.1$ and $c = 2$. (A) $w_1 = 0.53$, $w_3 = 0.4$: Killers and Sensitives are stable, no polymorphism. (B) $w_1 = 0.53$, $w_3 = 0.6$: $x_2 = 1$ (Sensitives) is the only stable state, but a quasi periodic orbit is also a stable solution. (C) $w_1 = 0.6$, $w_3 = 0.75$: no stable points: the result is a quasi periodic orbit. (D) $w_1 = 0.6$, $w_3 = 0.85$: one polymorphic stable point.

and if this lower fitness is compensated by an additional advantage after killing.

**Fitness differences during ascospore formation:** Suppose the fitnesses of the Killers and Resistants are different (probably lowered) if they are not functioning as killers and resistants. This might be caused by the useless and maybe even harmful production of some unused proteins. Let the fitness for unsuccessful attempt to kill (in a cross *Ks* × *-s*) be $w_1$, and for unnecessary resistance (in a cross *ks* × *k-*) be $w_3$. In the general scheme of Table 1 this means that $w_{11} = w_{13} = w_1$, $w_{12} = c(1 - r)$, $w_{23} = w_{31} = 1$ and $w_{32} = w_{33} = w_3$.

Then the following system of recurrence relations can be deduced:

$$\begin{aligned} Wx_1' &= x_1(w_1x_1 + c(1 - r)x_2 + w_1x_3) \\ Wx_2' &= x_1(x_2 + x_3) \\ Wx_3' &= x_3(x_1 + w_3x_2 + w_3x_3) + crx_1x_2 \end{aligned} \quad (6)$$

with

$$W = w_1x_1 + x_2 + w_3x_3 + x_1(x_3(1 - w_3) - x_2(1 + w_1 - c)) \quad (7)$$

In this model it is easy to see that with $r = 0$ and $x_3 = 0$, *i.e.*, without Resistants, the Killer will always invade the population and become fixed. (If $c = 1$, this invasion will proceed very slowly at first.) Polymorphism is always unstable.

Without these assumptions the analysis is more complicated and is discussed in the Appendix. It appears that Killers can now invade if

