Evolution of Genetic Systems in Filamentous Ascomycetes

Evolutie van genetische systemen in hyphenvormende zakjeszwammen

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aan mijn ouders

Voorwoord

Dit proefschrift is het resultaat van vier jaar onderzoek, verricht bij de vakgroep Erfelijkheidsleer van de Landbouwuniversiteit in Wageningen. In zekere zin valt zo'n proefschrift te vergelijken met een levend wezen. Uit de genetica is bekend dat de verschijningsvorm van elk levend wezen tot stand komt door een combinatie van erfelijke aanleg en invloeden uit de omgeving. Voor een proefschrift geldt eigenlijk hetzelfde: Zowel het werk van de auteur, als de bijdragen van zijn omgeving zijn onontbeerlijk om tot een verschijningsvorm te komen.

Ik wil dan ook graag iedereen bedanken die in de afgelopen jaren deel van mijn omgeving is geweest en, direct of indirect, heeft bijgedragen aan het resultaat. Daarbij denk ik natuurlijk met name aan de mensen van de vakgroep Erfelijkheidsleer, waar de gemoedelijke sfeer voor mij het ideale werkklimaat vormde.

Het verblijf in het theoretisch bolwerk, hoog op de tweede verdieping, werd bijzonder veraangenaamd door mijn kamergenoten Johan van Ooijen, Jan van Oeveren en Chris Maliepaard.

Een verdieping lager waren het vooral de mensen van Microbiële Genetica met wie ik met veel plezier heb samengewerkt. Ik ben blij dat ik mijn theorieën over schimmels vrijelijk op hen heb mogen loslaten. Het doet mij veel genoegen dat de schimmel *Podospora anserina* (en massa's konijnekeutels) hierdoor het lab zijn binnengedrongen. Ik wil met name Fons Debets bedanken voor het vele meedenken en zijn gezelschap tijdens twee werkbezoeken. Dankzij de stagiaires Marijke Ophorst, Inge Haspels en Robin Hartman heb ik het bezit van twee linkerhanden enigszins kunnen verbloemen en mee kunnen doen met experimenteel werk aan *Podospora*. De samenwerking met Klaas Swart, Edu Holub en Marijn van der Gaag heb ik hierbij als erg prettig ervaren. Dat er van de resultaten van dit experimentele werk weinig tot niets in dit proefschrift is terug te vinden, ligt zeker niet aan hen. Dit is geheel te wijten aan mijn overdreven voorkeur voor theoretisch werk en de halsstarrigheid van *Podospora*.

Mijn grootste dank gaat zonder enige twijfel uit naar mijn promotor, inspirator, discussiepartner en co-auteur, Rolf Hoekstra. Als hij me in 1983 niet op het spoor had gebracht van populatiegenetisch onderzoek aan schimmels, was ik nooit in deze fascinerende tak van wetenschap verzeild geraakt. Zijn onbezorgde en toch zorgzame manier van begeleiden heeft zeer veel bijgedragen aan de kwaliteit van mijn onderzoek en het plezier dat ik eraan heb beleefd.

Tenslotte wil ik alle mensen bedanken waar ik de laatste jaren 'thuis' ben geweest. Het is fantastisch dat jullie er waren, want als je alleen maar over schimmels kunt theoretiseren, heb je geen leven. En Jeanette, je weet dat ik jou voor geen goud als omgevingsfactor had willen missen. Ik hoop dat we nu maar snel samen kunnen gaan wonen.

Contents

CHAPTER 1

General introduction

The evolution of genetic systems is perhaps the most difficult and exciting topic in evolutionary genetics.

J. Maynard Smith (1989)

Above all, we should start to think about fungal evolution in terms of modern population genetics ...

C.E. Caten (1987)

Until recently the fungi have been almost completely neglected by evolutionary biologists and population geneticists. Population studies on fungi have mainly been performed by plant pathologists, interested in eliminating the pathogens and not in evolution (Sidhu, 1988). Genetical research on these large numbers of fungi, collected by mycologists, is scarce. The fungi studied by geneticists usually orgininate from one or a few natural isolates, showing little natural genetic variation. Ideas on the evolutionary biology of fungi (e.g. Rayner et al., 1987) are based on limited population genetic data, and the hypotheses lack the theoretical background that is abundantly available for animals and higher plants.

Probably many reasons for this lack of population genetic research on fungi exist. Important obstacles for evolutionarily oriented population biological studies are the complexity of their growth and life cycles, and the general inaccessibility of the vegetative 'body' of the fungus, the mycelium. This complexity has been widely recognized, and has led to the characterization of the fungi as 'a mutable and treacherous tribe' (Raper, 1966b). Moreover, not only the definition of a fungal 'individual' is unclear (Todd and Rayner, 1980), also the species concept is vague (Brasier and Rayner, 1987; Perkins, 1991). The variety of mating systems is enormous (Raper, 1966b) and the possibility of genetic exchange during vegetative growth is confusing.

Nonetheless, in this thesis some theoretical population genetic models on fungi are build and analysed. Until now, population genetic theory mostly deals with diploids that only reproduce sexually. This theory can partly be applied to the

fungi, but some important complications have to be taken into account. Haploidy, asexual reproduction, sexual reproduction of self-fertilizing haploids, and genetic exchange during vegetative growth are frequently found in the fungi and have to be incorporated into population genetic theory (Anderson et al, 1988; Barrett, 1987; Caten, 1987).

Some basic understanding of functional explanations for the diversity of genetic systems is necessary for research on the evolutionary biology of the fungi. Unfortunately this diversity is so great that it cannot be completely treated in one thesis. Therefore this thesis is focussed on a widespread group of higher fungi, the filamentous ascomycetes. This group comprises some of the genetically best studied fungi (*Neurospora crassa*, *Aspergillus spp.* and *Podospora anserina*), which gives access to essential information. The basics of their genetic systems are relatively simple compared to those of other groups of fungi.

Below, some introductory information will be given about the evolution of genetic systems and about filamentous ascomycetes, followed by an overview of several aspects of their reproductive systems. Heterokaryosis and vegetative incompatibility will be discussed as characteristic parts of the genetic system of the fungi. This introduction will conclude with some remarks about the definitions of 'individual' and 'species', the purpose of theoretical modelling and an outline of this thesis.

Genetic systems and their evolution

The term 'genetic system' has been widely used, in several contexts. According to Darlington (1958) and Carson (1987) 'genetic system' refers to the cellular, nuclear and chromosomal events that bring about the replication, proliferation and recombination of the DNA during the life cycle of the organism. Roughgarden (1979) refers to genetic parameters that are necessary to determine the evolution of a trait as the genetic system of that trait.

In this thesis 'genetic systems' are defined as all those mechanisms and processes that are directly involved in the transmission of genetic information. With this definition a genetic system contains more than a 'mating system' or a 'breeding system', which both imply the occurrence of sexual reproduction. In fungi, however, both sexual and asexual reproduction can occur. Moreover, transmission of genetic material does not only occur vertically, by (a)sexual reproduction, but also horizontally, following anastomosis during vegetative growth (see below).

Therefore a broader definition is nescessary.

Genetic systems take a central place in the study of evolution (Maynard Smith, 1989). It is known since Darwin, that the process of evolution is a consequence natural selection, which is the result of variation, reproduction and heredity. Genetic systems determine the mechanism of reproduction and heredity, and thus the way in which variation is established. They are not only essential in evolution, but also a product of evolution themselves, because they are variable (especially in 'lower' organisms) and genetically determined. The genetic system affects not only the fitness of the individual, but also the evolutionary potential of the population. So a study of the evolution of the genetic system hits the core of the evolutionary biology of a species.

The selective forces influencing the evolution of genetic systems are manifold. In general, they may be the same as those considered in the context of the evolution of sex and mating systems (Williams, 1975; Maynard Smith, 1978; Bell, 1982; Stearns, 1987; Michod and Levin, 1988). Roughly, these selective forces can be divided in two types. First, there are external forces, operating from outside the individual, like saturated and heterogeneous environments ('The Tangled Bank') (Bell, 1982; Bell, 1987) or coevolutionary arms races with predators or parasites ('The Red Queen') (Bell, 1982; Hamilton, 1980; Bremermann, 1987). Secondly, internal forces may play a role, like DNA repair (Bernstein et al., 1981), the selective pressure of variable numbers of mutations (Muller, 1932, 1964; Kondrashov, 1982) or intragenomic conflicts between competing (nuclear or cytoplasmic) genes (Cosmides and Tooby, 1981; Hurst, 1992).

Some of these arguments, used in explanations for the evolution of sex, are also used in this thesis. The question of how sex evolved, however, is not considered.

Filamentous ascomycetes

The ascomycetes form the largest subdivision of fungi, containing at least 15000 species (Webster, 1980; Ainsworth, 1973), which is some 45% of all fungi (Burnett, 1987). They are characterized by the fact that the sexually produced (asco-)spores are borne in a sac-like structure, the ascus. Filamentous ascomycetes are, in short, those ascomycetes that are not yeasts. Yeasts are then defined as those fungi which, in a stage of their life cycle, occur as single cells, reproducing by budding or fission (Kreger-Van Rij, 1973). Filamentous ascomycetes typically form a mycelium, a network of vegetative filaments, the hyphae. These hyphae are

normally not build up by neatly separated cells, containing one nucleus each. Instead, the hyphae are formed by segments, separated by cross walls or septae, containing a pore that allows the transmission of mitochondria and nuclei. So hyphal segments often contain more than one nucleus. If a mycelium originates from one uninuclear spore, all its nuclei will be genetically identical and the mycelium is called homokaryotic. If, however, genetically different nuclei coexist in a mycelium, it is called heterokaryotic.

The existence of such a heterokaryotic state is unique for the fungi. Heterokaryon formation can occur when two genetically different strains are grown together. Physical contact of the hyphae can sometimes lead to fusion, or anastomosis, which may lead to an exchange or invasion of cytoplasmic material and/or nuclei. Also, a (vegetative) incompatibility reaction may occur before or after such physical contact; in that case heterokaryon formation is prevented. The precise consequences of these phenomena for evolutionary genetics are still unclear.

The filamentous ascomycete species most frequently studied in genetics, is the well known bread mold *Neurospora crassa*. This ascomycete proved to be particularly suited to genetic analysis after it had been discovered by Shear and Dodge (1927). Some other species belonging to the same family of *Sordariaceae*, like *Sordaria fimicola* and *Podospora anserina*, are also well investigated. These species belong to the class of the *Pyrenomycetes*, which typically form a flask-formed fruiting body, the perithecium. Another well studied species that should be mentioned is *Aspergillus nidulans*, which belongs to another class, the *Plectomycetes*. Their fruiting bodies are completely closed and are called cleistothecia.

Taxonomy of fungi is mainly based on the modes of sexual reproduction (Ainsworth, 1973). Therefore the species where no sexual stage is known, the so called *Fungi Imperfecti*, are gathered in an artificial assemblage, the subdivision *Deuteromycetes*. This subdivision probably consists of many species closely related to the ascomycetes, that have simply lost the ability to reproduce sexually (Webster, 1980; Burnett, 1987). In a study on evolutionary relationships it may be confusing to investigate direct transitions of species belonging to different subdivisions. Therefore, in this thesis some asexual (or imperfect) species, like *Aspergillus niger*, will be regarded as filamentous ascomycetes.

Life cycles of filamentous ascomycetes

A description of the genetic system of a fungal species begins with a description

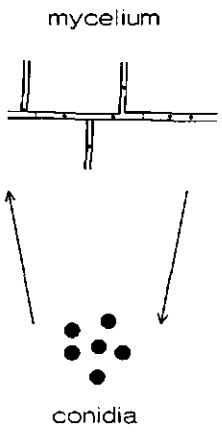

Figure 1. Life cycle of an asexual or imperfect ascomycete, like *Aspergillus niger*. The asexual propagules (the conidia) can germinate and grow out to a new mycelium.

of its life cycle. Roughly, the life cycles of filamentous ascomycetes can be divided into three types: asexual, homothallic and heterothallic. A fourth type, the pseudo-homothallic or secondarily homothallic life cycle, is probably derived from the heterothallics. Typically, these life cycles are mainly haploid. Sexual species have a very short diploid phase, just before meiosis. The individual mycelia are usually hermaphroditic, as they produce both male and female gametes. Additionally, mating type differentiation may be possible. After nuclear fusion and meiosis, normally eight ascospores are formed per ascus, in fruiting bodies that contain several asci.

- An asexual life cycle (figure 1) is characterized by the lack of a sexual cycle. In for example *Aspergillus niger* the mycelium forms asexual spores, the conidia, which can germinate and form a new mycelium.
- A homothallic life cycle is characterized by a sexual cycle with the capacity of self-fertilization (figure 2). Outbreeding, however, is usually possible too. As far as known, homothallic species lack any mating type differentiation. Some only reproduce sexually, like e.g. *Sordaria fimicola*, and others have an additional asexual cycle, like e.g. *Aspergillus nidulans*.
- A heterothallic life cycle is characterized by a sexual cycle without the capacity of self-fertilization (figure 3). Typically in a heterothallic ascomycete species two

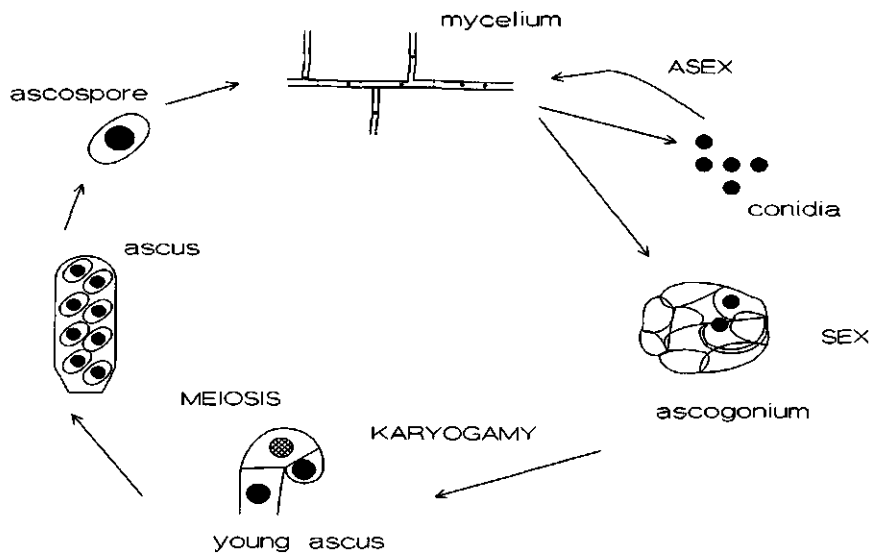

Figure 2. Life cycle of a homothallic ascomycete, like *Aspergillus nidulans*, with both sexual and asexual reproduction. (Self-)fertilization occurs in the ascogonium. There is no mating type differentiation.

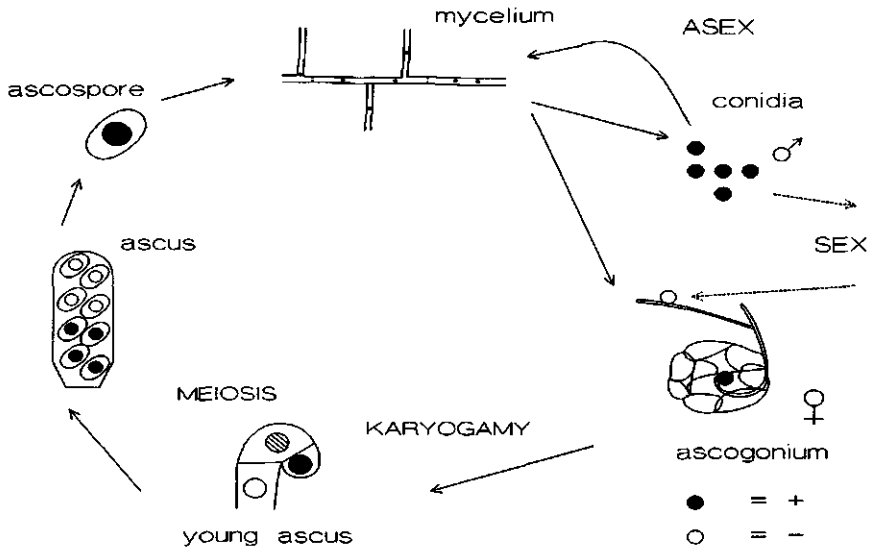

Figure 3. Life cycle of a heterothallic ascomycete, like *Neurospora crassa*. Self-fertilization is not possible, due to the action of two antagonistic mating types, '+' and '-'. Cross fertilization occurs by means of a conidium fusing with the trichogyne of an ascogonium of the opposite mating type.

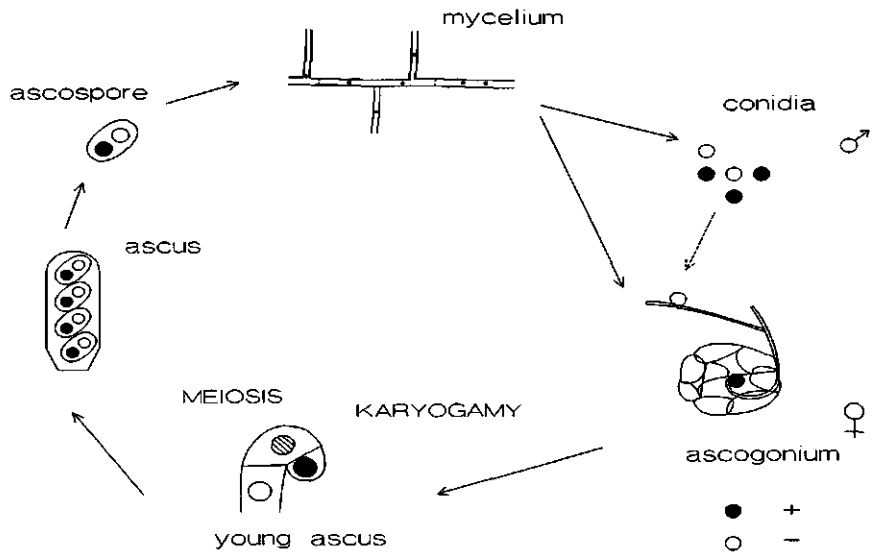

Figure 4. Life cycle of a secondarily homothallic (or pseudohomothallic) ascomycete like *Podospora anserina*, without asexual reproduction. Each ascospore contains two nuclei, with different mating types, so self-fertilization is possible. As a consequence only four ascospores are formed.

mating types exist. Strains with identical mating types cannot be crossed, whereas strains with different mating types can. Normally, like in *Neurospora crassa*, an additional asexual cycle exists.

- A pseudohomothallic or secondarily homothallic life cycle is characterized by the combination of self-fertilization and the possesion of mating types (figure 4). In for example *Podospora anserina* and *Neurospora tetrasperma*, binucleate ascospores are formed. As the two nuclei in a spore normally contain different mating types, the (dikaryotic) mycelia that grow out of the spores are self-fertile.

Apparently these different life cycles are found within many families of ascomycetes. This implies that evolution from one type to the other must have occurred frequently. Therefore the evolutionary transitions between the first three types are studied, as described in chapters 2 and 3.

Selfing

Homothallic fungi have the ability of self-fertilization, or selfing. It is fundamentally different from selfing in plants. This is illustrated in figure 5, where diploids

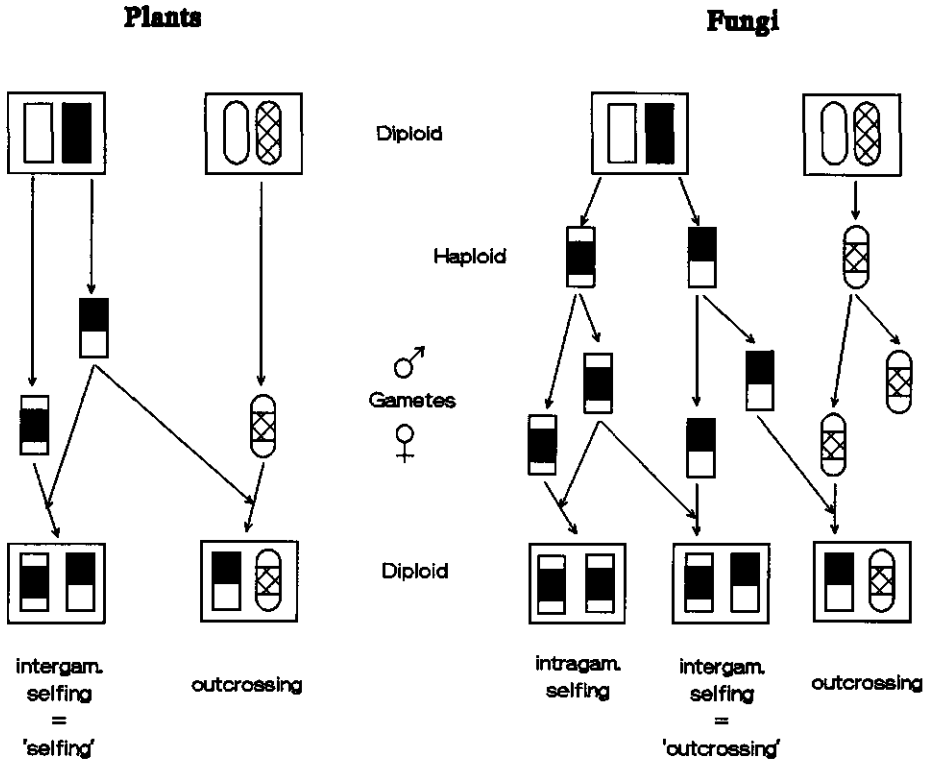

Figure 5. Comparison of outcrossing and selfing in diploids (plants) and haploids (fungi).
The diploid stages are indicated by boxes containing a single pair of 'chromosomes'. Haploids are produced after meiosis and are therefore combinations of these. In plants the gametes are produced by the diploid individuals, so each individual produces genetically different gametes. In fungi the gametes are produced by haploid hermaphroditic individuals and are therefore genetically identical. In plants intergametophytic selfing is called 'selfing', in fungi it is normally called 'outcrossing', because it is a crossing of two different individuals.

(plants) are compared to haploids (homothallic and heterothallic fungi). For these different types of organisms, different kinds of selfing are found. To distinguish them, the terms 'intergametophytic' and 'intragametophytic' have been proposed by Klekowski (1979) and Hedrick (1987), who were studying ferns.