$$w_1 > w_3 > 1 \quad \text{or} \quad c(1 - r) > 1 > w_3 \quad (8)$$

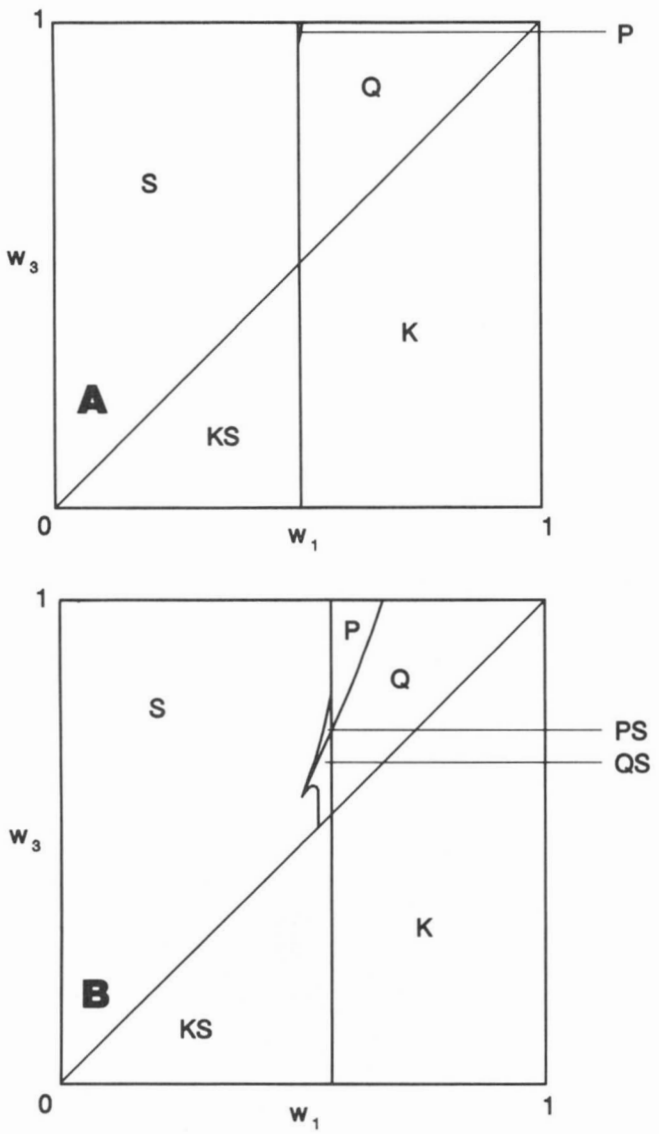


FIGURE 2.—Overview of the results of stability analysis in the first model. S: $x_2 = 1$ (only Sensitives) is stable; K: $x_1 = 1$ (only Killers) is stable; KS: both $x_1 = 1$ and $x_2 = 1$ are stable, polymorphism is unstable; Q: only a quasi periodic orbit is stable; P: a polymorphic point with Killers, Sensitives and Resistants is stable. QS: $x_2 = 1$ (only Sensitives) is stable, but a quasi periodic orbit can also be found. PS: both $x_2 = 1$ (only Sensitives) and a polymorphic point with the three types are stable. (A) Results for $r = 0.01$, $c = 2$ and $0 < w_1, w_3 < 1$. A stable polymorphic point is only found in a very restricted area. (B) Results for $r = 0.1$, $c = 2$ and $0 < w_1, w_3 < 1$. A stable polymorphic point is found in a larger area. Stable polymorphism next to only Sensitives as a stable point gets more frequent as $r$ increases. If $w_1, w_3 > 1$, $x_1 = 1$ (only Killers) is stable if $w_1 > w_3$ and $x_3 = 1$ (only Resistants) is stable if $w_3 > w_1$.

Several monomorphic stable states can exist: a Killer population is stable if $w_1 > 1$, a Sensitive population is stable if $c(1 - r) < 1$ and $w_3 < 1$, and a Resistant population is stable if $w_3 > 1$ and $w_3 > w_1$. A polymorphism of Killers and Resistants is stable if

$$1 > w_1 > w_3 > 2 - \frac{1}{w_1} \qquad (9)$$

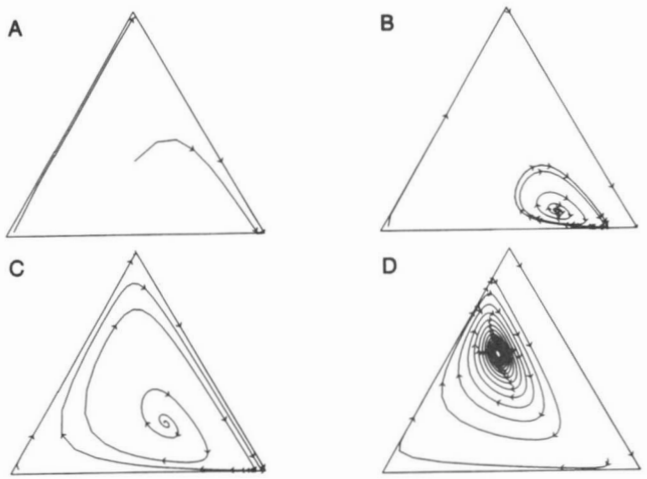


FIGURE 3.—Some examples of the dynamics in the second model with $r = 0.01$, $c = 1$ and $w3 = 0.6$. (A) $w_1 = 0.05$: $x_2 = 1$ (Sensitives) is the only stable state. (B) $w_1 = 0.25$: $x_2 = 1$ (Sensitives) is a stable state, but a quasi periodic orbit is also a stable solution. (C) $w_1 = 0.45$: $x_2 = 1$ (Sensitives) is the only stable state. (D) $w_1 = 0.65$: $x_2 = 1$ (Sensitives) is a stable state, but there also exists a polymorphic stable point.