A (intergametophytically) selfing diploid plant is usually supposed to suffer from inbreeding depression, caused by in increase of homozygosity, that allows the expression of deleterious recessive alleles. Avoidance of inbreeding depression is generally believed to be the functional explanation of the evolution of 'homogenic incompatibility', that is the inability of organisms to fertilize themselves (Darwin,

1877).

However, a haploid fungus that is (intragametophytically) selfing, combines two identical nuclei in a diploid, followed by meiosis. As the diploid is completely homozygous, recombination cannot occur. Strong selection against deleterious recessive mutants is expected, as these cannot 'hide' behind a dominant non-deleterious allele. Furthermore, haploids by definition cannot be hetero- or homozygous, and can therefore impossibly suffer from a loss of heterozygosity. In this respect only effects of heterozygosity in the short diploid stage after non-intragametophytic selfing can be important. That this may be the case is proposed by Leslie and Raju (1985).

Note also that heterothallics, which are usually regarded as outcrossers, are actually able to perform (intergametophytic) selfing (See figure 5; Fincham et al., 1979). This means that one has to be very careful with applying arguments derived from the study of breeding system evolution in plants, to the fungi (as Olive (1963) and Esser (1966) do).

The sexual mechanism

As described above, the reproductive systems of filamentous ascomycetes can most easily be divided in three types of life cycles: asexual, homothallic and heterothallic. A further division can be made by considering the sexual mechanism, that is the means by which the gametes and, after that, the nuclei fuse (Raper, 1966b). Remarkably, the exact course of events during fertilization is very often unknown.

Normally, in heterothallic fungi, the fertilizing (male) nucleus is present in a (micro-) conidium or spermatium, that functions as pollen in higher plants. However, like in *Neurospora crassa*, also hyphae can function as male. The female 'gamete' is located in the ascogonium, a structure that also holds the trichogyne. This is a hyphal thread that functions as a receptor of the male gamete. After the fusion of trichogyne and conidium, the proliferation of haploid nuclei of both mating types results in the formation of the dikaryotyic ascogeneous hypha. Nuclear fusion occurs in the penultimate cell of the ascogenous hypha and is followed immediately by meiosis. After an additional mitosis eight ascospores are formed (Glass and Kuldau, 1992).

In homothallic fungi the trichogyne is usually absent. There are normally no special structures that function as male gametes. The exact course of events is

rather mysterious, and even in a well studied species like *Aspergillus nidulans* it is still unknown (K. Swart, pers. comm.). Hemmons et al. (1952) described the occurrence of 'relative heterothallism' in this species, as it apparently showed more cross-breeding then expected on the basis of chance. However, this notion could not be confirmed in *Sordaria fimicola* (Olive, 1956) and has only been confirmed in some crosses of *A. nidulans* strains. (K. Swart, pers. comm.).

Far too few species have been studied to know how general the patterns described above are. An attempt to make a survey of the sexual mechanisms in a list of different species has failed by a lack of proper descriptions in the literature.

Exceptional systems

In general the reproductive systems of ascomycetes fit into the scheme described above. There are, however, some curious exceptions, that can illustrate the diversity of genetic systems mentioned in the first paragraph.

A probably rather general anomaly is the occurrence of spore killing, as found in *Neurospora spp.* (Turner and Perkins, 1991; Turner, 1993), *Gibberella spp.* (Kathariou and Spieth, 1982; Sidhu, 1984) and *Podospora anserina* (Padieu and Bernet, 1967; Nauta et al., 1993). It can be observed, when a strain with a Killer genotype is crossed with a strain with a Sensitive genotype. Such a crossing results in the death of four ascospores, all with the Sensitive genotype. So only the Killers survive. It can be considered as a form of segregation distortion, because Mendels law of segregation is severely violated. Chapters 6 and 7 of this thesis are devoted to this phenomenon.

Some species that do not neatly fit the classification *homothallic / heterothallic* are *Chromocrea spinulosa* (Mathieson, 1952), *Sclerotinia trifoliorum* (Uhm and Fujii, 1983; Fujii and Uhm, 1988), *Glomerella cingulata* (Wheeler, 1954), *Nectria haematococca* (Matuo and Snyder, 1973) and *Ceratostomella fimbriata* (Olson, 1949). In all these species (and probably many others) both self-fertility and self-sterility are found, although the genetics is mostly unclear.

In *Chromocrea spinulosa*, for example, the situation seems quite bizarre: Sixteen ascospores are formed, eight large ones and eight small ones. The large spores are self-fertile and the small ones are self-sterile. Selfed large spores do, again, produce the same pattern of sixteen ascospores, eight large ones and eight small ones. The genetic mechanism behind this system is not understood yet; both mutation (Mathieson, 1952) and mating type switching (Perkins, 1987; Anderson et

al., 1988) are suggested.

Another peculiar species is *Podospora arizonensis*, which is apomictic (Mainwaring and Wilson, 1968). It forms eight ascospores, four large ones and four small ones, from which only the large ones germinate in the lab. Before ascospore formation, the two nuclei in the penultimate cell of the crozier do not fuse, but instead they submit to two mitoses. So meiosis is skipped. It is completely unclear why.

Finally, Anagnostakis (1982) performed a crossing experiment in *Endothia parasitica*, in which she used a mixture of conidia, sampled from two different strains, to fertilize a third strain. She found that at least 17 out of 109 randomly isolated ascospores, collected from 4 perithecia found in a sample of 22, had three parents: two fathers and one mother. This was presumably the result of recombination between two paternal nuclei, before nuclear fusion between a 'male' and a 'female' nucleus. From the same (heterothallic) species it is reported that self fertilization can occur after cross fertilization has taken place (Anagnostakis, 1988; Milgroom et al., 1993).

It may be clear that the evolution of the 'general' pattern of genetic sytems in filamentous ascomycetes has to be understood, before the existence of all these exceptional cases can be explained.

Heterokaryosis and the parasexual cycle

For a long time heterokaryon formation has been considered to be beneficial (Pontecorvo, 1946; Jinks 1952a; Davis, 1966). Clearly, heterokaryosis can be regarded as the analogue of heterozygosity and a dikaryotic mycelium can be compared with a diploid. All benefits ascribed to being diploid (like the possibilty of heterozygous advantage and dominance over recessive deleterious alleles) can also be applied to originally haploid individuals if they are heterokaryotic. Moreover, heterokaryosis offers a plastic system which can quickly accomodate environmental changes by an overall change in nuclear ratio (Jinks 1952a; Roper, 1966).

Also, especially for the imperfect fungi, heterokaryon formation seems to offer an alternative for sex, by means of the parasexual cycle (Pontecorvo, 1956; Roper, 1966; Caten, 1981). This cycle allows genetic recombination without sex. Shortly, it consists of the following sequence of events: (1) heterokaryon formation; (2) fusion of two unlike haploid nuclei, forming a diploid; (3) segregation and mitotic recombination; (4) non disjunction and aneuploidy, finally leading to a recombined

haploid.

In imperfect fungi this parasexual, mitotic recombination offers the only possibility for genetic analysis and the construction of genetic maps (Debets, 1990). It may also be important in nature, because it offers opportunities for genetic recombination, which otherwise cannot occur in imperfect species (Pontecorvo, 1956).

However, heterokaryosis and parasexual processes probably only play a minor role in natural populations. Since Caten and Jinks (1966) examined the natural occurrence of heterokaryosis, it has become clear that, although it is shown to occur (Jinks, 1952b; Ming et al., 1966), the frequency and significance of heterokaryosis and parasexual recombination in nature were generally overestimated (Caten, 1981). This is due to the occurrence of vegetative incompatibility reactions, that block heterokaryon formation.

Vegetative incompatibility

Vegetative incompatibility (also referred to as somatic or heterokaryon incompatibility) shows up frequently between different natural isolates of a species (Carlile, 1987; chapter 4). As far as is known, a vegetative incompatibility reaction occurs when two strains differ at one or more alleles at their incompatibility loci. Only strains identical for all their vegetative incompatibility alleles are compatible.

Clearly vegetative incompatibility restricts the occurrence of heterokaryon formation and, as a consequence, parasexual recombination. Its wide occurrence suggests that there may exist a selective pressure favouring incompatibility and, therefore, a selective disadvantage of heterokaryon formation. This would imply that heterokaryosis is generally *not* advantageous.

In chapters 4 and 5 of this thesis, models on the evolution of vegetative incompatibility are discussed. Selective explanations, as suggested by Day (1968), Caten (1972) and Hartl et al. (1975) are studied, in particular the problem to explain the high frequency of vegetative incompatibility reactions between natural isolates.

Individuals and species

Defining the 'individual' is a general problem in biology (Buss, 1987), which evidently also applies to the fungi (Todd and Rayner, 1980; Rayner, 1991). Indiviuals can be defined both as genetically uniform entities and as physiological

entities, for which respectively the terms 'genets' and 'ramets' have been used in plants. (Harper, 1977; Stevens and Van Damme, 1988). If asexual reproduction is considered, the relevance of this distinction is clear: The progeny of one sporulating (imperfect) fungus consists of a large number of ramets, that all belong to the same genet.

Therefore, the mycelial unit, as an independent, physically distinct body-form within a population (Todd and Rayner, 1980) can probably best be regarded as the individual. However, the possibility of heterokaryosis, which allows the establishment of mixed mycelia, can then cause a mixing of 'individuals'. If heterokaryosis is a common feature, a network of interconnected mycelia can be formed, which gives rise to a community of genetically different units (Rayner (1991)).

So if an individual is defined as a genetically uniform entity, it can consist of several physiologically distinct 'ramets', and if it is defined as a physiologically distinct entity, it can be a collection of different genetical units. As the presumable high frequency of vegetative incompatibility predicts a low frequency of heterokaryosis in nature (Carlile, 1987; this thesis, chapters 4 and 5), the physiological entity is considered as the preferable description of the individual in this thesis.

Another problem arises for the definition of 'species'. Usually reproductive isolation is considered to be the best criterion for recognition of a species (Dobshansky, 1950; Mayr, 1970). As noticed by Maynard Smith (1989) and Perkins (1991) this immediately raises the problem, that a species can only be defined if the organisms reproduce sexually. It would be impossible to refer to an imperfect fungal species.

Therefore a species concept like that proposed by Lemke (1973), saying that 'the species is an integral system for genetic recombination, and members of a given species are expected to share a common gene pool', is probably the most adequate one. The possibility of heterokaryosis as a means to exchange genetic information between conspecifics, is included in this concept. However, its practical value is doubtful, as the impact of incompatibility systems in nature is unclear. Furthermore, in fungi also sexual incompatibility and intersterility between (groups of) individuals are widespread (Anderson et al., 1988), and crosses between 'species' are not unusual in fungi (Lemke, 1973; Perkins and Turner, 1988).

These problems are to some extend reflected in nomenclature. Many species that rarely have been reported to reproduce sexually, have a different name for the imperfect and the perfect stage (e.g. *Fusarium moniliforme* is the imperfect stage of *Gibberella fujikuroi*). Of some species the names have changed during the years

(e.g. *Endothia parasitica*, the chesnut blight, nowadays has to be called *Cryphonectria parasitica*), and for many others taxomists disagree (e.g., the thrush fungus, *Candida albicans*, a yeast, has 36 times been described as new and has 96 synonyms in 11 different genera (Lodder and Kreger-van Rij, 1952). Clearly, these taxonomic difficulties form an obstacle for anyone studying fungal literature.

Why using theoretical models ?

The research described in this thesis consists of theoretical models. These population genetic models have the purpose to make statements about the evolution of genetic systems in filamentous ascomycetes, that is, about real organisms. Such models are necessarily oversimplifications of what is happening in nature; they are an attempt to abstract from nature some significant aspects of the true situation (Crow and Kimura, 1970). Processes in nature are often extremely complex and under influence of many, and variable, environmental factors. Incorporating everthing that can be of importance in nature, can and will never be the intention of a theoretical model. When a model would be as complex as nature itself, it does not clarify anything.

The purpose of theoretical modelling in this thesis is therefore to find out to what extend (relatively) simple explanations can account for the phenomena observed in nature. Theoretical models are an excellent tool to investigate arguments, that are used to explain experimental findings. They can test the validity of such arguments, they can result in predictions and raise essential new research questions. One of the important aims of the research described in this thesis is to formulate such predictions and questions, which can stimulate experimental study of fundamental problems in the evolution of genetic systems in fungi.

Outline of the thesis

In this thesis attention is focussed in the first place on the evolution of reproductive systems. With population genetic models, comparable to those that have been build for higher plants (e.g. Charlesworth and Charlesworth, 1978a,b), conditions that can explain the evolutionary transitions of heterothallism to homothallism and vice versa are investigated (chapter 2). Also, explanations for the occurrence of hermaphroditism (in stead of separate male and females, or dioecy) and for asexual reproduction in filamentous ascomycetes are studied (chapter 3). Especially the fact

that hermaphroditic heterothallism has apparently evolved frequently and dioecy has not, seems remarkable.

The evolution of vegetative incompatibility and the high number of Vegetative Compatibility Groups (VCGs) is investigated by means of deterministic (chapter 4) and stochastic models (chapter 5). The role of parasitic nuclear genes and harmful cytoplasmic elements as selective agents is studied and compared with the assumption that vegetative incompatibility is selectively neutral. The influence of population size, mutation rate and random genetic drift is studied.

After these models on general and widespread genetic systems, spore killing is studied as a more aberrant case (chapter 6). The evolutionary dynamics of this form of segregation distortion is investigated, in an attempt to explain its existence.

Finally the special case of sexual non-allelic incompatibility in *Podospora anserina* is studied (chapter 7). As this genetically well-known form of incompatibility proves to be evolutionary enigmatic, a hypothesis is proposed that explains sexual incompatibility as a suppressor of spore killing.

CHAPTER 2

Evolution of mating types

with R.F. Hoekstra
Heredity 68 (1992) : 405-410.

Summary

In the ascomycete family of Sordariaceae both heterothallism (with two mating types) and homothallism (without mating types) are common. A population genetic model is made in an attempt to find out under which conditions evolution from one system to the other is conceivable.

Analysis shows that evolution from hetero- to homothallism is possible, but evolution from homo- to heterothallism is improbable. As in these haploid fungi self-fertilization has other consequences than in diploid organisms, homothallism seems to have little disadvantage.

It is found that polymorphism in homo- and heterothallism can be stable, although this has not yet been found in Sordariaceae in nature.

Introduction

Most population genetic models about the evolution of sex and mating systems concern animals and plants (e.g. Maynard Smith, 1978; Bell, 1982; Stearns, 1987; Michod and Levin, 1988). The fungi are largely overlooked. Some of the reasons for this may be the relative lack of knowledge about their population structure and genetics, the complex life cycle of many fungi and the puzzling variety in reproductive systems. This variation however also offers an opportunity for comparative studies of the evolutionary forces that shape the different mating systems.

This study presents a model of the evolution of mating types in filamentous Ascomycetes, exemplified by the family Sordariaceae. This family includes some genetically well-known species like *Neurospora crassa, Podospora anserina* and *Sordaria fimicola*, living on rotten plant material or herbivore dung. They show relatively simple life cycles (see below). Some population genetic (Perkins and Turner, 1988) and molecular (Glass et al., 1990) data are also available and

provide useful information. The model will probably also be valid for many other ascomycete species, but these are not treated explicitly here.

In the Sordariaceae (as in many other ascomycete families) roughly two mating systems exist: homothallism and heterothallism. Homothallic species are self-fertile and have no mating types, whereas heterothallic species are self-sterile and posses mating types. Here 'mating types' is defined as 'two different sexes without morphological sex-differentiation'. These mating types get different names in different species, but are called + and - in this study.

Note that the terms monoecy and dioecy are confusing in this context. In plants these terms refer to species in which individuals produce gametes of only one sex or of both sexes. All Sordariaceae make both, independent of mating type. (The implication of this fact will be discussed in chapter 3.

A remarkable phenomenon is the occurrence of both homo- and heterothallic species within many related ascomycete genera and families. This means that homo- and/or heterothallism must have evolved independently quite often. One may suspect, then, that the thresholds for switching from one system to the other cannot be too high.

The purpose of this study is to discover the conditions, defined in general fitness parameters, under which homothallism can evolve to heterothallism and vice versa.

The model

The model is based on a typical Sordariaceae life cycle as presented in figure 1. Note the following characteristics:

1) The life cycle is haploid. There is only a very short stage of diploidy (in the young ascus) which is immediately followed by meiosis.
2) Each individual mycelium forms both conidia and ascogonia, that is both male and female gametes. As stated above this is completely independent of mating type.
3) The conidia serve as both male gametes and asexual spores. (This is a simplification of the situation found in *N. crassa*, where micro- as well as macroconidia exist. The first seem to serve mainly as fertilizing agent and the second as asexual spore (Perkins and Turner, 1988). In the laboratory, however, both can perform both functions.)
4) Because of haploidy self-fertilization does not imply recombination. (A similar phenomenon in ferns is called intragametofytic selfing (Klekowski, 1979; Hedrick,

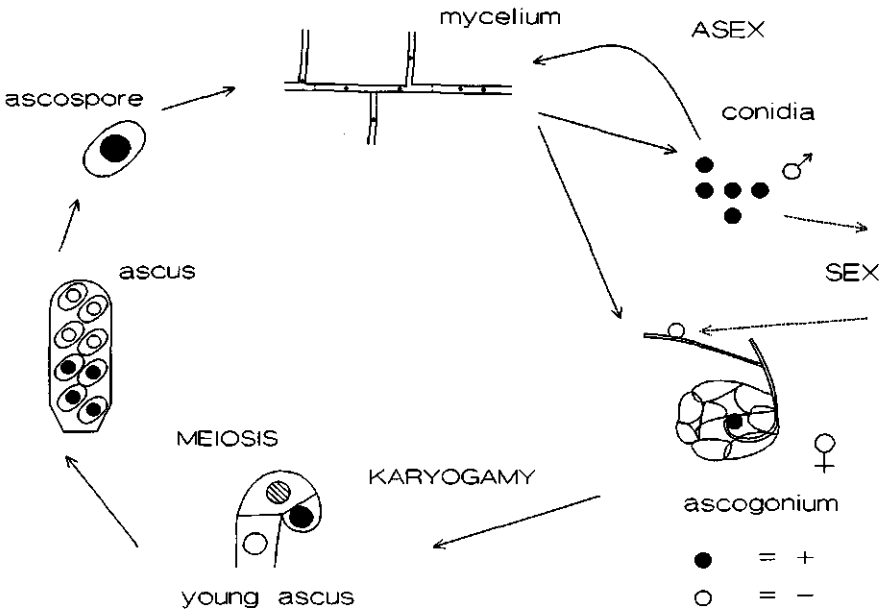

Figure 1. Life cycle of the heterothallic model organism. A haploid mycelium contains either nuclei of mating type + (black dots) or of mating type - (open dots). The conidia can develop asexually into a new mycelium or fertilize ascogonia of the opposite mating type. After karyogamy and meiosis an ascus with eight ascospores (four of each mating type) is formed. The homothallic model organism has the same life cycle, but has no mating types (self-fertilization is possible).

1987).) From a genetic point of view then the formation of selfed spores is equivalent to forming asexual spores.

Furthermore, the following assumptions are made in the model:

There are two heterothallic mating types, + and -, with frequencies x_1 and x_2, and one homothallic 'mating type' ± with frequency x_3 ($x_1 + x_2 + x_3 = 1$). These three types are assumed to be determined by three alleles at one locus. The fitness of a heterothallic cross + x - equals 1, the crosses ± x - and ± x + have a fitness w_1 ($w_1 \leq 1$). The homothallic crossing ± x ± has a fitness w_2 when it concerns outcrossing (frequency 1-s) and w_3 when selfing (frequency s).

All mycelia produce the same amounts of ascogonia and conidia. There is an excess of conidia formed, so all ascogonia are fertilized. (This can be compared with ovules and pollen in higher plants. (Charlesworth and Charlesworth, 1978b))

The conidia disperse randomly over the area. Some land on unoccupied substrate

and have a chance to germinate. Others land on a mycelium and may be able to fertilize ascogonia. There is an active attraction between unlike mating types as in, for example, *Podospora anserina* (Esser, 1959) and *Bombardia lunata* (Zickler, 1952). Identical mating types do not attract each other. This means that both a + conidium landing on a + mycelium, and a - conidium landing on a - mycelium will get lost. All ascogonia on a heterothallic mycelium will be fertilized, but of course not by conidia of its own mating type.

Both sexual spores (ascospores) and asexual spores (conidia) are formed. The difference in fitness between these spores (relating to differences in viability as well as frequency) is expressed in the parameter θ, denoting the fitness of an ascospore relative to that of a conidium. Since θ will appear of no importance in the analysis of this model, its precise definition will be discussed in chapter 3.

Taking all these assumptions into account the following recurrence relations can be derived:

$$Wx_1' = x_1[1+\tfrac{1}{2}\theta(\frac{x_2+w_1x_3}{x_2+x_3}+\frac{x_2}{x_1+x_3}+w_1(1-s)x_3)] \quad \textbf{(1a)}$$

$$Wx_2' = x_2[1+\tfrac{1}{2}\theta(\frac{x_1+w_1x_3}{x_1+x_3}+\frac{x_1}{x_2+x_3}+w_1(1-s)x_3)] \quad \textbf{(1b)}$$

$$Wx_3' = x_3[1+\tfrac{1}{2}\theta((1-s)(2w_2x_3+w_1(x_1+x_2))+w_1\frac{x_2}{x_1+x_3}+w_1\frac{x_1}{x_2+x_3}+2w_3s)] \quad \textbf{(1c)}$$

where

$$W = 1+\theta[x_3(1-s)(w_2x_3+w_1(x_1+x_2))+x_2\frac{x_1+w_1x_3}{x_2+x_3}+x_1\frac{x_2+w_1x_3}{x_1+x_3}+w_3s] \quad \textbf{(1d)}$$

When $x_1 = x_2$ (which can be shown to be the case in all equilibrium conditions, see Appendix) ,with $x_1 + x_2 = p$ and $x_3 = q$, this can be simplified to

$$Wp' = p[1+\tfrac{1}{2}\theta(\frac{p+w_1q}{\frac{1}{2}p+q}+w_1(1-s)q)] \quad \textbf{(2a)}$$

$$Wq' = q[1 + \tfrac{1}{2}\theta((1-s)(2w_2q + w_1p) + \frac{w_1p}{\frac{1}{2}p + q} + 2w_3s)] \quad \textbf{(2b)}$$

where

$$W = 1 + \theta[q(1-s)(w_1p + w_2q) + p\frac{\frac{1}{2}p + w_1q}{\frac{1}{2}p + q} + w_3sq] \quad \textbf{(2c)}$$

When (2b) is rewritten as

$$\frac{q'}{q} = \frac{F(q)}{W}$$

it is easy to see that q will increase when $f(q) = F(q)\text{-}W > 0$

It can be deduced that $f(q) = A.q^2 + B.q + C$, with

$A = (1\text{-}s)(w_2 - w_1)$
$B = 1 + w_2(1\text{-}s) + w_3s - \tfrac{1}{2}w_1(5\text{-}s)$
$C = \tfrac{1}{2}w_1(3\text{-}s) + w_3s - 1$

There will be an equilibrium when $f(q) = 0$, so two equilibrium points can be deduced, one stable:

$$\hat{q}_1 = \frac{-B - \sqrt{B^2 - 4AC}}{2A} \quad \textbf{(3)}$$

and one unstable:

$$\hat{q}_2 = \frac{-B + \sqrt{B^2 - 4AC}}{2A} \quad \textbf{(4)}$$

Using the Taylor expansion it can be shown that for $p \approx 0$: heterothallism can invade if $A+B+C < 0$, that is if

$$w_1 > 2\frac{w_2(1-s) + w_3s}{2-s} \quad \textbf{(5)}$$

and for $q \approx 0$: homothallism can invade if $C > 0$, that is if

$$w_1 > 2\frac{1-w_3 s}{3-s} \qquad (6)$$

There appear to be four possible states: homothallism (ho), heterothallism (he), stable polymorphism (po) and a frequency- dependent state (fd), where neither type can invade (heterothallism being stable if $q > \hat{q}_2$, and homothallism if $q < \hat{q}_2$). Conditions (5) and (6) describe which of these states is reached, except in cases when $0 < \hat{q}_1, \hat{q}_2 < 1$ where the final state depends on q. The four states occur in the following cases (for all q, except when stated otherwise) :

po: ($C > 0$ and $A+B+C < 0$) or ($C > 0$ and $A+B+C > 0$ and $A > -½B > 0$ and $q < \hat{q}_2$) or ($C < 0$ and $A+B+C < 0$ and $A < -½B < 0$ and $q > \hat{q}_2$)
fd: $C < 0$ and $A+B+C > 0$
he: $C < 0$ and $A+B+C < 0$ and not ($A < -½B < 0$ and $q > \hat{q}_2$)
ho: $C > 0$ and $A+B+C > 0$ and not ($A > -½B > 0$ and $q < \hat{q}_2$)

Before discussing some special cases, it should be noticed that the invasion of heterothallism in a homothallic population is very unlikely to happen with two mating types (two simultaneous mutations) at once. That is, one has to consider the introduction of one mating type first. This means that $x_1 = 0$ or $x_2 = 0$. Elaborating this case gives condition (1) again for heterothallic invasion. The frequency q must be 0.5 at least, because the ± mating type must also serve as heterothallic partner.

The expressions A, B and C in formulas (3) and (4) now become

$A = 2(1-s)(w_2-w_1)$
$B = 2sw_3 - (1+s)w_2$
$C = w_1$

After the right mutation the second mating type can invade under the same conditions as the first (condition (5)).

To achieve a better impression of these formulae, some special cases will be considered (See figure 2 for illustrations)

1) No selfing : $s = 0$

Homothallism can invade if $w_1 > 2/3$ and heterothallism if $w_2 < w_1$.

2) All homothallics are selfing : $s = 1$

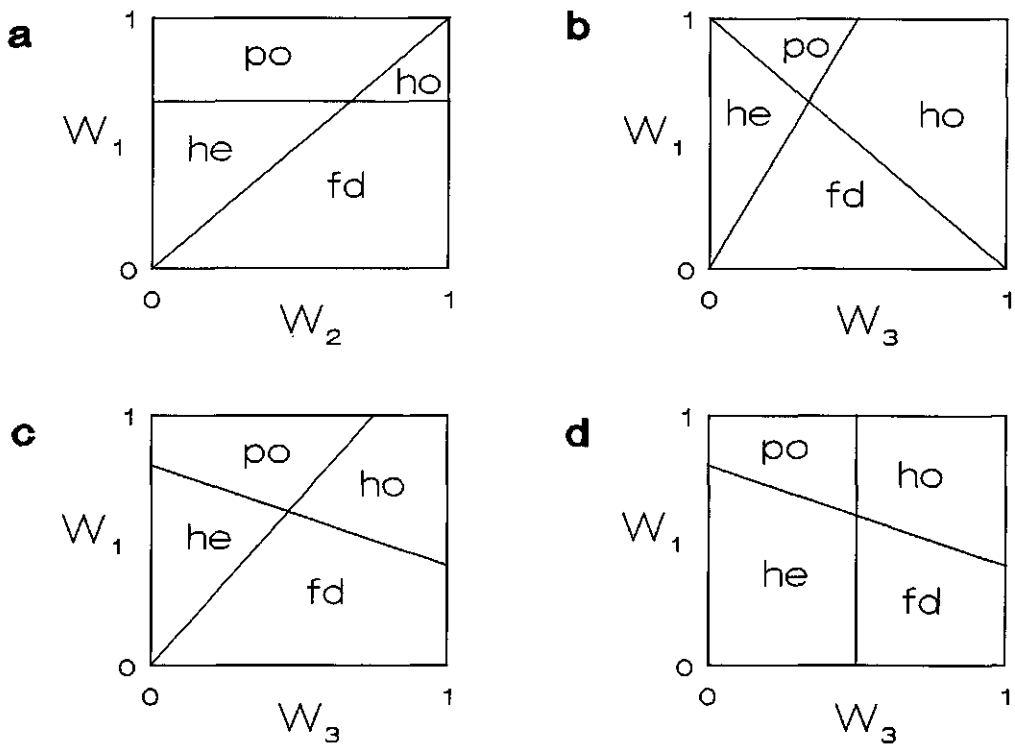

Figure 2. Equilibrium states in four different cases. po = stable polymorphism, he = heterothallism, ho = homothallism, fd = frequency dependent. a: s=0 (no selfing, discussed as case 1 in the main text), b: s = 1 (all homothallics are selfing, case 2), c: s = 0.5 and $w_2 = w_3$ (homothallic crosses are equally fit), d: s = 0.5 and $w_1 = w_2$ (outbreeding crosses with homothallics are equally fit).

Homothallism can invade if $w_1 > 1\text{-}w_3$ and heterothallism if $w_1 > 2w_3$.

3) All outcrossing sex has the same fitness : $w_1 = w_2 = 1$

(a) selfing mildly deleterious : $w_3 \geq 0.5$

Heterothallism can never invade, homothallism is stable.

(b) selfing strongly deleterious : $w_3 < 0.5$

Both homo- and heterothallism can invade, polymorphism is stable.

It is clear that conditions for homothallism to invade a heterothallic population will be much easier realized then conditions for heterothallism to invade. A heterothallic population can only be stable with strong selection pressure against homothallism and/or selfing.

The model seems to suggest, therefore, that evolution from hetero- to homothallism may be possible, but that evolution from homo- to heterothallism is

expected to be rare. At the same time it shows that, when evolution from homo- to heterothallism or vice versa occurs, one should also expect to find populations polymorphic for this trait.

Discussion

One of the few discussions on the evolution of heterothallism in ascomycetes has been given by Olive (1958, 1963). He assumes that in the early evolution of the fungi homothallism preceded heterothallism. In the homothallic species *Sordaria fimicola* a number of mutations affecting the sexual process have been found. In the laboratory 'heterothallism' could be created by selecting for self-sterile colonies that could be crossed with each other (El Ani and Olive, 1962). It is suggested that heterothallism might evolve by 'the occurrence and association of pseudo allelic self-sterility mutations in a compound locus of two or more subunits'.

The nature of the selective forces that promote the evolution of heterothallism u under natural conditions is not clear, but according to Raper (1968) and Esser(1971, 1974) these should mainly be the promotion of outbreeding and the prevention of inbreeding. This last argument, however, deserves a closer look. What is meant by inbreeding is actually intragametofytic selfing. (Selfing as it occurs in diploids, intergametofytic selfing, is possible with both homo- and heterothallism in these fungi.) This selfing does not imply any recombination and is in fact equivalent to asexual reproduction. This means the 'usual' disadvantages of inbreeding are not applicable here.

A model on the evolution of mating types in isogamous populations has been studied by Hoekstra (1982, 1987). Using comparable parameters (and with $s = 0$), he found that heterothallism can invade if $w_1 > w_2$ and homothallism if $w_1 > 1/2$. The difference with the present model is the second condition, which is less severe here ($w_1 > 2/3$). The reason for this is the gamete differentiation in the present model and the fact that no ascogonia get lost by incompatible fusions.

It does not mean, however, that the evolution of heterothallism has become easy. It is hard to find convincing reasons why w_1, w_2 and/or w_3 should be a lot smaller than 1.

The idea that heterothallism must have preceded homothallism in evolution is supported by DNA sequencing of the mating type genes of *Neurospora crassa* and the comparison of these sequences with other Sordariaceae (Glass et al., 1990; Metzenberg and Glass, 1990). It is found that + and - (called A and a in

Neurospora) are dissimilar and that most (but not all) homothallic species carry homologous sequences of both mating types in one haploid genome. Mating type switching like in yeast (Herskowitz, 1988) is very improbable in the Sordariaceae.

The fact that most homothallic Sordariaceae do not form conidia is not reflected in the model assumptions (Perkins and Turner, 1988), which means that outcrossing can only take place by occasional mycelial contact. So in homothallic species in nature, the frequency of selfing must be close to 100 per cent. This is supported by the finding that RFLP and mating type analysis show far less variation within homothallic species then within heterothallic species (Glass et al., 1990).

This possible lack of conidia may make the model less valid for the evolution of homo- to heterothallism. It is easy to see, however, that a more realistic model would put a heterothallic mutant at an even greater disadvantage than in the present model, because it decreases the chance of finding an appropriate mate. This will therefore only produce more severe conditions for the evolution of heterothallism.

The only explanation for the existence of heterothallic Sordariaceae seems to be that in some cases the fitness thresholds for intermediate stages are too high. More ecological research on these species is needed, to find out if and why that should be the case.

Note that the model suggests that polymorphism in homo- and heterothallism may very well be stable. In Sordariaceae no report of such polymorphism has been found. In some species of comparative ascomycete families both homothallic and heterothallic strains are described (e.g. *Glomerella cingulata* (Wheeler, 1954), *Gibberella zeae* and *Nectria haematococca* (Booth, 1971)), but the stability of these strains in nature is somewhat obscure. It may very well be that polymorphism occurs in some species but has never been reported.

The lack of conidia in many homothallic species, the differentiation in male and female gametes in addition to mating types, and the existence of related fungi imperfecti, offer some intriguing additional questions. These will be analyzed in a comparative model in the next chapter.

Appendix

Equal heterothallic frequencies are stable.

Formulas (1a) and (1b) can be rewritten to

$$\frac{x_1'}{x_2'} = \frac{x_1(a+bx_2)}{x_2(a+bx_1)}$$

with

$a = (1+½\theta w_1(1-s)x_3)(1-x_1)(1-x_2) + ½\theta w_1 x_3$

$b = ½\theta(1+x_3(1-w_1))$

It is easy to see that x_1 and x_2 are mutually interchangeable, so if x_1/x_2 converges to 1 for $x_1 < x_2$, it will also converge to 1 for $x_2 < x_1$.

If $x_1 < x_2$, then x_1/x_2 converges to 1 without oscillations if

$$\frac{x_2}{x_1} > \frac{a+bx_2}{a+bx_1} > 1$$

As both $a>0$ and $b>0$, these conditions hold if $x_1 < x_2$.

So x_1/x_2 always converges to 1 and equal frequencies of the heterothallic genotypes are stable.

CHAPTER 3

Hermaphroditism and other reproductive strategies

with R.F. Hoekstra
Heredity 68 (1992) : 537-546.

Summary

The evolution of different reproductive systems in filamentous ascomycetes is studied in a population genetic model. These fungi differ essentially from higher plants and animals, because mating types can exist in addition to male and female gametes, and the conidia serve as both male gametes and asexual spores; moreover selfing is genetically equivalent to asexual reproduction in these haploid organisms.

A variable fitness of ascospore production is predicted as the explanation for the evolution of two systems that abundantly exist in nature: hermaphroditism in heterothallic species, and the formation of both asexual and sexual spores in homothallic species. Imperfect fungi will evolve if sexual spores do not show a remarkably higher fitness than asexual spores.

Introduction

A great variety of reproductive systems exist in filamentous ascomycetes. Compared with the reproductive systems in higher plants, some specific differences attract attention. The first is the possible occurrence of mating types in addition to the existence of both male and female gametes (discussed in the previous chapter), where only crossings between male and female with unlike mating type are possible. The second is that both sexual and asexual spores exist, while the asexual spores (conidia) can often also serve as male gametes. Finally the fact that the gamete-producing individual is haploid has some special implications for selfing and inbreeding.

Mating systems such as monoecy, dioecy, gynodioecy and trioecy are known in plants. The evolutionary forces that can account for these different types have been thoroughly studied (Charlesworth and Charlesworth, 1978a, b; Charlesworth and

Ganders, 1979; Gregorius et al., 1982, 1983; Ross, 1982). For fungi, however, such studies appear to be lacking.

This paper is a theoretical analysis of the evolutionary relations between a number of fungal reproductive strategies. Attention is mainly limited to the family of Sordariaceae, which contains a number of well-studied species like *Neurospora crassa* and *Sordaria fimicola*; many ascomycete mating systems are represented.

All heterothallic Sordariaceae investigated to date have both conidia (male gametes) and ascogonia (structures holding the female gamete) and can therefore be considered to be hermaphrodite. No report on a natural dioecious filamentous ascomycete (i.e. with separate male and female individuals) is known to us. This leads to the question why, in heterothallic Sordariaceae, hermaphrodites with mating types do occur, whereas separate males and females without mating types do not.

It is also remarkable that in many homothallic species (such as the homothallic Neurosporas and *Sordaria fimicola*) the conidia are absent and ascogonia no longer form trichogynes (small hyphae growing towards fertilizing conidia). This means that no asexual spores are formed and outcrossing is probably very rare, being only possible after heterokaryosis.

Another phenomenon in fungi, incomparable to higher plants, is the existence of imperfect fungi, in which no sexual stage is found. Although taxonomically they are not classified as ascomycetes, most can be considered as such because they show all characteristics of ascomycetes except a sexual cycle (e.g. Ainsworth, 1973). These imperfect fungi can be considered as pure male ascomycetes, which only form conidia and no ascogonia. As there are no more females to fertilize, the conidia have ceased to function as male gametes and are now specialized as asexual spores.

A model is developed in this study in an attempt to find evolutionary pathways for all these different mating systems, as illustrated in figure 1. The important fitness parameters are the differences between sexual and asexual reproduction and between selfing and outbreeding.

The model

The model organism in this study is the idealized ascomycete described previously (chapter 2). It has a haploid life cycle and in principle can form both conidia and ascogonia. In the young ascus karyogamy and meiosis take place and

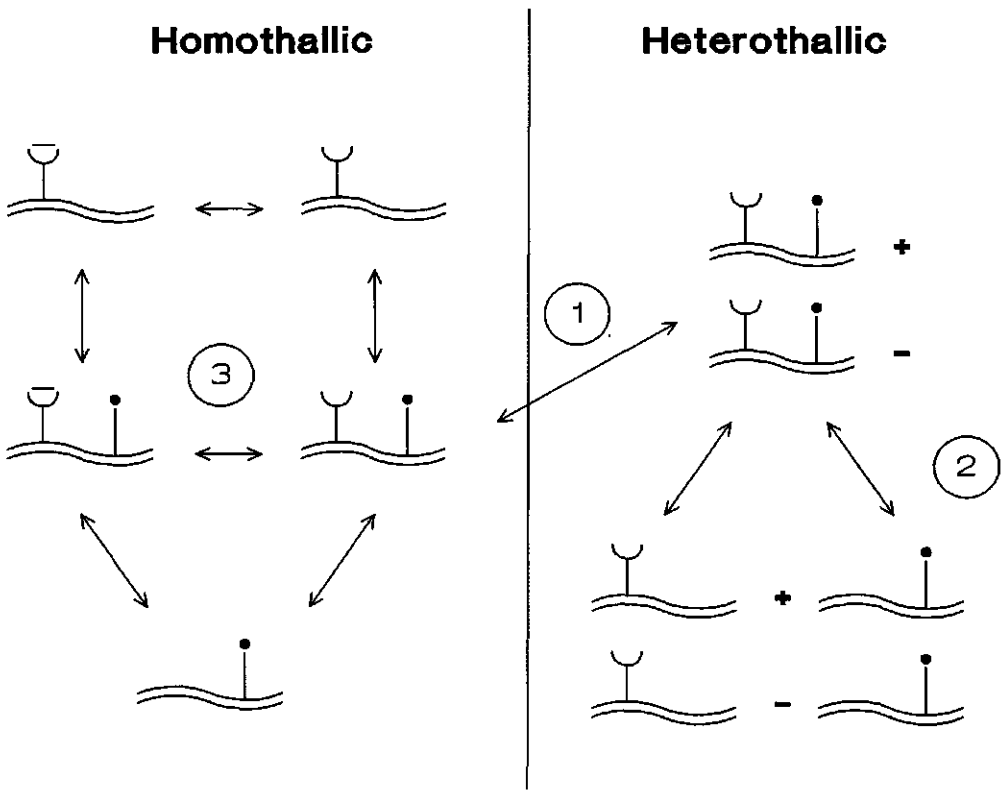

Figure 1. Overview of the evolutionary transitions considered. Conidia are symbolized by a dot above a stalk, ascogonia by a cup on a stalk (ready to catch airborne conidia). Strictly selfing ascogonia that do not get fertilized by conidia are symbolized by a cup covered with a lid.
Arrows indicate possible transitions between: (1): Homothallics and heterothallics (chapter 2). (2): Hermaphrodite, male and female heterothallics (Section A). (3): Different types of homothallics (Section B).

the ascospores are formed. Population size is assumed to be infinite and generations are separated.

It is assumed that the conidia and ascospores formed are dispersed randomly over the habitat of the population. They can land on three different kinds of substrate: (1) on an appropriate site where they can germinate, (2) on an unfit site where they cannot survive, or (3) on a site which is already occupied by another individual. In the last case a landing conidium can fertilize an ascogonium of that individual, whereas a landing ascospore will get lost. It is assumed that there are always sufficient conidia in the population to fertilize all available ascogonia.

The 'twofold disadvantage of sex' (Maynard Smith, 1971) or 'cost of genome dilution' (Lewis, 1987) is manifested in the production of ascospores. In the ascospores half of the genes are of paternal origin (the conidium) and half of maternal origin (the ascogonium), while in an asexual spore all genes are from 'paternal' origin. Although half of its genome is lost, a conidium may profit by sexual reproduction, because the female parent provides the resources for producing several (normally eight) ascospores out of one conidium. This female parent, however, experiences a twofold cost of sex, compared to a situation where she would produce asexual spores or selfed ascospores. It is essential, therefore, that ascospores, being more resistant and capable of remaining viable for long periods, have a higher fitness than asexual spores (Perkins and Turner, 1988). In addition, some kind of inbreeding depression must occur to explain outbreeding.

Several types of individual are considered. They differ in the production of male and female gametes and in the frequency of self-fertilization. An individual producing only conidia (a 'male') forms N conidia, and an individual producing only ascogonia (a 'female') forms n ascogonia. A hermaphrodite forms αN conidia and βn ascogonia. As the energy required to form a conidium (just a small cell) must be much lower then that needed to form an ascogonium (the receptive structure) plus a fruiting body filled with ascospores, we assume $n \ll N$. If all individuals can use the same amount of energy for reproduction, then $\alpha + \beta = 1$ for all hermaphrodites. This is assumed in the model, so we may define $\alpha = z$ and $\beta = 1\text{-}z$, where z is the 'maleness' of the individual.

The selfing rate for homothallic species is s. Outbred and selfed progeny have a relative fitness of 1 and d respectively.

As elaborated in Appendix I a general recursion equation can be derived from these assumptions. It is shown that the differences in fitness between sexual and asexual spores can be summarized by a single parameter θ for the fitness of ascospore production.

When a population contains different types of individuals, where type j has a frequency x_j, a maleness z_j and a selfing rate s_j, this general recurrence relation is given by:

$$Wx_j' = x_j[z_j(1+\tfrac{1}{2}\theta\frac{\sum_k (1-s_k)(1-z_k)x_k}{\sum_k z_k x_k})+\tfrac{1}{2}\theta(1-z_j)(1-s_j(1-2d))] \quad (1)$$

where

$$W = (1-\theta)\sum_k x_k z_k + \theta(1-(1-d)\sum_k (1-z_k)s_k x_k)$$

Note that the model covers both homo- and heterothallic populations, but not populations polymorphic for this trait.

In subsequent sections a number of specific models are analysed. The different types of individual and the possible transitions between them are shown schematically in figure 1. Both a constant and a variable ascospore fitness parameter θ are considered.

A1. Heterothallic population with males, females and hermaphrodites. Constant ascospore fitness θ

Consider a population with three types j, $j = 1$ representing males ($z_1 = 1$, $s_1 = 0$), $j = 2$ representing hermaphrodites ($z_2 = z$, $s_2 = 0$) and $j = 3$ representing females ($z_3 = 0$, $s_3 = 0$).

From the resulting recurrence relations (see Appendix II), it can be deduced that the population is in equilibrium if

$$x_1 + zx_2 = \frac{\frac{1}{2}\theta}{\theta - 1} \quad (2)$$

It is easy to see that for $\theta \leq 2$ a pure male population is stable. This implies that with $\theta \leq 2$ the evolution of imperfect fungi is expected. For $\theta > 2$ polymorphism is stable; the equilibrium values of x_j depend on the starting frequencies and are located on a straight line segment (figure 2). Thus, in an infinite population, one should expect to find trioecy: populations with hermaphrodites, males and females.