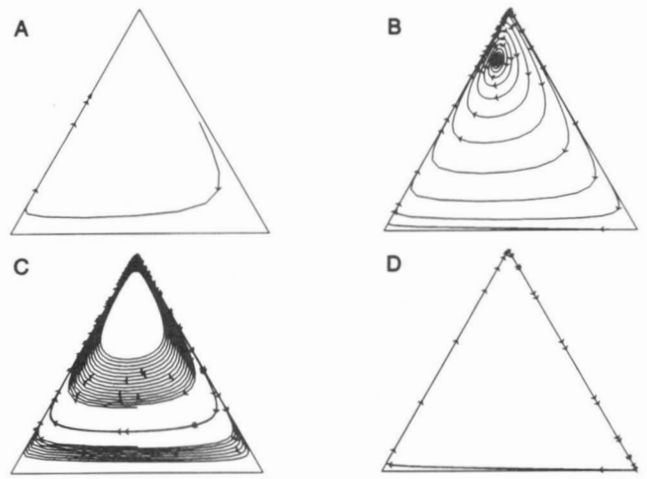


FIGURE 4.—Some examples of the dynamics in the second model with $r = 0.01$, $c = 2$ and $w_1 = 0.75$. (A) $w_3 = 0.6$: a polymorphism with Killers and Resistants at (11) is the only stable state. (B) $w_3 = 0.8$: one polymorphic stable point. (C) $w_3 = 0.85$: an unstable quasi periodic orbit occurs, so there are two possibilities: a polymorphic stable point inside it and another (stable) quasi periodic orbit around it. (D) $w_3 = 0.9$: no stable points: the result is a quasi periodic orbit.

If $c(1 - r) > 1 > w_1$ and $1 > w_3$, and Equation 9 is not true, a polymorphism of all three types is expected (either as a stable state or as a quasi periodic solution). This condition can easily be met: if $r$ is small, $c$ will only have to be a little larger than 1.

As in the model above, the stability of the steady states can be studied analytically (see Appendix), but numerical studies were necessary to find for which parameter values quasi periodic solutions occurred. Some examples of the dynamics are given in Figures 3 and 4, overviews for $r = 0.01$, $c = 1$ and $c = 2$ are given in Figure 5. Note that, unlike in the first model, also for $c = 1$ and $r > 0$ a polymorphism of the three