In a finite population random genetic drift may cause the frequencies in the population to change along the equilibrium line (2). As stated in Appendix II no specific tendency towards dioecy or hermaphroditism could be found, so in the

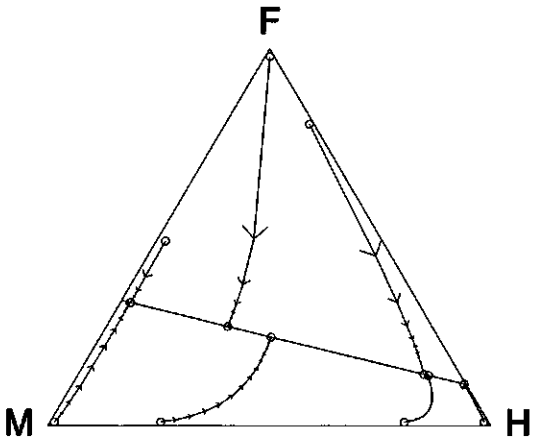

Figure 2. Results for heterothallism with constant ascospore fitness θ, as described in section A1, with $\theta = 4$ and $z = 0.75$. Frequencies of Males (M), Females (F), and Hermaphrodites (H) are given in a de Finetti diagram. Starting with different frequencies x_j, stability is reached when $x_1 + zx_2 = \frac{1}{2}\theta/(\theta-1)$, given as a solid line in the diagram. Arrows indicate the frequency every subsequent generation.

long-term a finite population may either become dioecious (males and females) or consist of hermaphrodites with either males or females.

A2. Heterothallic population with males, females and hermaphrodites. Variable ascospore fitness θ

Sexual spores are often formed under conditions of stress alone. In the model this can be represented by a varying θ. It is assumed that the fitness of ascospores is rather low for many generations, but occasionally very high. This situation has been studied numerically. An example of the results, where $\theta = 2.166$ for 9 generations and $\theta = 1000$ every 10th generation (so the geometric mean $\theta_G = 4$), is illustrated in figure 3. It is shown that due to the fluctuations the hermaphrodite frequency slowly increases.

It is found that, unlike with a constant θ, with a varying θ causes trioecy to be unstable. If θ_i represents the value of θ in generation i, the population will end up as pure male, i.e. imperfect if

$$\prod_i (z+\tfrac{1}{2}\theta_i(1-z)) < 1 \qquad (3)$$

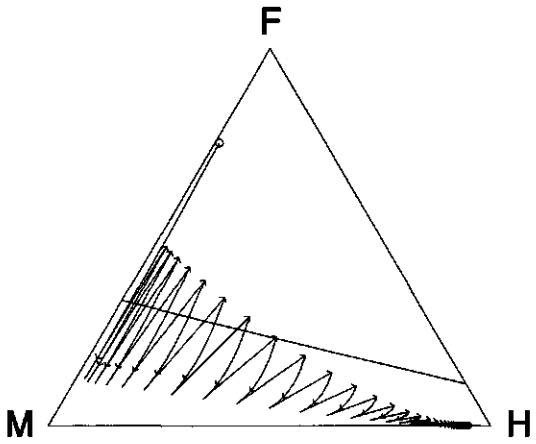

Figure 3. Results for heterothallism with variable ascospore fitness θ, as described in section A2, with $\theta_G = 4$ and $z = 0.75$. Every 10th generation $\theta = 1000$. The line $x_1 + zx_2 = \frac{1}{2}\theta_G/(\theta_G - 1)$ is given as in figure 2. Arrows indicate frequencies every 5th generation. Finally a polymorphism of hermaphroditism and males is reached.

If this is not true the population will become polymorfic hermaphrodite/male or hermaphrodite/female, or will become purely hermaphroditic. The conditions for these different results are given in Appendix II.

The fate of different hermaphrodites (with different values of z_j), occurring simultaneously in the population, has been investigated in some additional numerical studies. Our calculations show that such a situation is rather complex. The equilibrium frequencies for the different types of hermaphrodite partly depend on starting frequencies, and a number of hermaphrodite types can invade under a variety of circumstances. If hermaphrodites with any possible value of z_j can exist, the population will always become purely hermaphroditic, polymorphic for z_j.

B1. Homothallic population with selfing, non-selfing, conidiating and non-conidiating types. Constant ascospore fitness θ

Self-fertilization is possible in a homothallic population, so s_j will have values greater than 0. As emphasized before, most homothallic species do not form trichogynes, so the conidia (if present) will hardly be able to fertilize the ascogonia. It is assumed that there can be two types of pure selfer ($s_4 = s_5 = 1$), both with ($z_4 = z$) and without ($z_5 = 0$) conidia. Fertilization occurs internally in

these types by fusion of two (usually genetically identical) nuclei within an ascogenous hypha, as for example in *Sordaria fimicola* and *Aspergillus nidulans*.

In addition to these two types of individuals, males, females and hermaphrodites are also considered. The latter now have the ability to self fertilize ($s_2 = s$).

The five types j = 1 to 5 are symbolized by **i**, **ui**, **u**, **ûi** and **û** in accordance to the symbols used in figure 1; **i** stands for a conidiophore with a conidium and **u** stands for the receptive structure of the ascogonium, which is blocked in a strictly selfing **û**). An overview is given in table 1 and the recurrence relations are shown in Appendix III.

Analysis of the recurrence relations shows that the values of the parameters θ and d are important in determining which types of individuals can form a stable population.

If the fitness, d, of selfed offspring is smaller than 0.5, the strictly selfing types, **û** and **ûi**, will disappear; the situation is comparable to the cases described in section A1. All hermaphrodites with $s_2 > 0$ will disappear, however, and a stable population will contain only males (if $\theta \leq 2$) or only males and females (with possibly hermaphrodites with $s_2 = 0$) (if $\theta > 2$).

It is easy to see, from a consideration of the recurrence relations in Appendix III, that for $d > 0.5$ the strictly selfing types **û** and **ûi** always have a higher fitness then the types fertilized by conidia, **u** and **ui**. Competition between **û**, **ûi** and **i** will

Table 1. Survey of the types used in the model. (The genus *Aspergillus* does not belong to the Sordariaceae, but the species mentioned seem to fit into the model quite well).

j	Name	s_j	z_j	symbol	example species
1	male/imperfect	0	1	i	*Aspergillus niger*
2	hermaphrodite	s	z	**ui**	*Neurospora crassa*
3	female	0	0	**u**	(none)
4	conidiating selfer	1	z	**ûi**	*Aspergillus nidulans*
5	non-conidiating selfer	1	0	**û**	*Sordaria fimicola*

result in a population either monomorphic for **û** (if $\theta d > 1$) or monomorphic for ***i*** (see Appendix III). So the model suggests that a homothallic species like *Aspergillus nidulans*, which forms conidia that serve as asexual spores, can never be stable if the ascospore fitness is constant.

B2. Homothallic population with selfing, non-selfing, conidiating and non-conidiating types. Variable ascospore fitness θ

As in the heterothallic population considered in section A, the model with a variable θ gives results that differ from those of the constant θ model.

As in section B1 where $d<0.5$ the selfing types **û** and **ûi** are never stable in this population. However, some types of partly selfing hermaphrodites (**ui**) can now invade in the population. (The conditions for this are given in Appendix III). As hermaphrodites with $s_2 = 0$ are again the optimal hermaphrodite type, the situation for $d < 0.5$ is apparently identical to the one described in section A2 for heterothallic populations.

The competition between **û**, **ûi** and **i** can be won for $d > 0.5$ by each of the three types this time. As calculated in Appendix III, it can be deduced that with a variable θ (with $\theta = \theta_i$ in generation i), a population will become monomorphic for **ûi** if

$$\prod_i (z+\theta_i d(1-z)) > \prod_i \theta_i d \quad \text{and} \quad \prod_i (z+\theta_i d(1-z)) > 1 \qquad \textbf{(4)}$$

If these inequalities are not satisfied, the population will become monomorphic for **i** if

$$\prod_i \theta_i d < 1 \qquad \textbf{(5)}$$

and monomorphic for **û** if

$$\prod_i \theta_i d > 1 \qquad \textbf{(6)}$$

A summary of the results is given schematicly in table 2.

Table 2. Summary of the results described in sections A and B. (The notation is explained in table 1.)

Heterothallic (Section A)	Homothallic (Section B)
constant θ	
θ < 2 : **i**	d < 0.5:
	θ < 2 : **i**
	θ > 2, $s \neq 0$: **i** + **u**
	$s = 0$: **i** + **ui** + **u**
θ > 2 : **i** + **ui** + **u**	d > 0.5:
	$\theta d < 1$: **i**
	$\theta d > 1$: **û**
variable θ	d < 0.5:
i (eq. (3))	as Heterothallic, but disadvantage **ui**
or	if s ≠ 0 (eq. (19))
ui(+**i**)(+**u**)	
	d > 0.5:
	i, **ûi** or **û** (eq. (4))

Discussion

The model suggests that in heterothallic populations hermaphroditism can only be stable if the fitness of ascospore formation, expressed in the parameter θ, is variable. With a constant θ no advantage was found for hermaphroditism above dioecy or trioecy. However, as is apparent from the precise definition of θ as given in Appendix I, a constant θ is biologically quite improbable. Variation in θ will result not only from environmental heterogeneity affecting ascospore fitness, but also from variation in fractions of germinating and fertilizing conidia. Therefore a variable θ is the most relevant case and the evolution of hermaphroditism is expected.

The model shows for homothallism that only for a varying ascospore fitness are there conditions for stability of hermaphroditism (**ui**) or selfing with conidia formation (**ûi**). No report has been found of the first in the homothallic Sordariaceae, and the second seems to be rare (Perkins and Turner, 1988). This is in accordance with the model, where these outcomes are only found for rather

restricted parameter combinations.

The occurrence of 'conidiating selfers' like *Aspergillus nidulans* is an interesting phenomenon, since both the sexual and the asexual spores are genetically identical to the parent. This coexistence of two types of spores is comparable to the seed heteromorphism found in higher plants, which can also only be explained by some environmental variation in space or time (Venable and Brown, 1988). Whether meiosis, preceding ascospore formation, still has a function in these species, or should be considered a phylogenetic artifact remains to be questioned.

In the previous chapter the evolution of mating types and the competition between homo- and heterothallic types has been studied. It was found that if all outcrossing sex has equal fitness, homothallism is stable if the fitness of selfing $d > 0.5$ and polymorphism for the two types is stable if $d < 0.5$. Heterothallism is only stable if homothallism has some additional disadvantage.

In the current model (B1 and B2) the condition $d < 0.5$ reappears. If this condition is satisfied, a non-selfing hermaphrodite is the optimal type if the ascospore fitness θ varies. It could be that heterothallism is the only way for a species to prevent selfing. A problem is that with heterothallism the frequency of potential mates is lowered. A polymorphic homo- and heterothallic population is expected (chapter 2), which is unknown to exist in nature.

The 'cost of genome dilution' (Lewis, 1987), is revealed twice in the model. Both selfing and asexual reproduction are forms of uniparental inheritance, where the progeny is genetically identical to the parent. The advantage of these modes of reproduction over sexual reproduction are twofold in principle (Maynard Smith, 1971). This factor two shows up for the parameters d (for selfing) and θ (for asexual reproduction), $d = 0.5$ and $\theta = 2$ being the threshold values in the model.

Charnov et al. (1976) and Maynard Smith (1978) studied a model for resource allocation in hermaphrodites, where males produce N sperm, females produce n eggs and hermaphrodites αN sperm and βn eggs. They find that hermaphroditism is stable if $\alpha + \beta > 1$ and dioecy if $\alpha + \beta < 1$. The case of $\alpha + \beta = 1$, as is assumed in the current model, is a neutral case in their model. (Their findings are comparable to what is found in section A1, where all types are neutral if the population has reached the equilibrium $x_1 + zx_2 = \frac{1}{2}\theta / (\theta\text{-}1)$.) The main difference from our model is that they do not consider the dynamics of the relative frequencies of the different types in an infinite random mating population, but discuss why the fitness sets for the allocation of resources to male and female functions will normally not be linear ($\alpha + \beta = 1$, as in our model). One of the main reasons for assuming a convex

fitness set (which makes hermaphroditism the optimal strategy) are the diminishing returns for male and female function. However, as random mating is assumed and frequency dependent aspects of the returns for males and females are covered implicitly in the formulas of our model, we could find no reason to assume such a fitness set here.

Charlesworth and Charlesworth (1978a, b) have modelled the evolution from monoecy to dioecy via gynodioecy in angiosperms. The general formula used in their studies is :

$$Wx_j' = x_j\left[\tfrac{1}{2}b_j\frac{\sum_k (1-s_k)e_k x_k}{\sum_k b_k x_k} + \tfrac{1}{2}e_j(1-s_j) + e_j s_j(1-\delta)\right] \qquad (7)$$

where

$$W = \sum_k (1-s_k)e_k x_k + \sum_k (1-\delta)s_k e_k x_k$$

Here e_j is the ovule production of type j, b_j is the pollen production, s_j is the fraction selfing and 1-δ is the fitness of offspring from selfing.

Equation (7) is very similar to the general recurrence relation (1) in this study. In fact, by excluding asexual reproduction by means of male gametes, and by putting:

$e_j = (1-z_j)$
$b_j = z_j$ and
$1-\delta = d$,

both formulae become identical.

The simplest situation Charlesworth and Charlesworth discuss is $b_j=0$ and $e_j = 1+k$ for male-steriles and $b_j=1$ and $e_j=1$ for hermaphrodites. They find that a male-sterile mutant can invade a hermaphrodite population if $k > 1-2\delta s$. This can be compared with our model if $1+k = 1/(1-z)$ and $\theta = \infty$, using types **u** (male-sterile) and **ui** (hermaphrodite) only. As the condition $\theta = \infty$ (meaning a fitness 0 for asexual reproduction) is not very realistic, this is a situation of little biological relevance in Sordariaceae.

An important difference between angiosperms and the ascomycetes considered here is that pollen differ from conidia in their inability to transport cytoplasmic genes and to germinate asexually. In addition, in the plants sex determination occurs in the diploid stage. These differences make a comparison with models

concerned with cytoplasmic inheritance (Charlesworth and Ganders, 1979) and different types of biallelic sex determination (Gregorius et al., 1982, 1983) less useful.

The main selective force considered by Charlesworth and Charlesworth (1978a, b) is inbreeding depression, which shows up after selfing. They found this necessary for gynodioecy to evolve, just as it is necessary for the evolution of heterothallism (chapter 2). One problem is that because of haploidy in fungi, the disadvantage of selfing is hardly comparable with that in higher plants and animals. Probably this disadvantage is much lower in haploids. The haploidy and the existence of mating types in addition to hermaphroditism make it difficult to compare the evolution of hermaphroditism in angiosperms and ascomycetes.

The ability of conidia to germinate as an asexual spore makes the evolution of imperfect fungi possible. It has been found that the fitness θ of ascospore production must be more than twice the fitness of asexual spores, for imperfect fungi not to evolve. Whether (and if so, why) this presumed higher fitness of ascospores is related to sexual reproduction is unclear.

Finally we may conclude that if a species is heterothallic the evolution of hermaphroditism can be explained. But for a homothallic species, even if it is suffering from inbreeding depression, both the evolution to heterothallism (chapter 2) and to dioecy (defined as the existence of males next to females), are hard to explain. So there seems no reason why heterothallism should be more common than dioecy.

It is a problem that the many experimental data, needed for a better understanding of the evolutionary aspects of reproductive systems in ascomycetes, are lacking. More knowledge about natural populations of ascomycetes is necessary to obtain reliable estimates of the values of the parameters related to the degree of 'maleness' of hermaphrodites (z), rates of selfing (s), and to fitness differences between the various types of spores (d and θ). More species should be investigated to get more information on the reproductive processes, the factors that initiate conidia and ascogonium production, the frequency of outcrossing in homothallic and heterothallic species and the occurrence of inbreeding depression. It would be very interesting to carry out a thorough search for polymorphisms in homo- and heterothallism or in sex types. The current models predict that such polymorphisms may be found in nature.

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Appendix

I. Derivation of the general formula (1).

Consider an individual model organism of type j, which produces $\alpha_j N$ conidia and $\beta_j n$ ascogonia. Then, if each ascogonium produces c ascospores, an individual of type j produces $\beta_j cn$ ascospores. (As ascospore production requires higher energy expenditure than conidium production, $cn < N$.)

As described in the main text, the different spores can land on different substrates. From the produced ascospores a fraction p_a germinates and a fraction $1-p_a$ is lost. A fraction $1-p_c$ from trhe conidia is lost, a fraction $p_c E$ germinates as asexual spore and a fraction $p_c(1-E)$ serves as a male gamete. (As ascospores normally have a greater survival ability $p_a > p_c E$)

It is assumed that a fraction s_j of the ascogonia are self-fertilized and that the remaining ascogonia are (randomly) cross-fertilized. The amount of conidia used for selfing is assumed to be negligible.

In the whole population there is a total of M conidia and m ascogonia that participate in outcrossing. When the relative fitness of an asexual spore is scaled equal to 1, the fitness of a sexual spore is assumed to be equal to F if it results from outcrossing, and Fd if it results from selfing.

Thus the genetic contribution by this individual to the next generation is the number of successful asexual spores ($= \alpha_j N p_c E$)
+ the number of successful own ascospores from cross-fertilization
($= \frac{1}{2} F p_a \beta_j cn(1-s_j)$)
+ the number of successful ascospores from self-fertilization ($= F p_a d \beta_j cns_j$)
+ the number of successful ascospores (from other individuals) resulting from fertilization by conidia from the individual considered ($= \frac{1}{2} F p_a p_c (1-E) \alpha_j Ncm/M$).

Thus the change in frequency x_j ($\Sigma x_j = 1$) of type j will be given by

$$Vx_j' = x_j \cdot [\alpha_j N(p_c E + \tfrac{1}{2} F p_a p_c (1-E) c \frac{m}{M}) + \tfrac{1}{2} F p_a \beta_j cn(2s_j d + 1 - s_j)] \qquad (8)$$

where V equals the sum of the right hand sides of all j.
By putting

$$\theta \equiv \frac{Fp_a cn}{Np_c E} \tag{9}$$

and noting that

$$\frac{m}{M} = \frac{n \cdot \sum_j (1-s_j)\beta_j x_j}{Np_c(1-E) \cdot \sum_j \alpha_j x_j}$$

equation (8) can be rewritten as

$$Wx_j' = x_j \cdot [\alpha_j (1 + \tfrac{1}{2}\theta \frac{\sum_k (1-s_k)\beta_k x_k}{\sum_k \alpha_k x_k}) + \tfrac{1}{2}\theta \beta_j (2s_j d + 1 - s_j)] \tag{10}$$

where W equals the sum of the right hand sides.

For $\alpha_j = z_j$ and $\beta_j = (1-z_j)$ formula (10) is the same as (1).

In the analysis the parameter θ appears to be very important. From equation (9) it follows that

$$\theta = \frac{F_{ra} \times P_{ea}}{P_{ec}}$$

where F_{ra} is the relative ascospore fitness, P_{ea} and P_{ec} are the effective ascospore and conidium production, respectively. So θ can be called the fitness of ascospore production.

II. The dynamics of the system of recurrence relations in a heterothallic population.

For the three types j considered in section A1 of this study (j=1,2 and 3 representing respectively males, hermaphrodites and females) formula (1) becomes:

$$Wx_1' = x_1 (1 + \tfrac{1}{2}\theta \frac{(1-z)x_2 + x_3}{x_1 + z x_2}) \tag{11a}$$

$$Wx_2' = x_2\left(z\left(1+\tfrac{1}{2}\theta\frac{(1-z)x_2+x_3}{x_1+zx_2}\right)+\tfrac{1}{2}\theta(1-z)\right) \quad \textbf{(11b)}$$

$$Wx_3' = x_3\tfrac{1}{2}\theta \quad \textbf{(11c)}$$

where

$$W=(1-\theta)(x_1+zx_2)+\theta$$

Defining $x_1 + zx_2 = y$ and $\frac{1}{2}\theta/(\theta-1) = z^*$, these equations can be rewritten as:

$$\Delta x_1 = x_1\frac{(y-1)(y-z^*)}{y(2z^*-y)} \quad \textbf{(12a)}$$

$$\Delta x_2 = x_2\frac{(y-z)(y-z^*)}{y(2z^*-y)} \quad \textbf{(12b)}$$

$$\Delta x_3 = x_3\frac{y-z^*}{2z^*-y} \quad \textbf{(12c)}$$

It is now easy to see that the population is in equilibrium if $y = z^*$, so if $x_1 + zx_2 = \frac{1}{2}\theta/(\theta-1)$. ($z^*$ can be interpreted as the 'maleness' of the whole population in equilibrium.) Furthermore, it is easy to see that

x_1 decreases if $y > z^*$ and else increases,

x_3 decreases if $y < z^*$ and else increases and

x_2 decreases if $z < y < z^*$ or $z^* < y < z$,and else increases.

This means the equilibrium line $y = z^*$ is stable (see figure 2).

It should be noticed that the population in the model is infinite. In nature random processes such as genetic drift will cause small perturbations from the equilibrium state. It is therefore important to ask whether the population is expected to move along the equilibrium line towards hermaphroditism or dioecy. We have studied this both in simulations and analytically, but no evidence was found for a systematic tendency towards either hermaphroditism or dioecy. The thorough analysis of this problem is rather complex and falls beyond the scope of this paper.

In section A2 a variable θ is considered. We could not find analytic expressions for the equilibria in this situation. Numerical studies showed that with a variable θ

trioecy was never stable, and that the final population is always either pure male, polymorphic male/hermaphrodite, pure hermaphrodite or polymorphic female/hermaphrodite.

By linearizing equations (11) in some special cases, a qualitative prediction of the equilibrium state of the population can be made. If we denote the value of θ in generation i by θ_i, then it can be shown by equation (11), that, in a population with only males, females can invade the population if

$$\prod_i \tfrac{1}{2}\theta_i > 1 \qquad \textbf{(13)}$$

and hermaphrodites can invade if

$$\prod_i z+\tfrac{1}{2}\theta_i(1-z) > 1 \qquad \textbf{(14)}$$

In a population with only hermaphrodites, males can invade the population if

$$\prod_i \frac{z+\frac{1}{2}\theta_i(1-z)}{z(z+\theta_i(1-z))} > 1 \qquad \textbf{(15)}$$

and females can invade if

$$\prod_i \frac{\frac{1}{2}\theta_i}{z+\theta_i(1-z)} > 1 \qquad \textbf{(16)}$$

If condition (14) is not true, (13) cannot be true either and a pure male (i.e. imperfect) population is expected. If both (15) and (16) are untrue a pure hermaphroditic population (as found abundantly in nature) is expected.

III. The dynamics of the system of recurrence relations in a homothallic population.

For all types j considered in section B of this study (see table 1 and figure 1) formula (1) becomes

$$Wx_1' = x_1\left[1+\tfrac{1}{2}\theta\frac{(1-s)(1-z)x_2+x_3}{z(x_2+x_4)+x_1}\right] \quad \textbf{(17a)}$$

$$Wx_2' = x_2\left[z\left(1+\tfrac{1}{2}\theta\frac{(1-s)(1-z)x_2+x_3}{z(x_2+x_4)+x_1}\right) + \tfrac{1}{2}\theta(1-z)(1-s(1-2d))\right] \quad \textbf{(17b)}$$

$$Wx_3' = x_3\tfrac{1}{2}\theta \quad \textbf{(17c)}$$

$$Wx_4' = x_4\left[z\left(1+\tfrac{1}{2}\theta\frac{(1-s)(1-z)x_2+x_3}{z(x_2+x_4)+x_1}\right) + \theta(1-z)d\right] \quad \textbf{(17d)}$$

$$Wx_5' = x_5\theta d \quad \textbf{(17e)}$$

where

$$W = (1-\theta)[z(x_2+x_4)+x_1] + \theta[1 - (1-d)((1-z)(sx_2+x_4)+x_5)]$$

It is easy to see that for $d < 0.5$ types 2 (**ui**) and 3 (**u**) will always do better then types 4 (**ûi**) and 5 (**û**), and vice versa for $d > 0.5$.

With a variable θ, as considered in section B2, for $d < 0.5$ conditions comparable to (13) - (16) in Appendix II can be derived. The same stable population compositions are possible as in a heterothallic species.

Denoting by θ_i the value of θ in generation i, it can be derived from (17a), (17b) and (17c) that, in a population with only males, females can invade the population if

$$\prod_i \tfrac{1}{2}\theta_i > 1 \quad \textbf{(18)}$$

and hermaphrodites can invade if

$$\prod_i [z+\tfrac{1}{2}\theta_i(1-z)(1-s(1-2d))] > 1 \quad \textbf{(19)}$$

In a population with only hermaphrodites, males can invade the population if

$$\prod_i \frac{z+\frac{1}{2}\theta_i(1-s)(1-z)}{z(z+\theta_i(1-z)(1-(1-d)s))} > 1 \qquad (20)$$

and females can invade if

$$\prod_i \frac{\frac{1}{2}\theta_i}{z+\theta_i(1-z)(1-(1-d)s)} > 1 \qquad (21)$$

It is easy to see that in the case of $d > 0.5$ there is competition between the types **i**, **îi**, and **î** only. If $x_2=x_3=0$, formulas (17a), (17d) and (17e) can be rewritten as

$$Wx_1' = x_1 \qquad = a_1 \cdot x_1 \qquad (22a)$$

$$Wx_4' = (z+(1-z)\theta d)\cdot x_4 \qquad = a_4 \cdot x_4 \qquad (22b)$$

$$Wx_5' = \theta d \cdot x_5 \qquad = a_1 \cdot x_1 \qquad (22c)$$

where all a_j are constant if θ is constant.

If $\theta d > 1$ then $a_5 > a_4 > a_1$ and if $\theta d < 1$ then $a_1 > a_4 > a_5$. Parameter a_4 can never have the highest value. Therefore either type **i** or type **î** will go to fixation in the situation described in section B1.

If θ_i is varying (as described in section B2), then $a_{i,j}$ (the value of a_j in generation i) also varies and after T generations :

$$Vx_j^{(T)} = \prod_{i=0}^{T} a_{i,j} \cdot x_j = b_j \cdot x_j \qquad (23)$$

As the b_j, the product of the $a_{i,j}$ over T generations, are new constants, a system analogous to equation (22) is obtained. This time, however, each of the three types may become fixed, because, with variable $a_{i,j}$ all b_j can have the largest value.

CHAPTER 4

Vegetative incompatibility I : Deterministic models

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Summary

Vegetative incompatibility (VI), the prevention of heterokaryon formation in ascomycetes, has to occur very frequently in natural populations, since in all species studied so far, many vegetative compatibility groups (VCGs) are found. Using a population genetic approach, this chapter explores two possible selective explanations for the evolution of VI: selection by a nuclear parasitic gene and selection by a harmful cytoplasmic element. It is found that both forms of frequency dependent selection cannot explain the large number of VCGs as found in nature because the selective pressure for more VCGs disappears once a limited number of VCGs exist. In comparing the two, selection by a cytoplasmic element seems a more plausible explanation than selection by a nuclear gene.

Introduction

Somatic incompatibility is known to exist in animals, protists and fungi (Buss, 1982; Grosberg, 1988). It is expressed by an incompatibility reaction following tissue contact between genetically different conspecifics. Well known examples are the vertebrate immune systems (like HLA in man (Klitz et al., 1992)) and the allorecognition systems in invertebrates (like the colonial ascidian *Botryllus schlosseri* (Scofield et al., 1982)). In fungi somatic incompatibility is also very common, and is most often called vegetative incompatibility (VI), although the terms somatic, protoplasmic or heterokaryon incompatibility are also used.

Heterokaryon formation, i.e. the formation of a coenocytic state where two (or even more) genetically different haploid nuclei are present, is typical for the fungi. Its occurrence has for a long time been considered as evolutionary beneficial. It can give the dikaryotic haploid organism some kind of 'heterozygous advantage', and clear the

way for the parasexual cycle as an alternative for sex in imperfect species (Pontecorvo, 1946; Snyder, 1961; Davis, 1966; Caten, 1987). But later it appeared not only that heterokaryons are rarely isolated from nature (Caten and Jinks, 1966), but also that the formation of heterokaryons between different wild isolates of a species is normally impossible due to VI, which prevents the formation of heterokaryons in nature.

Many examples of VI in filamentous ascomycetes (the group of fungi to which this study is restricted) are known. As shown in an overview in Table 1, in many of them a large number of vegetative compatibility groups (VCGs) are found (See also Carlile, 1987). Within such groups heterokaryon formation and the exchange of genetic material by means of heterokaryosis are possible, but not between groups. In the species studied so far, it has been found that the incompatibility reaction is always mediated by many nuclear gene loci (Puhalla and Spieth, 1985), mostly with two and occasionally with multiple alleles. Since one allelic difference can cause incompatibility, very many VCGs can be formed.

The widespread occurrence of VI means that the disadvantages of heterokaryon formation will probably be bigger than the advantages. The reason for VI, however, is still unknown. Protection of the 'genetical integrity' of the individual seems to be the most plausible (Todd and Rayner, 1980). This can be interpreted as the prevention of a conflict between two different nuclear genes in a heterokaryon (Hartl et al., 1975), and the prevention of an invasion by harmful cytoplasmic elements (plasmids, viruses, mitochondria) (Day, 1968; Caten, 1972). It may also be possible however that VI is an unregulated expression of cell death and a consequence of independent evolution of natural isolates (Davis, 1966; Bernet, 1992). The restriction of outbreeding, which is seen as a possible explanation for VI by Esser and Blaich (1973) and Boucherie and Bernet (1980), is probably merely a result of, rather then an evolutionary cause of VI.

Until now the only theoretical model on the evolution of VI has been made by Hartl et al. (1975). They concluded that a parasitic gene can give adaptive significance to the evolution of VI in *Neurospora crassa*; however, they used some rather restrictive assumptions and they did not study more then two VCGs. Their model is extended in this study. Next to that, a model is made assuming the evolution of VI to be driven by harmful cytoplasmic elements. In these models we try to find an answer to the question why so many VI-genes have evolved, i.e. how selection, caused by a parasitic nuclear gene or a harmful cytoplasmic element, may lead to the evolution of many VCGs, as found in nature.

Our model organism is an imperfect species like *Aspergillus niger*, where heterokaryon formation between VCGs and therefore recombination between VI-genes is impossible. This means that no specific assumptions have to be made about the number of loci, number of alleles per locus and linkage, and that new VCGs arise by mutation only. The differences with sexual species will be discussed afterwards.

The model of Hartl et al. (1975)

In their model, Hartl et al. (1975) consider a parasitic nuclear gene in *Neurospora crassa*, comparable with a gene found by Pittenger and Brawner (1961). It has an advantage in a heterokaryotic, but a disadvantage in a homokaryotic state. They assume only vegetative reproduction in discrete non-overlapping generations and random dispersion of conidia, which germinate and grow out to new mycelia. These mycelia meet in pairs and if they are compatible, they fuse to form a heterokaryon. If they are incompatible they survive normally and grow along side by side as two homokaryons.

Supposing there are two types of nuclei, H and h, a homokaryon with only (parasitic) h nuclei has a fitness $1-w$ ($0 < w < 1$), whereas $h + H$ heterokaryons and H homokaryons have a fitness 1. In a $H + h$ heterokaryon the h nucleus has a slight proliferative advantage over the H nucleus so that the ratio of $h : H$ conidia from such heterokaryons is $\theta : 1-\theta$, where $0.5 < \theta < 1$.

Let x be the frequency of h and y the frequency of H (so $x + y = 1$). Then analysis of the recurrence relations describing the dynamics of the model (which can easily be recovered from system (1) given below, by putting i = 1, 2) shows that there is a stable equilibrium $x = x^* = (2\theta-1) / w$ if $x^* < 1$ or $x = 1$ if $x^* \geq 1$. This means h can always invade the population and consequently lower its mean fitness.

Next it is supposed that there is a gene for VI with two alleles, c_1 and c_2, creating two VCGs which are incompatible. Then in a population with only H or only h nuclei VI is neutral. But in a population with four types Hc_1, Hc_2, hc_1 and hc_2, selection will operate. There are essentially three situations:

1) $2\theta-1 < w$: h will disappear, the population can get polymorphic for c_1 and c_2 by (repeated) introduction of H

2) $w < 2\theta-1 < 2w$: no stable equilibrium, the population can get polymorphic for c_1, c_2, h and H

Table 1. An overview of filamentous ascomycetes where Vegetative Compatibility Groups are found.

The numbers of biallelic VI loci, the number of VCGs found and the number of isolates studied in the experiments are given. The numbers of loci and groups are minimal values, real values are expected to be higher in all cases. The last five species are imperfect, from the other species perfect stages have been found.

Species	loci	groups	isolates	references
Aspergillus nidulans	8 [a]	19	100	Croft and Jinks, 1977 Croft, 1985
Neurospora crassa	10	very many		Perkins and Turner, 1988
Endothia = *Cryphonectrica parasitica*	7	73	258	Anagnostakis, 1984
Podospora anserina [b]	5	very many		Boucherie and Bernet, 1980
Diaporthe phaseolorum		29	297	Ploetz and Shokes, 1986
Diaporthe eres = *Phomopsis oblonga*		10 29 22 12	10 [c] 59 [d] 49 [d] 37 [d]	Brayford, 1990
Phomopsis subordinaria		89	134	G. Meyer, B. Megneneau, and E.G.A. Linders, unpublished results.
Gibberella fujikuroi [e] = *Fusarium moniliforme*	10 9	 13 13	 38 79	Puhalla and Spieth, 1985 Sidhu, 1986 Lamondia and Elmer, 1988

Ceratocystis = *Ophiostoma ulmi*	44	51	Brasier, 1984
Leucocytospora kunzei	44	487	Proffer and Hart, 1988
Gaumannomyces graminis var. tritici	18	31	Jamil et al., 1984
Aspergillus terreus	6	7	Caten, 1971
Aspergillus flavus [e]	22	32	Papa, 1986
	16 - 57	105	Bayman and Cotty, 1991
Aspergillus niger [e]	11	12	A.J.M. Debets, pers. comm.
Verticillium dahliae[e]	16	86	Puhalla and Hummel, 1983
Fusarium	16		Puhalla, 1985
oxysporum	14	110	Correll et al., 1986
	29	100	Gordon and Okamoto, 1991
Fusarium oxysporum f.sp. asparagi	42	97	Elmer and Stephens, 1989
Fusarium oxysporum f.sp. melonis	8	188	Jacobson and Gordon, 1990

[a] 2 loci known with > 2 alleles (Croft, 1985)
[b] Only the allelic system is considered. The additional non-allelic incompatibility system is far more complicated.
[c] isolates from different trees
[d] isolates from the same tree
[e] Also *HSI* (= Heterokaryon Self Incompatible) strains are known

3) $2w < 2\theta\text{-}1$: H will disappear, the population can get polymorphic for c_1 and c_2 by (repeated) introduction of h

Although Hartl et al. (1975) could not find any limit cycle in situation 2, our simulations show that limit cycles do occur (See Appendix I). In some cases this limit cycle passes through values very close to zero, which may imply extinction in a finite population. A new mutation to the extinct type however will be able to invade into the population again.

The main conclusion of Hartl et al. (1975) was that VI could have adaptive significance in a situation as described in the model, so polymorphism in VCGs could be explained.

As also noticed by Hartl et al. (1975), some assumptions in the model deserve some discussion. Firstly the fusion of the mycelia in pairs is highly unrealistic: Heterokaryon formation is no sexual process, and its occurrence will depend on the density of the population. Some mycelia will meet no fusing partner, others will meet 1,2,3 or even more fusing partners. The consequences of this fact will be analyzed in the extended model below.

Further it is assumed that some recombination occurs between the *h* / *H*- and the *c*-locus. In *N. crassa* (for which the model was made) this can take place after a (rare) sexual crossing, but in imperfect species this can never happen. This means that in that case a situation with four types (situation 2. above) can only occur after two independent mutations, both to a new VCG and H, which have to occur nearly simultaneously, because a situation with only three types is always unstable .

Finally only the situation with one or two VCGs is considered. As it is found that there are always many more VCGs, the question how many VCGs will be selected for can only be answered in an extended model.

An extended model for a parasitic nuclear gene

If VI has any adaptive significance, it is clear that contact between different mycelia must occur regularly. This contact will take place when two or more mycelia on a certain substrate grow out and meet each other. To model this for different situations the following is assumed: Each individual mycelium can potentially have n neighbours, i.e. there are n neighbouring spots for each individual. ($n = 4$ for a model individual living on some kind of squared paper, $n = 1$ in the Hartl et al.-model.) Since not every spot will be occupied (by a member of the same species) it is assumed that a fraction p of all these spots is empty. ($p = 0$ in the Hartl et al.-model.) For

simplicity it is further assumed that all individuals and empty spots are randomly spread.

Since in nature many VCGs are found, it is assumed that mutations to new VCGs (denoted as c_i) can occur, every mutation being a mutation to a new VCG.

In some species (Table 1) Heterokaryon Self Incompatible (*HSI*) strains are found. These strains are incompatible with every other strain they meet, even with clonally related strains. The fate of this type of individual (c_{SI}) is also considered, giving them a lower fitness value f_{SI} (f_{SI} <1), because *HSI* strains probably have difficulties in forming anastomoses, which may lower the fitness of the mycelium (Correll et al., 1989; A.J.M. Debets, pers. comm.).

Finally an 'omnicompatible' type (*OC*) is introduced, i.e. individuals which are compatible with all others (except *HSI*s). Although to our knowledge such a type has not been reported, it is studied for theoretical purposes. It might be interesting to know whether and when such a type might be able to invade a population if it could arise by mutation.

Based on all these assumptions a system of recurrence relations can be derived, relating the frequencies of all types between consecutive (discrete, non-overlapping) generations. The derivations and the general form of these equations are given in Appendix II.

With this system the fate of mutations to new VCGs can be studied for different parameter values. The question is whether some kind of stability will be reached, when random mutations to new VCGs and to *H*- or *h*-nuclei (with constant values of θ and w) do occur and how many VCGs will be selected for.

The simplest case

At first the case with $n = 1$ and $p = 0$, without *HSI*s and *OC*s, will be discussed. This differs from the Hartl *et al.*-model only in that more than two VCGs are allowed.

Equation (14) (Appendix II) becomes:

$$\begin{aligned} Wx_i' &= x_i((1-w)(1-y_i)+2\theta y_i) \\ Wy_i' &= y_i(1 + x_i(1-2\theta)) \end{aligned} \qquad (1)$$

with

$$W = 1 - w\sum_j x_j(1-y_j)$$

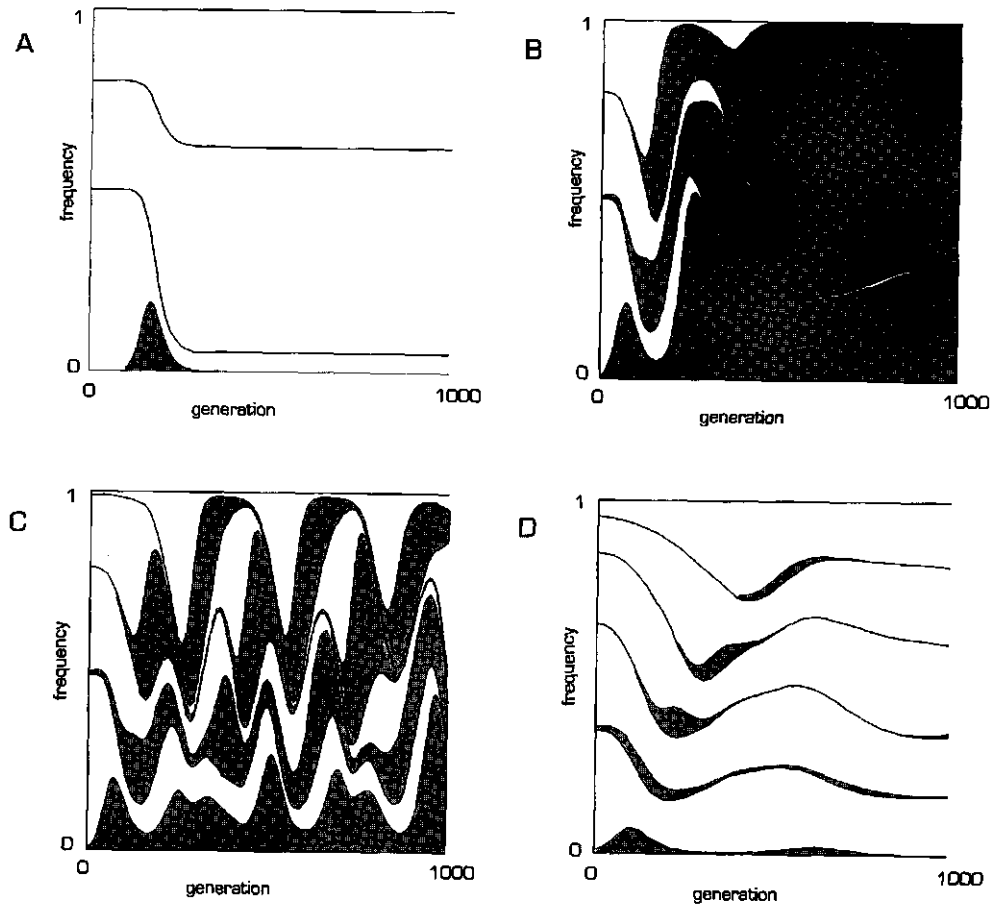

Figure 1. The fate of *h*-mutants invading populations with only *H*-nuclei.

The cumulative frequencies of the VCGs are given by the frequencies of the *h*-nuclei (filled area) and *H*-nuclei (blank area) within each VCG, for a population with $w = 0.055$ and $\theta = 0.6$ over 1000 generations. The critical values for invasion are $z_i = 0.2157$ and $z_i = 0.275$ according to equations (2) and (3).

A: A population with three VCGs with frequencies 0.5, 0.3 and 0.2 at generation 0. Mutation to *h* at generation 80 leads to invasion in the first VCG. As a consequence the fitness and thus the frequency of this VCG decreases, making it more difficult for the *h*-nuclei to be maintained. Eventually the *h*-nuclei are lost and at generation 1000 the frequencies of the three VCGs have become 0.051, 0.569, 0.380.

B: In a population with the same initial conditions as in **A**, mutations to *h* in all VCGs lead to a population without *H*-nuclei, as in this case all VCGs suffer fitness loss due to the presence of *h*-nuclei. Frequencies after 1000 generations tend to be equal: 0.333, 0.281, 0.385.

Figure 1 (continued). **C**: A population as in **B** with a fourth VCG at frequency 0.01 at generation 0, leads to chaotic dynamics and a polymorphism of *h* and *H*.
D: In a population with five VCGs with frequencies 0.35, 0.3, 0.2, 0.1 and 0.05 at generation 0, and allowing *h* mutations in all VCGs, the mean VCG-size is to small to support the presence of *h*-nuclei. Selection against *h*-nuclei in large VCGs leads to a convergence of VCG-frequencies: 0.17, 0.168, 0.258, 0.222, 0.182 at generation 1000.

with x_i representing the frequency of VCG c_i individuals with *h* nuclei and y_i those with *H* nuclei (so the frequency of c_i equals $x_i + y_i = z_i$, and $\Sigma z_i = 1$).

From this it follows that in a population without *h*-nuclei ($\Sigma x_i = 0$) *h* cannot invade VCG c_i if

$$z_i < \frac{w}{2\theta + w - 1} \qquad (2)$$

and *H* cannot invade VCG c_i in a population without *H*-nuclei ($\Sigma y_i = 0$) if

$$z_i > \frac{w}{2\theta - 1} \qquad (3)$$

This means that *h* will spread in a common VCG, but not in a rare one. Only if neither of the inequalities (2) and (3) hold, polymorphism of *H* and *h* will be found, and selection for more VCGs can take place.

To provide an intuitive understanding of these results, the course of events in a population with only *H* nuclei after mutation(s) to *h* is illustrated in figure 1. As stated above, *h* is unable to invade the population if (2) holds for all VCGs c_i. In such a population each mutation to a new VCG is neutral. But if, in a population without *h*-nuclei, inequality (2) does not hold in a certain VCG, *h* will invade there after mutation. This will lower the fitness and thus the frequency of that VCG, until (2) holds. Since $\Sigma (x_i + y_i) = 1$, the frequencies of the other groups will rise, possibly so far that some of them will reach a frequency high enough for inequality (2) not to hold any more. Then the process will continue indefinitely if the number of VCGs is too small for (2) to hold for all VCGs. (This will lead to chaotic behaviour of VCG-frequencies, as an equivalent of the limit cycle found with only two VCGs.) New VCGs with *H* nuclei will be able to invade the population, until the process stops once enough VCGs exist. As VCGs with high frequencies will be 'punished', all frequencies will tend to be equal at equilibrium.