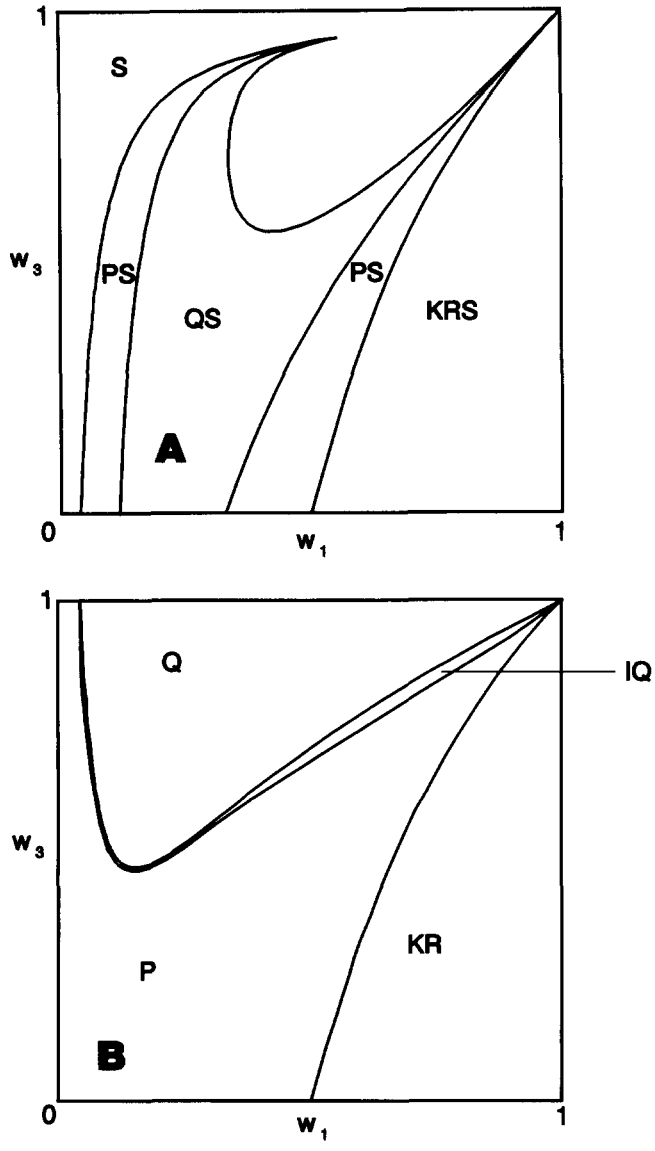


FIGURE 5.—Overview of the results of stability analysis in the second model. S: $x_2 = 1$ (only Sensitives) is stable; Q: only a quasi periodic orbit is stable; P: a polymorphic point with Killers, Sensitives and Resistants is stable. QS: $x_2 = 1$ (only Sensitives) is stable, but a quasi periodic orbit can also be found. PS: both $x_2 = 1$ (only Sensitives) and a polymorphic point with the three types are stable. KR: a polymorphism of Killers and Resistants is stable. KRS: both $x_2 = 1$ (only Sensitives) and a polymorphism of Killers and Resistants are stable states. IQ: an unstable quasiperiodic orbit occurs: both a polymorphic point and a quasi periodic orbit can be stable. (A) Results for $r = 0.01$, $c = 1$ and $0 < w_1, w_3 < 1$. Polymorphism may evolve, but if so, a Sensitive population is also stable. (B) Results for $r = 0.01$, $c = 2$ and $0 < w_1, w_{3i} < 1$. Polymorphism is frequent. Only if a population without Sensitives is stable (KR) spore killing is not detected. If $w_1, w_3 > 1$, $x_1 = 1$ (only Killers) is stable if $w_1 > w_3$ and $x_3 = 1$ (only Resistants) is stable if $w_3 > w_1$.

types is possible, be it in combination with a monomorphic Sensitive population as an additional stable state.

As most probably $w_1, w_3 < 1$, a comparison of formulae (4) and (8) shows that the criteria for the invasion of Killers will be more easily met in this model than in the first model. This was expected, because a cross of Killers and Sensitives yields a higher fitness here. Also, polymorphism showing spore killing can be established for a broader range of parameter values. This is because the conditions $c(1 - r) > 1$ and $w_3 < 2 - 1/w_1$ are more easily met than $w_1c(1 - r) > 1$ and $w_3 > w_1$.

## DISCUSSION

The models above show that the appearance of spore killing cannot be explained as a stable polymorphism of only Killers and Sensitives. However, a polymorphism with Killers, Sensitives and Resistants is possible. When fitness differences in the vegetative haploid stage of the life cycle occur, polymorphism is possible if Killers have a lower fitness than Resistants but an additional advantage resulting from killing ($c$), due to less local sib competition. If fitness differences occur during ascospore formation or in the short diploid stage, polymorphism can easily be established. In that case a polymorphism may be possible due to some kind of "heterozygous" advantage in Killer × Sensitive and Killer × Resistant crosses. Then the "local sib-competition advantage" is not a necessary condition.

These results partly differ from what has been found for meiotic drive genes in diploid organisms, like SD in Drosophila (PROUT, BUNDGAARD and BRYANT 1973; CHARLESWORTH and HARTL 1978; FELDMAN and OTTO 1991) and the $t$-haplotype in mice (LEWONTIN and DUNN 1960; TEMIN *et al.* 1991). In these organisms a strong directional selection against the driving alleles (due for instance to linked recessive lethals or sterility) can cause a stable polymorphism. But this makes no sense in haploid organisms like the ascomycetes considered here. Spore killers actually seem to need an additional advantage to invade into a population.

The model assumption of two genes, a killer gene and a sensitivity gene, is consistent with the findings in Neurospora (TURNER and PERKINS 1979, 1991). Due to the recombination block found there, recombination between the two loci does not occur in crosses where killing takes place (CAMPBELL and TURNER 1987). So the model might be valid for these spore killers, with $r$ being zero or approximately zero. The recombination frequency between the two loci may have been higher when the Killer first arose. As the recombination block is only found in combination with killing and the resistance locus is found at the end of the block, the blocking of recombination between these two loci is probably its only function.

However, if one assumes two different loci (and therefore two different genes), it is hard to understand how a Sensitive type can mutate to a Killer, as this can only be the result of two independent mutations.

Resistance might be an intermediate stage in this process, but it is clear that there will be no selection for Resistants in the absence of Killers. So the genetics may be even more complicated than assumed here, although there is no experimental evidence for this.

The fitness parameters used in these models are purely hypothetical. They can not be based on experimental findings, as fitness studies in natural populations of ascomycetes are simply lacking. TURNER and PERKINS (1991) did not notice any lowered viability in progeny from a Killer × Killer crossing, but this does not exclude any such thing in natural populations. Some preliminary experimental studies at our laboratory with *Podospora anserina* (M. J. NAUTA, A. F. M. DEBETS and R. F. HOEKSTRA, unpublished data) indicate that Sensitive strains may have a selective advantage in competition with Killer strains.

Field data on the occurrence of spore killing in natural populations show rather different results for different Spore killers. In Neurospora (PERKINS and TURNER 1988; TURNER 1993), among 400 isolates of *N. sitophila* both Sensitives and Killers have been found for the Spore killer gene *Sk-1*, in monomorphic as well as in polymorphic populations. Resistants for *Sk-1* have not been found. In *N. intermedia* two Spore killers (*Sk-2* and *Sk-3*) have been found in a sample of more than 2500 isolates. Isolates of the Killer type were extremely rare; populations were polymorphic for Sensitives and Resistants or monomorphic for Sensitives. In *N. crassa* in 450 isolates no Killers were detected, but some isolates were resistant to *Sk-2*, when introgressed from *N. intermedia*. Finally, among 47 isolates of *N. celata*, both Killers, Sensitives and Resistants for another Killer (*Sk-4*) have been found. In *Fusarium moniliforme* KATHARIOU and SPIETH (1982) found a Spore killer frequency of 80%, higher than in any of the Neurospora species.

In this study we searched for conditions for a stable polymorphism of Killers and Sensitives, because such a polymorphism can be found in nature. It might be, however, that this polymorphism is unstable, and that the populations where Spore killers are found, are just on the way to fixation of one of the types. This would require frequent introduction of Spore killers (either by mutation or migration), and it would mean that many "hidden" Spore killers (which do not show up by the lack of Sensitives) should exist in natural populations. If so, new mutations to Sensitives can also lead to a new polymorphism. The only report of resampling a population on a site where Spore killers had been found is from TURNER and PERKINS (1991). In New Guinea they were unable to find the two Spore killers they found 15 years before, and in Borneo they could only find one Killer strain after extensive collecting, on a spot where it had been present 25 years earlier. It is clear that much more field data on frequencies of Killers in the course of time are necessary to make a statement about the stability of the polymorphism.

If the spore killing polymorphism *is* stable, the models presented in this paper predict that Resistants should be present in all species where spore killing occurs. However, in *Fusarium moniliforme* no fully resistant types were collected in a sample of 225 strains (KATHARIOU and SPIETH 1982). Also, Resistants have never been reported in *N. sitophila* and *Podospora anserina*. This absence of Resistants may be an indication that the spore killing polymorphism is *not* stable in these species, although it is also possible that this absence is a consequence of restricted sampling.