Therefore, with approximately all $z_i = z$ in equilibrium, the number of VCGs

selected for can be estimated as $1/z = N$. That is, the number of VCGs where h can no longer invade (and selective pressure for more VCGs has disappeared) is the smallest integer N that satisfies

$$N \geq \frac{2\theta + w - 1}{w} \tag{4}$$

In an imperfect species however, this need not be the number of VCGs selected for. When inequality (3) holds for all VCGs in the population, H will not be able to invade into the population. A double mutation to both H and a new VCG is needed for this new VCG to be selected for, and this is expected to be an extremely rare event. Additionally, if such a double mutation occurs, the frequency of the new VCG may rise to a value that satisfies inequality (3), which may lead to a population monomorphic for h again. Therefore subsequent double mutations both to H and to a new VCG will be necessary: there appears to be a threshold in the number of VCGs that must exist before selection to new VCGs can act.

This threshold can be calculated with (3), assuming equal frequencies of all VCGs, as the largest integer that satisfies

$$N_{Thr} \leq \frac{2\theta - 1}{w} \tag{5}$$

In figure 2 critical numbers N and N_{Thr} as given by (4) and (5) are given. As $N - N_{Thr} = 2$, there are only two numbers of VCGs which are susceptible for selection.

Figure 2 also shows that N and N_{Thr} are very high for values of w close to zero. Simulations showed that the selection process slows down remarkably if w is very low and that it takes a very long time before equilibria are reached.

The general case

If n and p can assume larger values, the model becomes much more complicated to analyze. Qualitatively however the results do not differ much from those presented above. Again there are three kinds of populations possible: Those with few VCGs and without H-nuclei, those showing chaotic behaviour with more VCGs and both h- and H-nuclei, and those with many VCGs and without h-nuclei. An example of the results of the regular introduction of a new VCG for $n = 4$ and $p = 0.4$ is given in figure 3. Critical values for the number of VCGs can be calculated numerically with the

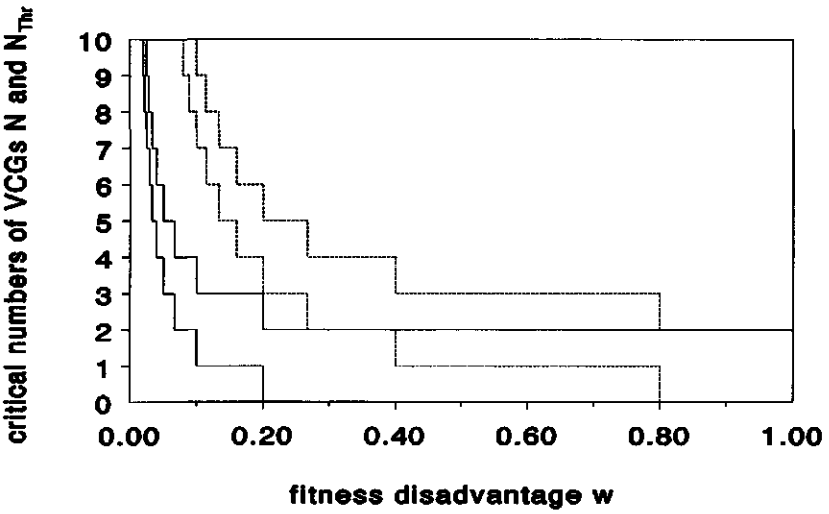

Figure 2. Critical numbers of VCGs for $n = 1$ and $p = 0$ in the nuclear parasite model. The solid line gives the values of N (above) and N_{Thr} (below) as in (4) and (5) for $\theta = 0.6$, the broken line for $\theta = 0.9$. Selection occurs in the area between the two lines, below the N_{Thr}-line monomorphism for h and above the N-line monomorphism for H will be found.

formulae (18) and (20) given in Appendix II.

It is easy to see that higher values of n and lower values of p cause a higher threshold and allow selection for more VCGs, as soon as this threshold is passed. The expected number of neighbours $n(1\text{-}p)$ appears to be the most decisive factor and the intermediate number of VCGs $N - N_{Thr}$ gets larger when $n(1\text{-}p)$ increases. (See figure 13 in Appendix II for an example.)

A Heterokaryon Self Incompatible (HSI) type

The fate of a *HSI* mutant is greatly dependent on its fitness f_{SI}. If $f_{SI} = 1$, the *HSI* type will always take over the whole population, as long as mutations to h occur. If $f_{SI} < 1\text{-}w$ the *HSI* type will always have the lowest fitness, and it will be unable to invade any population. More precisely, as shown in Appendix II, a *HSI* type can invade the population if

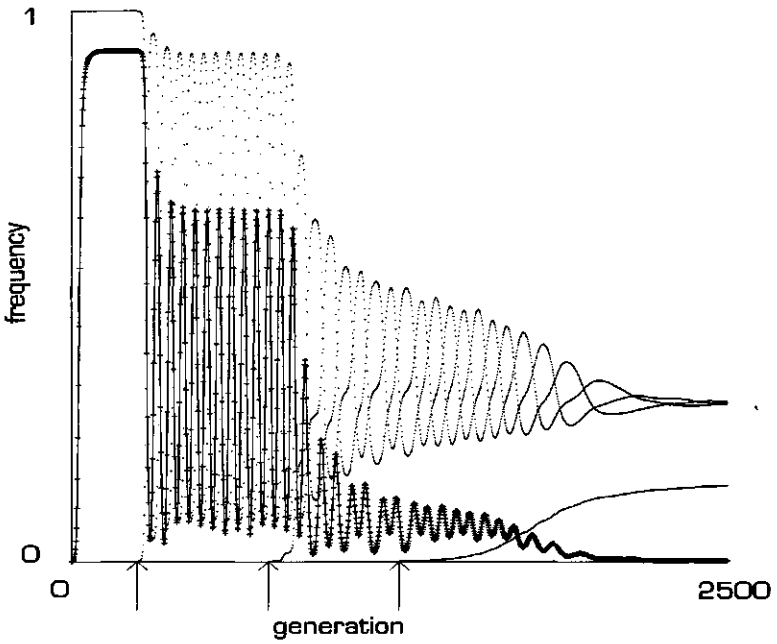

Figure 3. The fate of a parasitic *h* type in a population with $n = 4$, $p = 0.4$, $\theta = 0.6$ and $w = 0.3$, over 2500 generations.

The frequency of each VCG (z_i) is given by dots (which may show up as a solid line if they stand close together). The frequency of all *h*-nuclei (Σx_i) is given by crosses connected with a solid line. At generation 0 there is only 1 VCG. The arrows on the x-axis indicate mutation to a new VCG (at generation 250, 750 and 1250), so at the end there are 4 VCGs. There is a mutation frequency from *h* to *H* or vice versa of 0.1 per generation per population (so e.g. a mutation frequency of 10^{-6} in a population with size 10^5).

It shows that with one VCG there is a stable *h*/*H* polymorphism, with two VCGs a limit cycle shows up, with three VCGs the mean frequency of *h* decreases and the results become chaotic, and finally with four VCGs *h* gets lost and there is no longer selection for more VCGs.

$$f_{SI} > 1 - w\Sigma x_i(1-(1-p)y_i)^n \qquad (6)$$

With only one VCG a stable equilibrium can be found, as in the example given in figure 11 in Appendix I. As the number of VCGs grows, the frequency of *h* nuclei in the population decreases, as will the *HSI* frequency. Finally the *HSI* type will disappear.

The fate of an *HSI* mutant with $f_{SI} = 0.99$ in a population as in figure 3, is given

in figure 4.

An Omnicompatible (OC) type

As in the example shown in figure 5, a hypothetical *OC* type will be able to invade a population. As it is compatible with all other types, a *h-OC* type will behave as a *h* type in a population with only one VCG: it can always invade the population (no matter how many VCGs already exist), and can eventually cause the total disappearance of the whole VI phenomenon. The reason for this is that only few homokaryons with only h-nuclei will be formed by the *OC* type, because it is compatible with far more neighbours than the other types. So its disadvantage is very low compared to h-nuclei in other groups.

The fact that this type can destabilize the system of incompatibility, plus the fact that is not known to exist, suggests that this type of mutation may be impossible.

A model of the evolution of VI driven by a harmful cytoplasmic element

As mentioned above, harmful cytoplasmic elements may also be considered to contribute to selection for VI (Day, 1968; Caten, 1972). In this section we analyze a model, in which a cytoplasmic element is assumed in stead of a nuclear gene. It gives its carrier a lowered fitness 1-*c* ($0 < c < 1$), and is passed on in a fraction α of the (asexual) spores. Further, all compatible neighbours (as in the nuclear parasite model) are infected by the carrier; the fitness of newly infected individuals is unaffected.

Working out these assumptions, without considering the *HSI* and *OC* types, leads to the following system of equations, with x_i standing for the frequency of the infected individuals of VCG c_i and y_i for the frequency of the uninfected individuals of VCG c_i.

$$Wx_i' = \alpha\,[x_i(1-c) + y_i(1-(1-(1-p)x_i)^n)]$$

$$Wy_i' = (1-\alpha)(y_i + x_i(1-c)) + \alpha\, y_i(1-(1-p)x_i)^n \qquad (7)$$

with

$$W = 1 - c\sum_i x_i$$

from which it is easy to see that if $\Sigma x_i = 0$ a stable equilibrium $x_i = 0$ is found if

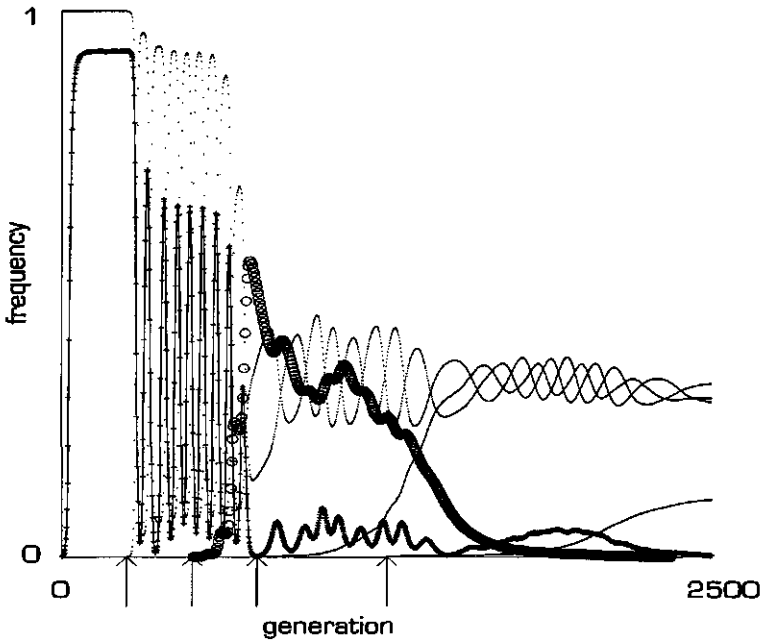

Figure 4. The fate of a *HSI* mutant in a population with the same parameter values as in figure 3.

At generation 500 a mutation to a *HSI* type with $f_{SI} = 0.99$, indicated by open circles, occurs. When the frequency of h lowers, the frequency of the *HSI* type lowers too. It finally disappears, and the situation ends up as in figure 3.

$$y_i < \frac{1-\alpha(1-c)}{\alpha n(1-p)} \tag{8}$$

and $y_i = 0$ is only stable if

$$\alpha = 1 \quad \wedge \quad (1-(1-p)x_i)^n > 1-c \tag{9}$$

This means that the population can only get monomorphic with infected individuals if $\alpha = 1$, which is highly improbable. Therefore in this model the parasitic type can never reach a frequency of 1 like in the previous model and consequently no threshold in the number of VCGs necessary for the operation of selection exists. Whenever a cytoplasmic element as described in this model occurs, and (8) is not true, there will be selection for more VCGs.

As figure 6 shows, during the process of mutations to new VCGs, the frequencies

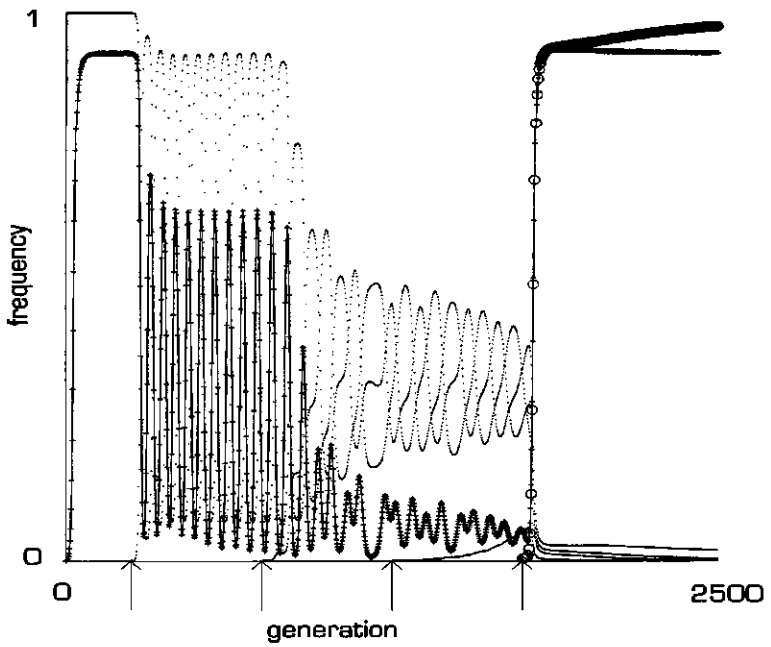

Figure 5. The fate of an *OC* mutant in a population as in figure 3.
At generation 1750 a mutation to an *OC* type, indicated by open circles, occurs. It invades rapidly and as a consequence the frequency of *h* nuclei is also rapidly increasing. Finally the population ends up with the *OC* type in high frequency with one VCG and a stable *h/H* polymorphism, just as in the case with one VCG only.

of all types tend to equalize, so $x_i + y_i = 1/N$ if there are N VCGs. Then for n=1 the expected value of Σx_i in equilibrium can be calculated as

$$\Sigma \hat{x}_i = N\hat{x}_i = \frac{N(\alpha(c-1)+1) - \alpha(1-p)}{Nc - \alpha(1-p)} \qquad (10)$$

The expected number of VCGs selected for is the smallest integer N that satisfies

$$N \geq \frac{1}{\hat{y}_i} = \frac{\alpha n(1-p)}{1-\alpha(1-c)} \qquad (11)$$

What this means for different values of α and c if $n(1\text{-}p) = 2.4$ (e.g. $n = 4$ and $p = 0.4$) is shown in figure 7. By adapting the y-axis, expected numbers of VCGs can be calculated for other values of $n(1\text{-}p)$. It shows that for large n, small p, small c and high α the expected number of VCGs increases. As in the case of a parasitic nuclear

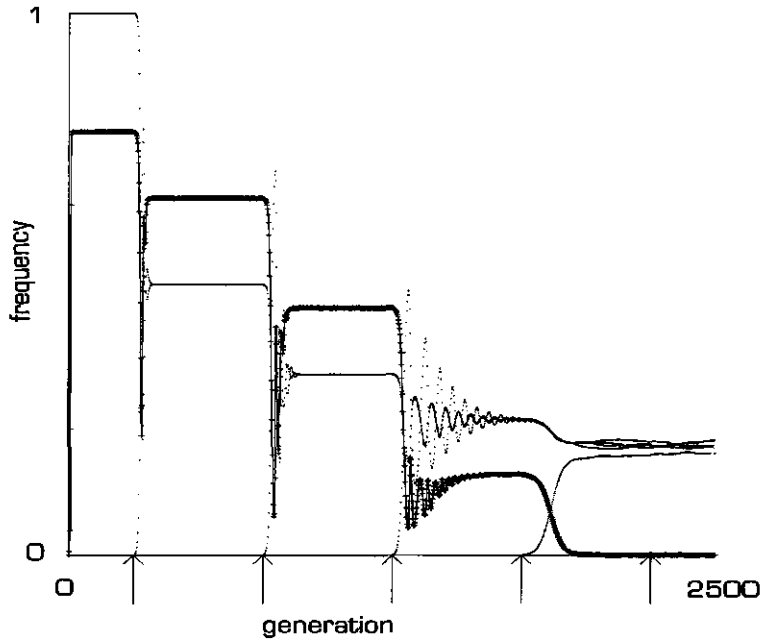

Figure 6. The dynamics in case of a harmful cytoplasmic element, in a population with $n = 4$, $p = 0.4$, $c = 0.3$ and $\alpha = 0.8$ over 2500 generations. Representation and conditions as in figure 1., the crosses connected with a solid line now representing the frequency of the cytoplasmic element.

For N VCGs the frequency of each VCG tends to $1/N$ as long as the cytoplasmic element can maintain itself in the population. When five VCGs exist it disappears and there is no longer selective pressure for new VCGs to invade into the population.

gene, the selection process is slowed down for small values of c. (For c=0 inequality (11) would predict a high number of groups, but it is easy to see that no selection on VCGs can act in that case.)

If the *HSI* and *OC* types are considered, the system of equations can be derived as given in Appendix III.

When a *HSI* type is introduced, the results are essentially the same as in the nuclear parasite model. As shown in Appendix III (equation (25)) it can be deduced that *HSI* can invade the population if $f_{SI} > 1 - c\Sigma x_i$. So only if $1-c < f_{SI} < 1$ an equilibrium with the *HSI*-type can exist. When the number of VCGs rises, Σx_i will decrease and it will be harder for the *HSI*-type to be maintained in the population. An example of the fate of an introduced *HSI* type is given in figure 8.

The fate of an *OC* mutant is essentially different as in the previous model: As there

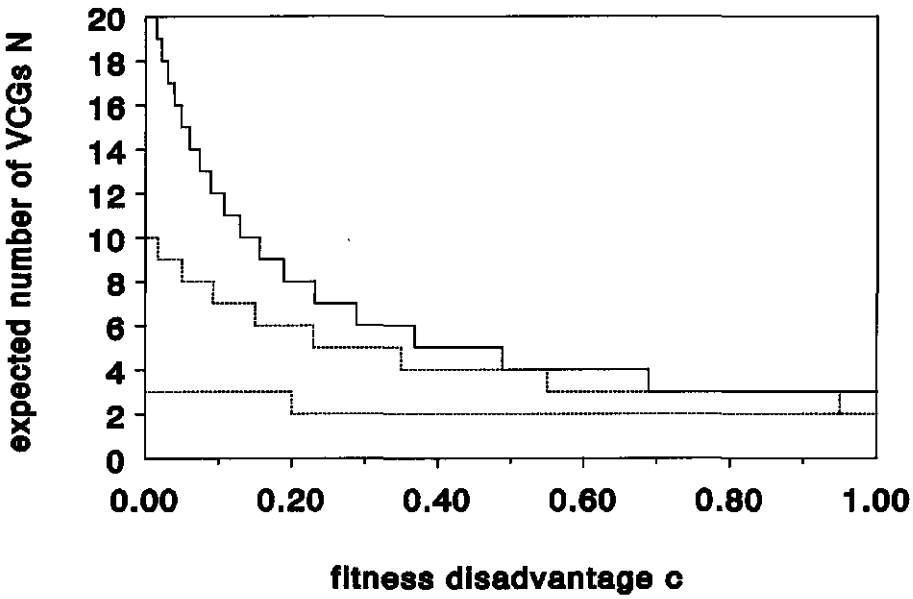

Figure 7. Expected numbers of VCGs after selection due to a cytoplasmic element for $n(1\text{-}p) = 2.4$.
From the top downwards for $\alpha = 0.9$, 0.8 and 0.5.

is no coupling of (nuclear parasitic) *h*- and c_{OC} genotype in the nucleus, the *OC* type can never invade the population.

Discussion

This paper explores the possibility that vegetative incompatibility (VI) in filamentous ascomycetes has evolved as a defense mechanism against invading parasitic genetic elements. The first theoretical analysis of this problem has been carried out by Hartl et al. (1975), who studied a population genetic model based on the occurrence of a parasitic nuclear gene in *Neurospora crassa*. This gene is competitively superior in heterokaryons but lowers the fitness in homokaryons. They concluded that VI could evolve as a consequence of the occurrence of such a parasitic gene. But whereas their model allows only two vegetative compatibility groups (VCGs), in reality (many) more VCGs are found in ascomycete species.

The extended model presented in this study shows that selection for more than two VCGs will occur under rather restricted conditions. Only when the population is

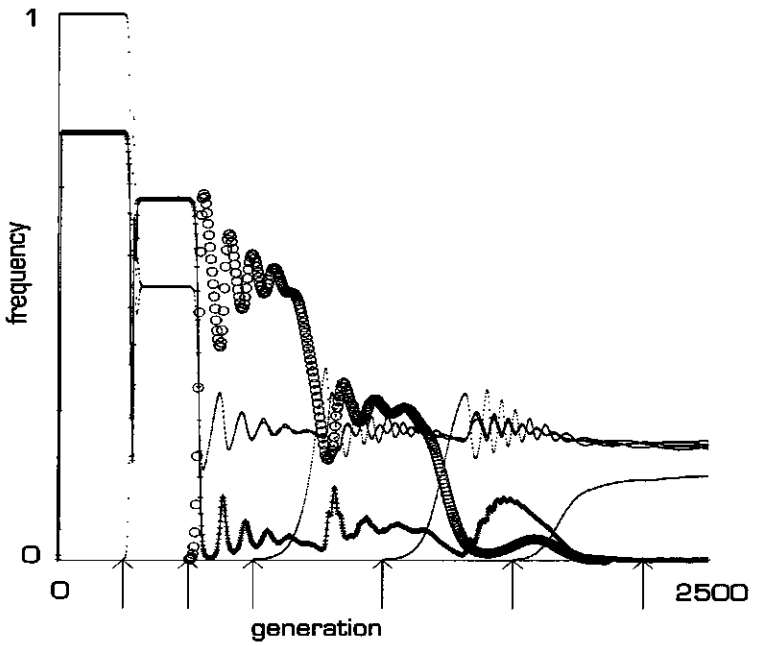

Figure 8. The fate of a *HSI* mutant in a population as in figure 6.
At generation 500 a mutation to a *HSI* type with $f_{SI} = 0.975$, indicated by open circles, occurs. When more VCGs invade, the frequency of the *HSI* type decreases, until it is finally lost when the cytoplasmic element has disappeared, and the population ends up as in figure 6.

polymorphic for a parasitic nuclear gene (in the sense defined above) and its non-parasitic allele, a new VCG will increase in frequency. The conditions for such a polymorphism are narrow, depending on the frequencies of the VCGs already present in the population, the fitness effects of the parasitic gene and population structure. If one or more new VCGs invade in a polymorphic population, the parasitic nuclear gene will not be able to maintain itself, and the polymorphism (*and* the selective pressure for more VCGs) will disappear.