In most ascomycetes, like in *Neurospora crassa*, each ascus normally contains eight ascospores, with each ascospore containing only one type of nucleus. In pseudohomothallic species like *Neurospora tetrasperma*, however, only four ascospores are formed in an ascus, each spore containing two different nuclei. In *N. tetrasperma* a locus showing first division segregation after meiosis, will end up heterokaryotic in the ascospore and a locus showing second division segregation will end up homokaryotic. The mating type locus, for example, is very closely linked to the centromere, leading to ascospores heterokaryotic for mating type, giving rise to self-fertile progeny. If one of the nuclei in the ascospore carries a Killer and the other is Sensitive, killing is suppressed and both nuclei survive (RAJU and PERKINS 1991). TURNER and PERKINS (1991) and LYTTLE (1991) therefore suggest that pseudohomothallism may have evolved as a defense mechanism against the action of Spore killers. But the problem with this argument is that killing is only suppressed in heterokaryotic spores. A Killer-gene located at the distal end of the chromosome in *N. tetrasperma* and thus ending up homokaryotic in a spore, will not suffer from this defense mechanism at all: homokaryotic Sensitive ascospores will get killed by the homokaryotic Killers. Such a situation, (although different in genetic detail) has been found in another pseudohomothallic species, *Podospora anserina* (PADIEU and BERNET 1967; NAUTA *et al.* 1993). So selection affecting the particular location of Spore killer loci on the chromosome in pseudohomothallic species may be expected, but evolution of pseudohomothallic species as a consequence of spore killing is highly improbable.

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## APPENDIX

Below the criteria for invasion of a Killer into a population and the stability analysis of the models will be analyzed. The stability of the steady states of the nonlinear systems of difference Equations 1 and 6 can be found by using Taylor expansion and determining the eigenvalues of the linearized systems, as for example described by EDELSTEIN-KESHET (1988). As $x_1 + x_2 + x_3 = 1$, both systems can be reduced to a system of two equations, by putting $x_3 = 1 - x_1 - x_2$.

It is clear that a monomorphic population of one type $X$ will be stable if the type that yields the highest fitness in a cross with type $X$, is type $X$ itself: in that case no other type can invade a population consisting of type $X$ only. So Killers are stable if $w_{11} > w_{31}$, Sensitives are stable if $1 > w_{12}, w_{32}$ and Resistants are stable if $w_{33} > w_{13}, w_{23}$. On the other hand, if this is not valid for any of the types, no monomorphic population will be stable. In that case polymorphism of two or three types is expected, which can be a stable point or frequencies showing cycling behavior.

A complicating factor is recombination, which always leads to the formation of Resistant types if both Sensitives and Killers are present and $r > 0$: in the model a polymorphism, where killing can be detected, must always consist of all three types. But even if $r = 0$, a polymorphism of only Killers and Sensitives can never be stable, because always $w_{11} > 0$.

**Fitness differences in the vegetative stage of the life cycle:** In a population without Killers a polymorphism of Sensitives and Resistants is never stable (if $w_3 \neq 1$). If $w_3 > 1$, there will only be Resistants, and Killers can only invade if $w_1 > w_3$. If $w_3 < 1$, there will only be Sensitives and Killers can invade if $w_1 c(1 - r) > 1$.

A polymorphism of Sensitives and Killers is always unstable and produces Resistants by recombination. A polymorphism of Killers and Resistants is also never stable (if $w_3 \neq w_1$). So if none of the monomorphic steady states are stable, Equation 5 is true and polymorphism is expected.

Analysis of system (Equation 1) shows that there are two potential polymorphic steady states for

$$\hat{x}_1 = \frac{A \pm \sqrt{B}}{C} \tag{10a}$$

where

$$A = 2 + w_1(1 - 2c(1 - r)) - w_3(1 + r) - w_1 w_3(1 - c)$$

$$B = (1 - cr)^2 w_3^2 - 2w_1 w_3(1 - cr)(1 - w_3(1 - c + 2cr)) + w_1^2((1 - (1 - c)w_3)^2 - 4c^2 w_3 r(1 - r))$$

$$C = 2(1 + w_1(1 - c + cr) - crw_3)$$

and

$$\hat{x}_2=\frac{1-w_1-\hat{x}_1}{w_1(c(1-r)-1)} \tag{10b}$$

It is clear that $\hat{x}_1$ does not exist if the function within the square root of Equation 10a is negative; also we require $0 \leq \hat{x}_1, \hat{x}_2, \hat{x}_3 \leq 1$. If this is true, stability can be determined by calculating the eigenvalues.

If no steady state is stable, the only possible solution left is a (quasi-) periodic orbit. As illustrated in Figures 1 and 2, polymorphism is found if Equation 5 is true. Also, in a population where Sensitives are stable, a polymorphic stable point or a quasi periodic orbit may be found, especially for higher values of $r$. This orbit can only be found by numerical computations.

**Fitness differences during ascospore formation:** Like in the previous model, in a population without Killers a polymorphism of Sensitives and Resistants is never stable if $w_3 \neq 1$. If $w_3 > 1$, there will only be Resistants, and Killers can only invade if $w_1 > w_3$. If $w_3 < 1$, there will only be Sensitives and Killers can invade if $c(1-r) > 1$.

If $1 > w_1 > w_3$ a steady state can be found for a polymorphic population with only Killers and Resistants at

$$(\hat{x}_1, \hat{x}_2)=\left(\frac{(w_1-w_3)}{(1-w_3)}, 0\right) \tag{11}$$

Calculation of the eigenvalues shows that this is a stable equilibrium point if

$$w_3<2-\frac{1}{w_1} \tag{12}$$

So a stable polymorphism at Equation 11 is expected if Equation 9 is true.

Moreover, analysis of system (6) shows that two potential steady states can be found for a polymorphism with all three types for:

$$\hat{x}_1=\frac{A \pm \sqrt{B}}{C} \tag{13a}$$

$$A=(w_3-1)(2(1-c+cr)+w_1)-w_1cr-1+c$$

$$B=(1-c)^2+2w_1((w_3-1)(1-c+2c^2r(1-r))+cr(1-c)) + w_1^2((1-w_3+cr)^2$$

$$C=2((w_3-2)(1-c+w_1)+(w_3-1)cr)$$

and

$$\hat{x}_2=\frac{1-w_1-\hat{x}_1}{c(1-r)-w_1} \tag{13b}$$

The function within the square root of Equation 13a must be positive, and $0 \leq \hat{x}_1, \hat{x}_2, \hat{x}_3 \leq 1$. If so, stability can be determined by calculating the eigenvalues.

Again, it is easy to see that if there does not exist any stable state and no polymorphic stable state with two types, all three types will be expected to coexist in the population. If stability analysis shows that one of the steady states (Equation 13) is stable, there will be a stable point, otherwise there must be a (quasi-) periodic orbit.

These events can be complicated by the existence of an unstable (quasi-) periodic orbit, as illustrated in Figure 3. Here both a polymorphic point and a quasi periodic orbit can be stable. In this case perturbations in gene frequencies can easily cause a shift from the periodic orbit toward the stable point, as the trajectories of the two are very close, especially near the axes.

**Fitness differences in the diploid stage of the life cycle:** If fitness differences in this stage are assumed, $w_{ij} = w_{ji}$ for the fitness parameters in Table 1 (except in case of killing). Putting the fitness of *kkSS* (the homozygote Sensitive) at 1, this leaves five fitness parameters $w_{ij}$. There are, however, no data on realistic values of these parameters. Investigating all possibilities falls beyond the scope of this paper.

If it is assumed that $w_{ij} = w_i \cdot w_j$, *i.e.*, assuming multiplicative fitness of haplotype fitnesses, it can be shown that the model becomes identical to our first model with selection in the haploid stage of the life cycle.

If heterozygote advantage is assumed, it is likely that conditions can be found for a stable polymorphism of the three types considered. In this model a cross Killer × Sensitive yields a double heterozygote (*KkSs*) and crosses of Killer × Resistant (*KkSS*) and Sensitive × Resistant (*kkSs*) yield single heterozygotes. This means that the fitness parameters $w_{ij}$ with $i \neq j$ will have the highest values under this assumption, and no monomorphic population will be stable: polymorphism of two or three types is expected.