One of the consequences of the process is that all frequencies of the different VCGs tend to be equal. This is something which is also found in the case of evolution of self-sterility alleles in plants (e.g. Wright, 1969). The latter phenomenon is highly comparable to the evolution of VCGs as described in our model. Remarkable differences however are that they operate in opposite directions, and that a similar model considering frequency dependent selection predicts selection for an infinite number of alleles in the case of self-sterility alleles and a restricted number in the case

of VCGs.

Our analysis shows that for purely asexual (so-called imperfect) species the conditions for polymorphism of parasitic and non-parasitic nuclear genes will be harder to fulfil than for sexual species. This is because in an asexual species a double mutation (to a new VCG and to a new (non-)parasitic gene) has to occur, whereas in a sexual species recombination can generate the suitable combination of alleles at two loci at which single, independent mutations have occurred.

Another result of our study is that when VI is selected as defense against parasitic *nuclear* genes, incompatibility will break down after introduction of an omnicompatible (*OC*) mutant. This is a (hypothetical) type showing vegetative compatibility with all individuals in the population. Such *OC* mutations are not known to exist. Perhaps their occurrence is highly unlikely, for example if several independent mutations in the same individual are required. This can be judged only when detailed information will be available on the mechanisms and genetics of VI.

Selection for large numbers of VCGs (as found in nature) requires rather extreme parameter values in our model. The mean number of neighbours $n(1\text{-}p)$ must be high and the selective disadvantage of the parasitic types must be low. Apparently there seems no reason why these parameters should meet these conditions, and estimates based on empirical findings cannot be made, as these are not known to us. It is therefore important to consider possible situations not covered by the model that are favourable for the selection of many VCGs. One possibility would be the occurrence of many parasitic *h*-genes with different fitness parameters. Then the population could 'walk' through different areas where selection occurs (like 'walking up the stairs' from right to left in figure 2 and in figure 13). The number of VCGs would get higher and higher, as there is no selection against VCGs. However, this requires at every step at the right moment the presence of a mutant *h*-gene with the right fitness parameters.

Another possibility requires a highly skewed VCG frequency distribution, as for example with a subdivided population structure, in which many subpopulations occur. The model predicts that at equilibrium all VCGs will have equal frequency, none of them carrying parasitic *h* nuclei. If a few of these VCGs set up a subpopulation, this can possibly give a situation where *h* can invade, which will lead to *H,h* polymorphism. Consequently selection for additional VCGs will be possible in such a subpopulation.

Both possibilities seem to require rather special conditions and do not seem very likely on *a priori* grounds. Clearly more information concerning the nature and occurrence of parasitic nuclei and on the structure of natural populations of

filamentous fungi is required for a better evaluation of these issues.

The impossibility of recombination in imperfect species between individuals belonging to different VCGs considerably lowers the ecological importance of the parasexual cycle. As mitotic recombination will only occur within VCGs, and therefore with high probability between (nearly) clonally related individuals, it will only have minimal effects in terms of the generation of new genetic combinations.

The results from the model of a cytoplasmic parasitic element differ from the nuclear case in mainly two respects. First, the threshold in number of VCGs that need to be present in a population before selection for more VCGs becomes possible, is absent. This means that the difference between sexual and asexual species referred to above in the nuclear case, does not exist in the cytoplasmic case. Second, an omnicompatible (*OC*) mutant cannot invade the population. These findings suggest that cytoplasmic parasitic genetic elements are perhaps more likely candidates for causing selection for VI than nuclear elements. However, a problem with this idea is that VI not always forms an absolute barrier to cytoplasmic replicons (Caten, 1972; Gordon and Okamoto, 1991). Further analysis is needed to explore possibilities for selection for VCGs in situations where VI can be leaky and harmful cytoplasmic elements can occasionally slip through.

The existence of self-incompatible (*HSI*) types (see table 1 for documented cases) can only be understood with the present model if the self-incompatibility causes no fitness loss. In that case a *HSI* type will invade and subsequently go to fixation. A stable coexistence of *HSI* and self-compatible types is not possible. If the fitness loss caused by *HSI* is less than a specified amount (given by inequality (6)), a *HSI* type can invade the population, but will disappear again when the number of VCGs has increased. A study of the genetics and ecology of vegetative self-incompatibility may well contribute to a better understanding of the phenomenon of VI.

The present models do not consider the specific role of population size and mutation rate. Since mutation to new types is essential in the model (and is assumed to occur occasionally at low rates), and random drift in small populations can cause the loss of VCGs, a specific study on these aspects is carried out (chapter 5).

In his overview Grosberg (1988) mentions three hypotheses invoking natural selection for the evolution of very many 'VCGs', as they have also been found in several sessile, clonal invertebrates: Frequency dependent selection, overdominance and spatial or temporal variation in selective pressures. He argues that the first hypothesis is the probably the best, though not really appropriate to explain the very large amount of groups that is found. This agrees with our results, as our models

clearly are examples of frequency dependent selection. In haploid organisms overdominance can impossibly play a role, but spatial and temporal selective pressures, working on hypothetical varying pleiotropic effects of different VCGs may be of some importance, though this can never explain large numbers of VCGs.

There are possible alternative or additional selective explanations for VI. It may be important in interactions with other species, it may be necessary to occupy a territory for living, or it may be a pleiotropic effect of genes having their main effect in other functions. An example of this are the findings by Bernet (1992) on *Podospora anserina*, where some incompatibility genes also play part in regulating the formation of fruiting bodies. But in the same species Turcq et al. (1991) could not find another function for a different VI gene. But it is clear that, whatever the function of VI may be, regular contact between and within different VCGs is necessary for selection for VI to occur.

At present there is a conspicuous lack of information concerning the occurrence, genetics and physiology of VI. Differences in ecology and population structure are expected to be important, but again data from natural fungal populations are very scarce. A unified nomenclature should be welcome, and characteristics like homo- or heterothallism, (absence of) perfect stages, and origins of isolates are critical information, which unfortunately is lacking for many species. We hope that this paper will also stimulate others to study the genetics and ecology of natural fungal populations.

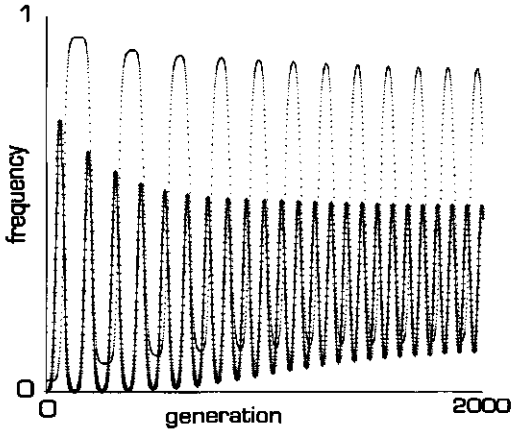

Figure 9. A stable limit cycle arises in a population with two VCGs. The frequencies of VCG c_1 (dots) and the frequency of h, Σx_i (crosses connected by a solid line) are given for $n = 1$, $p = 0$, $\theta = 0.6$ and $w = 0.15$ over 2000 generations. No mutations occur (neither to new VCGs, nor to H or h).

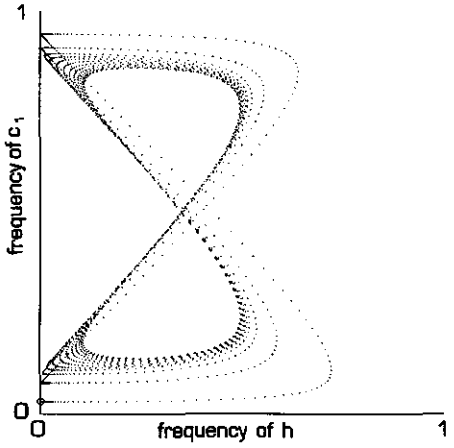

Figure 10. The same limit cycle as in figure 9. The frequency of h (Σx_i) is plotted against the frequency of c_1 ($x_1 + y_1$) every generation, starting with $x_1 = x_2 = 0.0005$, $y_1 = 0.03$ and $y_2 = 0.969$ (open circle).

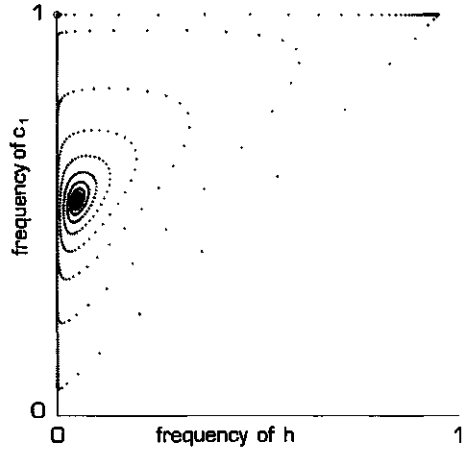

Figure 11. In a population with one VCG c_1 and a *HSI* type, a stable equilibrium is reached.

The frequency of h (Σx_i) is plotted against the frequency of c_1 in a population with $n = 1$, $p = 0$, $\theta = 0.6$, $w = 0.21$ and $f_{SI} = 0.99$ in a simulation over 4000 generations. Mutation to *HSI* occurs at generation 500.

Appendix

I. The dynamics of the model of Hartl et al. (1975).

Hartl et al. (1975) wrote that they 'performed extensive simulations looking for limit cycles and other kinds of attractors' in a population with two VCGs, but that 'no such instances were found'. Our simulations of their model however show that such instances can be found.

Figure 9 shows simulation results of a case where $2w > 2\theta\text{-}1 > w$ for two VCGs, so there is a polymorphism of h and H as described in the main text. Figure 10 shows the same simulation, with Σx_i, the sum in frequency of all h-nuclei plotted against the frequency of one VCG. This shows a clear limit cycle, which is the 'stable state' of all simulations, independent of starting frequencies.

A Heterokaryon Self Incompatible (*HSI*) type (as described in the third section of the nuclear parasite model) can invade the population if its fitness f_{SI} is not too small, and if h-type nuclei are not removed from the population by selection. Figure 11 shows that a stable equilibrium point exists if *HSI* is introduced in a population with only one VCG. From (14) (Appendix II) this point can be calculated for n=1 as the point at which:

$$\hat{y}_{SI} = 1 - \frac{w(2\theta - f_{SI})}{(1-p)(2\theta - 1 + w)(2\theta - 1)}$$

$$\hat{y} = \frac{f_{SI} - 1 + w}{(1-p)(2\theta - 1 + w)} \qquad (12)$$

$$\hat{x} = 1 - \hat{y}_{SI} - \hat{y}$$

In a population with two VCGs, where a limit cycle occurs, this cycle will be shifted by the introduction of a *HSI* type, as shown in figure 12. After the introduction of more (and more) VCGs, the *HSI* type will finally disappear.

II. The dynamics of the extended model for a parasitic nuclear gene

The general system of equations for the nuclear parasite model can be derived as follows:

In the model (with n neighbouring spots, from which a fraction p is empty) a VCG with frequency x has a probability

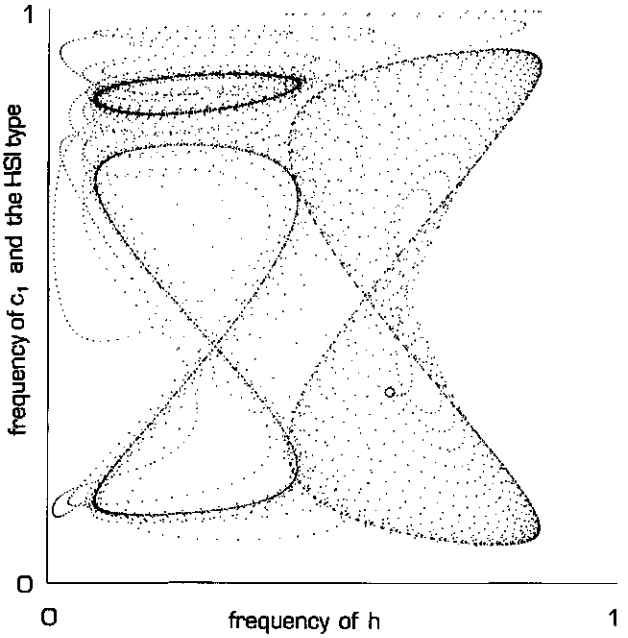

Figure 12. A population with two VCGs and $n = 1$, $p = 0$, $\theta = 0.6$, $w = 0.12$ for 8000 generations with mutation to a *HSI* type with $f_{SI} = 0.975$ after 4000 generations. The frequency of h (Σx_i) is plotted against the frequencies of VCG c_1 and $1\text{-}y_{SI}$, the latter showing up as the upper cycle left. Before mutation the population ends up in the limit cycle at the right, after the mutation to a *HSI* type the limit cycle shifts to the left.

$$\phi(x) = (1 - (1-p)x)^n \qquad (13)$$

of not forming a heterokaryon with any neighbour, and consequently staying homokaryotic.

It is assumed that heterokaryons are only formed with direct neighbours. So with n potential neighbours an individual grows as a homokaryon (consisting of one individual) when its has no compatible neighbours, or forms a heterokaryon consisting of 2,3.. n+1 individuals when it has 1,2.. n compatible neighbours. If such a heterokaryon consists of X h-individuals and Y H-individuals, the spores will produce a fraction $X\theta$ / ($X\theta$ + Y(1-θ)) h-spores and a fraction Y(1-θ) / ($X\theta$ + Y(1-θ)) H-spores.

To calculate the frequency of the different VCGs in the next generation, the frequencies of spore genotypes from all possible homo- and heterokaryons, weighted by the frequency of occurrence of the latter, must be added. Each VCG c_i only forms

heterokaryons with members of the same VCG and with the omnicompatible type *OC*. The self incompatible type *HSI* forms no heterokaryons at all. This leads to the following system of equations, with x representing the frequencies of individuals with h nuclei and y those of individuals with H nuclei, and the subscripts i, OC and SI standing for VCG c_i, the omnicompatible and the self incompatible individuals:

$$Wx_i' = x_i(1-w)\phi(y_i+y_{OC}) + x_i\Theta_i \tag{14a}$$

$$Wx_{OC}' = x_{OC}(1-w)\phi(\Sigma y_i+y_{OC}) + x_{OC}\Theta_{OC} \tag{14b}$$

$$Wy_i' = y_i\phi(x_i+x_{OC}) + y_i\mathrm{T}_i \tag{14c}$$

$$Wy_{OC}' = y_{OC}\phi(\Sigma x_i+x_{OC}) + y_{OC}\mathrm{T}_{OC} \tag{14d}$$

$$Wy_{SI}' = f_{SI}y_{SI} \tag{14e}$$

with:

$$\Theta_i = \sum_{r=0}^{n-1}\sum_{s=0}^{n-r-1} \frac{n!}{s!\,r!\,(n-r-s)!}(1-(1-p)(x_i+y_i+x_{OC}+y_{OC}))^s$$
$$(1-p)^{n-s}\,(y_i+y_{OC})^{n-r-s}\,(x_i+x_{OC})^r\frac{(n+1-s)\theta}{(r+1)\theta + (n-r-s)(1-\theta)} \tag{15a}$$

$$\Theta_{OC} = \sum_{r=0}^{n-1}\sum_{s=0}^{n-r-1} \frac{n!}{s!\,r!\,(n-r-s)!}(1-(1-p)(1-y_{SI}))^s$$
$$(1-p)^{n-s}\,(\Sigma y_i+y_{OC})^{n-r-s}\,(\Sigma x_i+x_{OC})^r\frac{(n+1-s)\theta}{(r+1)\theta + (n-r-s)(1-\theta)} \tag{15b}$$

$$T_i = \sum_{r=1}^{n}\sum_{s=0}^{n-r} \frac{n!}{s!\,r!\,(n-r-s)!}(1-(1-p)(x_i+y_i+x_{OC}+y_{OC}))^s$$
$$(1-p)^{n-s}\,(y_i+y_{OC})^{n-r-s}\,(x_i+x_{OC})^r \frac{(n+1-s)(1-\theta)}{r\theta + (n-r-s+1)(1-\theta)} \qquad \textbf{(15c)}$$

$$T_{OC} = \sum_{r=1}^{n}\sum_{s=0}^{n-r} \frac{n!}{s!\,r!\,(n-r-s)!}(1-(1-p)(1-y_{SI}))^s$$
$$(1-p)^{n-s}\,(\Sigma y_i+y_{OC})^{n-r-s}\,(\Sigma x_i+x_{OC})^r \frac{(n+1-s)(1-\theta)}{r\theta + (n-r-s+1)(1-\theta)} \qquad \textbf{(15d)}$$

and with mean fitness W standing for the sum of the right hand sides of (14).

In formulae (14a) to (14d), the first part of the equation right of the '='-sign stands for the fraction of that genotype produced by homokaryons and the second part the fraction produced by heterokaryons. The parts of the latter given more explicitly in (15a) to (15d) can be derived from the multinomial distribution, leaving out the non-heterokaryotic types.

For $x_{OC} = y_{OC} = 0$ the mean fitness W becomes:

$$W = 1 - w\Sigma x_i\phi(y_i) - y_{SI}(1-f_{SI}) \qquad \textbf{(16)}$$

The system of equations (14) is to complicated to solve analytically. Some kind of stability analysis is possible however, as it can be shown under which conditions $\Sigma x_i = 0$ or $\Sigma y_i = 0$ are stable, that is when h or H are unable to invade.

If $x_{OC} = y_{OC} = y_{SI} = 0$, for $f(x_i) = x_i'$, equation (14a) can be rewritten as

$$f(x_i) = x_i \frac{(1-w)(1-(1-p)y_i)^n + \Theta_i}{1 - w\sum_j x_j(1-(1-p)y_j)^n} \qquad \textbf{(17)}$$

Now it is easy to see that $f(0)=0$ is an equilibrium.

This is stable if $|f'(0)| < 1$, i.e. for $\Sigma x_i = 0$, if

$$|f'(0)| = |(1-w)(1-(1-p)y_i)^n + \sum_{s=0}^{n-1}\binom{n}{s}(1-(1-p)y_i)^s((1-p)y_i)^{n-s}\frac{(n+1-s)\theta}{\theta+(n-s)(1-\theta)}| < 1 \qquad \textbf{(18)}$$

Therefore if (18) is true, a h type will not be able to invade in VCG c_i if there are

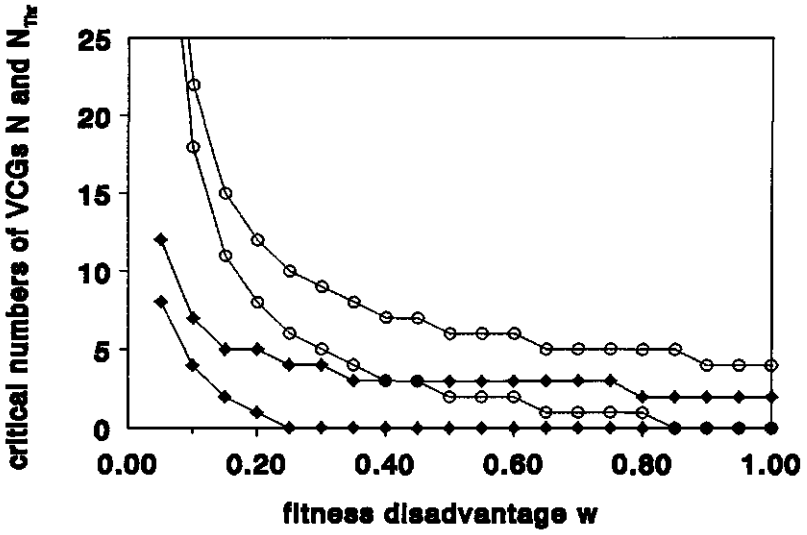

Figure 13. Critical numbers of VCGs for $n = 4$ and $p = 0.4$ in the nuclear parasite model, calculated numerically by formulae (18) and (20). The black squares give N (above) and N_{Thr} (below) for $\theta = 0.6$, the circles for $\theta = 0.9$.

no h nuclei present in the population.

Also, for $g(y_i) = y_i'$, equation (14c) becomes

$$g(y_i) = y_i \frac{(1-(1-p)x_i)^n + T_i}{1 - w\sum_j x_j(1-(1-p)y_j)^n} \tag{19}$$

Here again $g(0) = 0$ is a stable equilibrium if $|g'(0)| < 1$ for $\Sigma y_i = 0$, so if

$$|g'(0)| = \left| \frac{(1-(1-p)x_i)^n + \sum_{r=1}^{n} \binom{n}{r} (1-(1-p)x_i)^{n-r} ((1-p)x_i)^r \frac{(r+1)(1-\theta)}{(r-1)\theta + 1}}{1-w} \right| < 1 \tag{20}$$

If this condition is satisfied, H will not be able to invade in VCG c_i.

It is easy to see that (18) solved for $n = 1$, $p = 0$ gives (2), and solving (20) gives (3). For larger n, (18) and (20) can only be solved numerically. As an example the results of these calculations in case of $n = 4$ and $p = 0.4$ are given in figure 13.

For the *HSI* type it can be deduced that it increases in frequency if

$$f_{SI} > 1 - \frac{w\Sigma x_i \phi(y_i)}{1 - y_{SI}} \tag{21}$$

and decreases otherwise.

III. The dynamics of the model for a harmful cytoplasmic element.

The general system of equations in case of a cytoplasmic element can be derived in the same way as the system above:

With Φ defined as in Appendix II, x_i standing for the frequency of the infected individuals of VCG c_i and y_i that of the uninfected ones, the system of recurrence relations for all types in case of a harmful cytoplasmic element becomes:

$$\begin{aligned}
Wx_i' &= \alpha[(1-c)x_i + y_i(1 - \phi(x_i + x_{OC}))] \\
Wx_{OC}' &= \alpha[(1-c)x_{OC} + y_{OC}(1 - \phi(\Sigma x_i + x_{OC}))] \\
Wy_i' &= (1-\alpha)(y_i + x_i(1-c)) + \alpha y_i \phi(x_i + x_{OC}) \\
Wy_{OC}' &= (1-\alpha)(y_{OC} + x_{OC}(1-c)) + \alpha y_{OC} \phi(\Sigma x_i + x_{OC}) \\
Wy_{SI}' &= f_{SI} y_{SI}
\end{aligned} \tag{22}$$

with

$$W = 1 - c(\Sigma x_i + x_{OC}) - y_{SI}(1 - f_{SI}) \tag{23}$$

For the *HSI* type it is easy to see that y_{SI} will increase if

$$f_{SI} > 1 - \frac{c(\Sigma x_i + x_{OC})}{1 - y_{SI}} \tag{24}$$

so *HSI* cannot invade the population if

$$f_{SI} < 1 - c(\Sigma x_i + x_{OC}) \tag{25}$$

CHAPTER 5

Vegetative incompatibility II : Stochastic models

Summary

Vegetative incompatibility (VI), the prevention of heterokaryon formation, occurs frequently in ascomycetes. In all species studied, a large number of Vegetative Compatibility Groups (VCGs) is found. In this study the evolution of these large numbers of VCGs is investigated by using stochastic models, assuming random dispersal. Both the role of random genetic drift and selection for VI are studied. In asexual populations it is found that the product of population size and mutation rate for new VCGs has to be larger than 1 to get realistic numbers of VCGs, independent of selection. If the number of VCGs is large, selection does not lead to a significant increase in the number of VCGs. Sexual reproduction, however, enlarges the number of VCGs, due to recombination of VI-genes. Here strong selective pressure does give an extra increase VCG numbers. These results suggest that VI may be a neutral trait, although that does not explain the ubiquity of VI. Further research, incorporating population structure, is suggested.

Introduction

The phenomenon of vegetative incompatibility (VI) is known to occur frequently in filamentous ascomycetes (Carlile, 1987, see chapter 4 for overview). It is comparable to somatic incompatibility in invertebrates and the histocompatibility system in mammals, as it is a reaction preventing fusion of genetically different tissues, following a self / non-self recognition (Grosberg, 1988). A common feature of these incompatibility systems is the existence of a genetically determined, extremely high number of different incompatible types

In ascomycetes VI prevents the exchange of genetic material between conspecifics, which get in contact in the vegetative stage of the life cycle. Heterokaryon formation is therefore prevented by VI, but sexual fusion is not

affected. Generally VI is genetically regulated by a number of different loci with two alleles each (Puhalla and Spieth, 1985). One or more allelic difference(s) over all these loci causes VI.

In a previous study (chapter 4) we have tried to explain the large numbers of Vegetative Compatibility Groups (VCGs) that are found in natural populations. (Within a VCG all members are vegetatively compatible, whereas between VCGs VI occurs.) In that study deterministic models were used, assuming an infinite population size. A parasitic ('selfish') element (either nuclear or cytoplasmic) was considered as an agent generating selective pressure for the evolution of VI, as has been proposed by various authors (Pittenger and Brawner, 1961; Caten, 1972; Hartl et al., 1975). It was found that the cytoplasmic element is a better candidate for effective selective pressure, but that neither of the two could explain the existence of large numbers of VCGs as found in nature.

As an alternative to selection, it might be that VI has to be considered as a neutral trait, perhaps a pleiotropic effect of genes performing another function (Davis, 1966; Bernet, 1992). Therefore the evolution of VI under both neutral and selective hypotheses will be studied in this paper. This will be done in a stochastic approach, in order to assess the importance of the effects of random genetic drift, with population size and mutation rate as the main parameters. As in the previous study (chapter 4) the main goal is to find out which conditions can explain the large numbers of VCGs found in nature.

At first the evolution of VCGs in an imperfect (clonal) species will be studied, assuming that VI is a neutral trait. In the second part the effects of selection in such a species will be treated, and in the final part some of the effects of sexual reproduction will be considered.

Methods

The stochastic models on the evolution of VI are treated both by analysis and by computer simulation. In analysis the diffusion equation method (Wright, 1938; Crow and Kimura, 1970; Ewens, 1979) is used, which only gave results in the less complicated models. Simulation, however, is performed in all cases, so that the results of analysis can be compared with it.

Computer simulation was performed using a program written in PASCAL, assuming a population of N individuals. Reproduction was imitated by sampling N new individuals from the old population with replacement. Selection was

effectuated by adjusting the probability of being sampled, depending on the strength and the type of selection. Mutation occurred at random each generation, after the number of VCGs was counted.

An asexual population without selection

In a species without sexual reproduction (an imperfect species like *Aspergillus niger*) recombination can occur in a parasexual cycle following heterokaryosis (Pontecorvo, 1946). But because heterokaryosis does not occur between VCGs, recombination between different VI-alleles is always impossible in such species. So if one only considers the VI-loci, the whole genome can be regarded as one large locus with (approximately) infinitely many alleles, each allele rendering a different VCG.

With this assumption, population genetic theory on one locus with infinitely many alleles can be applied to the evolution of VI. Therefore, the formula for the expected number of alleles in a population with size N and a mutation rate u, as derived by Ewens (1964) and Kimura (1968) (see also Appendix II), can be used. Noting that fungi are haploid organisms (so there exist not $2N$ but only N haploid genomes in the population) and that in this case the mutation rate u is the rate over all VI-loci in the genome, the expected number of VCGs n, is given by:

$$n = 2Nu \int_{N^{-1}}^{1} \frac{(1-x)^{2Nu-1}}{x}\, dx \qquad (1)$$

The solutions of equation (1) for different values of N and u are given in table 1. It is shown that realistic values for n (like $n > 30$, see chapter 4) are only found if $Nu > 1$.

A computer simulation of the process was performed for different values of N and Nu. As table 2 shows, the results of this simulation agree fairly well with the analytic results.

An asexual population with selection

It is shown above that neutral theory can explain high numbers of VCGs as long as Nu is high. Selection for VI (against compatibility) is expected to enlarge the number of VCGs in the population. To get an impression of the quantitative effect

Table 1. Expected numbers of VCGs calculated by (1).

N Nu	10^3	10^4	10^5	10^6
0.001	1.014	1.018	1.023	1.028
0.01	1.137	1.184	1.230	1.276
0.1	2.324	2.784	3.245	3.705
1	11.82	16.42	21.03	25.63
10	67.58	113.3	159.3	205.4
100	244.9	671.4	1131	1592

Table 2. Numbers of VCGs in a population without sex and without selection. For $N = 100$, $N = 1000$ and $N = 5000$, analytical predictions (above) can be compared with the mean numbers ± standard deviation found in computer simulations over 50000 generations (below).

Nu →	0.001	0.01	0.1	1	10	100
N = 100	1.01	1.09	1.86	7.23	24.78	
	1.01 ± 0.07	1.11 ± 0.27	1.89 ± 0.92	7.73 ± 2.31	31.43 ± 3.81	
N = 1000	1.01	1.14	2.32	11.82	67.58	244.9
	1.05 ± 0.22	1.12 ± 0.34	2.42 ± 1.18	12.26 ± 3.05	71.92 ± 7.18	311.11 ± 12.10
N = 5000	1.02	1.17	2.65	15.035	99.46	536.7
	1.13 ± 0.34	1.19 ± 0.41	2.80 ± 1.06	14.90 ± 3.56	102.98 ± 8.90	577.29 ± 19.81

of such selection, two models will considered. The first model assumes selection by a cytoplasmic element, the second assumes severe fitness disadvantage of all somatic fusions between different (compatible) individuals.

In a previous study (chapter 4) two types of selection, by a parasitic nuclear gene and by a harmful cytoplasmic element, were compared. These selective forces are considered realistic (Caten, 1972; Hartl et al, 1975). It was found that a cytoplasmic element was more effective in maintaining high numbers of VCGs than a nuclear gene. This 'cytoplasmic selection' is caused by the transmission of a harmful cytoplasmic element (e.g. a suppressive mitochondrion or a virus), which lowers the fitness of its carrier by a factor c. In the previous study a fraction α of the spores was assumed to transmit the element to the next generation. As the strongest selective pressure was found for high values of α, it is assumed that $\alpha = 0.9$ throughout this paper. Further it is assumed that, unless stated differently, each individual meets exactly one conspecific with which it undergoes an (in)compatibility reaction. The recurrence relations for an infinite population as given in the previous study (chapter 4) are shown in Appendix I.

In addition a stronger, hypothetical type of selection is studied, which has been given the name 'compatibility selection'. The purpose of this model is to assess by how much the number of VCGs may be enhanced by very strong selection forces, in finite populations. Such strong selection is almost certainly unrealistic, but the model serves as a 'limiting case', to study the maximum effect of selection on the number of VCGs. 'Compatibility selection' assumes that individuals meeting a compatible partner suffer a fitness disadvantage s. So if $s = 1$, all individuals meeting a compatible partner die, which supplies about the strongest selective pressure one can imagine. Again, for simplicity it is assumed that each individual meets exactly one conspecific. For comparison with 'cytoplasmic selection', in Appendix I the recurrence relations for an infinite population in case of 'compatibility selection' are given. It shows that a model with that assumption would predict an infinite number of VCGs.

Only for 'compatibility selection' it was possible to get analytical results for the number of expected VCGs in a finite population. As shown in Appendix II it can be derived that the average number of VCGs in equilibrium will be

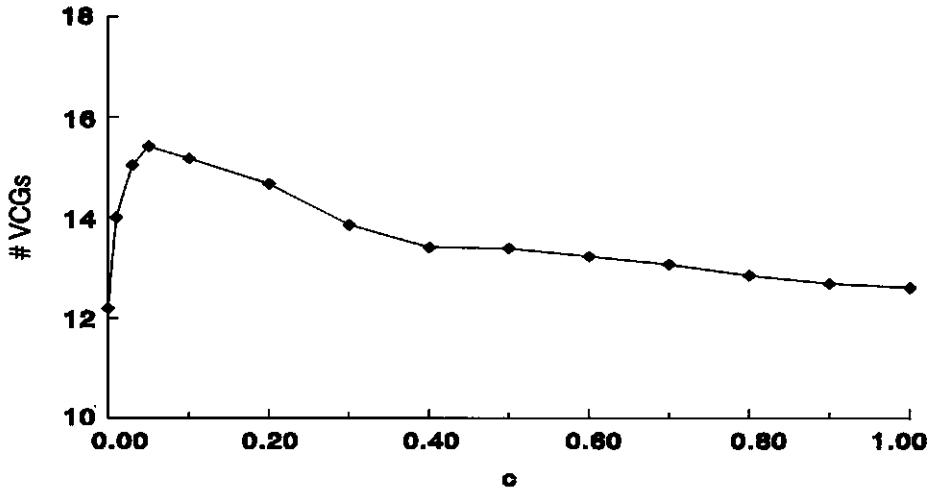

Figure 1. Mean numbers of VCGs in case of 'cytoplasmic selection'. The results of computer simulations over 100000 generations per given *c*-value. N = 1000 and Nu = 1.

$$n = 2Nu \int_{N^{-1}}^{1} e^{\frac{2Nsx}{1-s\Sigma x^2}} (1-x)^{\frac{2N(s+u-s\Sigma x^2(1+u))}{1-s\Sigma x^2}-1} x^{-1}\, dx \qquad (2)$$

where Σx^2 stands for the sum of squares of the frequencies of all VCGs. (This sum can be estimated as given in Appendix II).

For 'cytoplasmic selection' a similar formula could not be found.

In addition computer simulations were performed. The number of VCGs found for different values of c in case of 'cytoplasmic selection' is given in figure 1. It shows that the largest number of VCGs (15.4 ± 2.96 if N = 1000 and Nu = 1) is found when $c \approx 0.05$. The same is done for 'compatibility selection', where selection is maximal for s = 1 (with 31.1 ± 3.24 VCGs if N = 1000 and Nu = 1), as given in figure 2. This shows again that the results of analysis and simulation do agree.

The results of selection for various values of Nu, as found in computer simulations, are given in table 3 and figure 3. Maximal impact of selection is assumed, that is c = 0.05 for 'cytoplasmic' and s = 1 for 'compatibility selection'. Surprisingly, the difference between the number of VCGs in populations with and

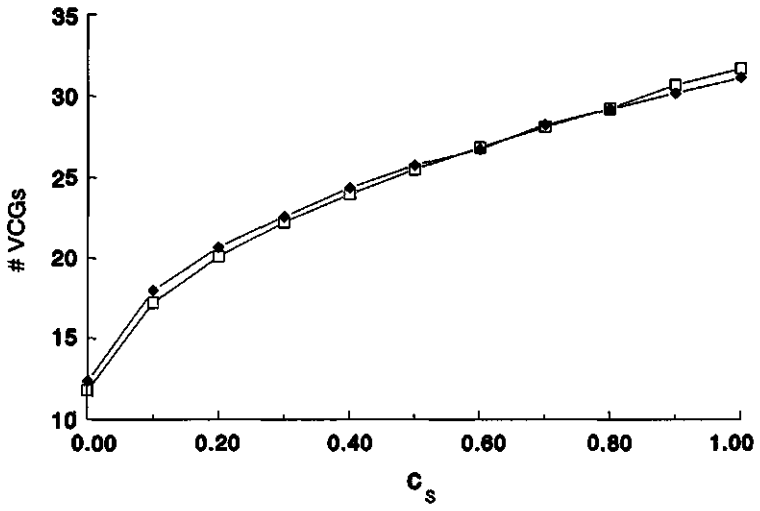

Figure 2. Numbers of VCGs in case of 'compatibility selection'. The mean values found in computer simulations over 100000 generations are given for different values of s (♦) and can be compared with the results of analytical predictions (□). $N = 1000$ and $Nu = 1$

Table 3. Numbers of VCGs in populations without sex, with $N = 1000$. The results of computer simulations over 50000 generations of populations without selection (neut) can be compared with those of populations with 'cytoplasmic selection' (cyt) and 'compatibility selection' (com). The analytic predictions for the first and the last are added in the rows on top.

Nu →	0.001	0.01	0.1	1	10	100
neut	1.01	1.14	2.32	11.82	67.58	244.9
	1.05 ± 0.22	1.12 ± 0.34	2.42 ± 1.18	12.26 ± 3.05	71.92 ± 7.18	311.11 ± 12.10
cyt	1.05 ± 0.22	2.17 ± 0.90	5.14 ± 1.21	15.08 ± 2.963	72.36 ± 7.02	307.55 ± 12.09
com	11.88	14.07	18.54	31.68	84.08	
	6.46 ± 2.25	12.66 ± 2.40	17.78 ± 1.64	31.3 ± 3.30	86.8 ± 6.83	313.4 ± 11.99

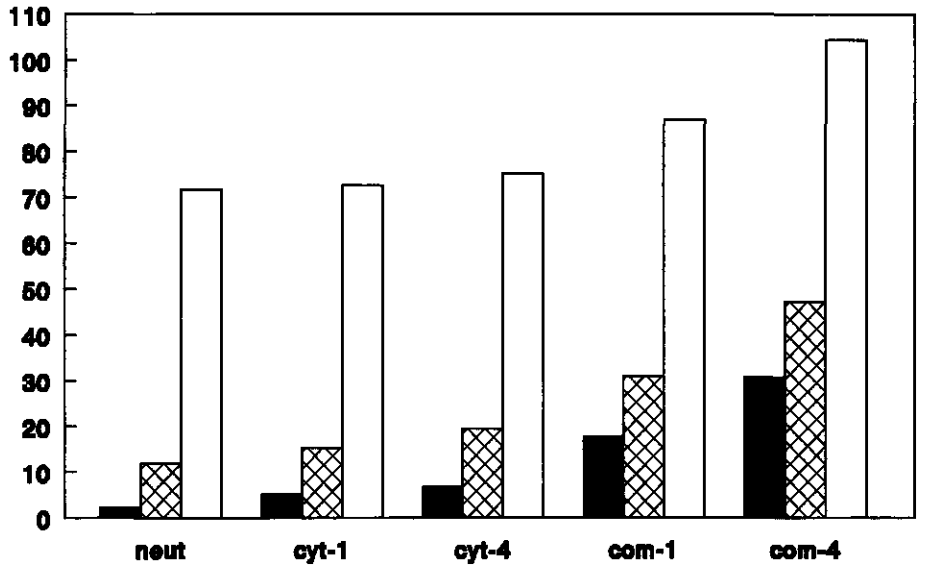

Figure 3. Numbers of VCGs found in computer simulations over 100000 generations, for N = 1000 and different values of *Nu* : 0.1 (left), 1 (middle) and 10 (right). In case of selection the results for always meeting one (n = 1) and four (n = 4) conspecifics are given separately (in the latter case selection is stronger, see Appendix I). *neut* = no selection; *cyt-1* = 'cytoplasmic selection', n = 1; *cyt-4* = 'cytoplasmic selection', n = 4; *com-1* = 'compatibility selection', n = 1; *com-4* = 'compatibility selection', n = 4;

without selection is small, when there are many VCGs. Therefore, selection does not have a large influence on the number of VCGs in a population with realistic numbers of VCGs. The number of VCGs is mainly determined by *Nu*.

A population with sexual reproduction

In many ascomycetes both sexual and asexual reproduction can occur. In a heterothallic species (like *Neurospora crassa*) self-fertilization is impossible, so sexual reproduction always implies outcrossing. In a homothallic species (like *Aspergillus nidulans*) however, selfing is probably very frequent, and outcrossing may be rare. As selfing and asexual reproduction are genetically identical in haploids, the terms sexual and asexual reproduction will be used here as equivalents of respectively outcrossing and vegetative reproduction or selfing.

With sexual reproduction recombination between VI-loci is possible.

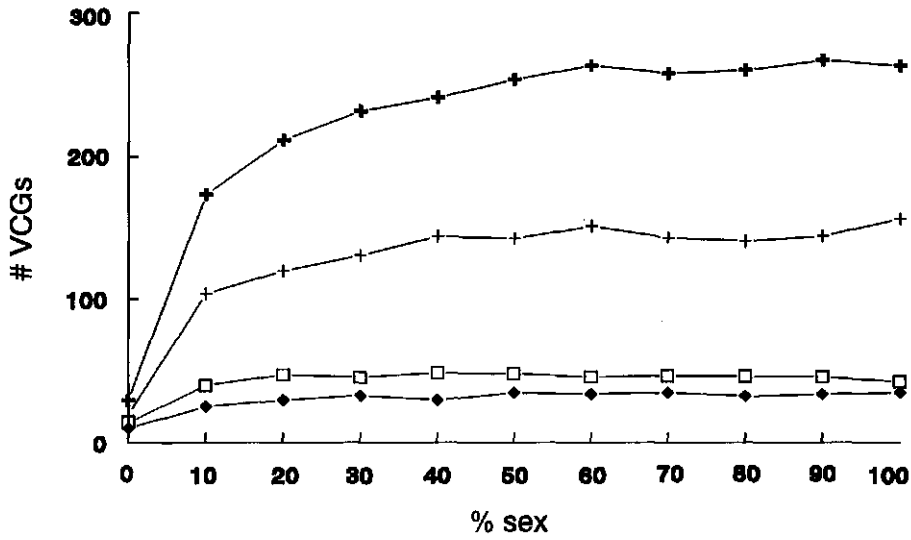

Figure 4. Numbers of VCGs found in computer simulations over 100000 generations, for N = 1000, Nu = 1, with different percentages of sexual reproduction along the horizontal axis. Results are given in case of 'compatibility selection' with s = 1 (+), 'compatibility selection' with s = 0.2 (+), 'cytoplasmic selection' with c = 0.05(□) and no selection (♦).

Consequently, the approach of considering VI as determined by one large locus with infinitely many alleles can no longer be used. Therefore VI was assumed to be determined by 10 unlinked loci with 2 alleles each in the simulation studies, allowing a maximum of 1024 VCGs. (This is comparable to the number of loci that has been found in *Neurospora crassa* (Perkins and Turner, 1988).) Further, it was assumed that in each generation a fixed proportion reproduces sexually, and the remainder reproduces asexually. Sex was simulated by sampling two random parents (with replacement) from the population, which produce one descendant for the next generation. The genotype of this descendant was a random combination of the parental genes, similar as in real life. Like in the two models above, the mutation frequency u was considered to be the frequency per genome, not per locus.

The results of simulation studies with different percentages of sex, all with N = 1000 and Nu = 1 are given in figure 4. It shows that sexual reproduction gives a considerable increase in the number of VCGs. Only a small percentage of sex is sufficient to achieve this. As in the models without sex, 'cytoplasmic selection'

only gives a low selective pressure for more VCGs, whereas 'compatibility selection' can cause a much larger increase.

Discussion

In this study we show that the effects of random genetic drift are extremely important for an explanation of the high number of VCGs found in natural populations of ascomycetes. The absence of selection is compared with two potential selective regimes: mild and presumably realistic selection by the action of a harmful cytoplasmic element (as in chapter 4), and strong and probably unrealistic selection, by assuming a (severe) fitness disadvantage for every compatible interaction. Generally, selection cannot explain large numbers of VCGs, unless the product of population size and mutation frequency *Nu* is larger than 1. However, if *Nu* is this high, selection is not necessary to explain large numbers of VCGs. Only extreme ('compatibility'-)selection in a sexual population, can give a considerable increase in the number of VCGs. Therefore it can be concluded from this study that the role of selection in the evolution of large numbers of VCGs may be limited.

This conclusion might support the idea that VI is selectively neutral, and therefore probably not essential. It might be that the genes causing VI are pleiotropic, and that other functions are more important (Davis, 1966; Bernet, 1992). Such a neutral hypothesis, however, is not really convincing. In their studies on some incompatibility genes in *Podospora anserina*, Turcq et al. (1991) could not find any pleiotropic effect of these genes. Furthermore, it is hard to believe that an identical neutral side effect is found in probably all known ascomycete species. Also, the specific biallelic interaction of the VI-alleles suggests significance of VI. The number of loci determining VI seems rather high for an insignificant trait.

Also, the relevance of the conclusion for the situation in the field is not clear yet. An important assumption in the model is the constant fixed population size with random sampling of descendants and random mating when sex occurs. Although hardly anything is known about population structure of most ascomycetes, it is improbable that this assumption resembles the situation in nature. Data on population structures are necessary and models including spatial and temporal variation based on such data will give a better insight in the situation in nature. It should be stressed here, that the present study certainly does not show that VI has no selective value, but only that selection is not necessary and

insufficient to explain *large numbers* of VCGs. In small local populations, selection may operate and induce the origin of different VCGs or a higher mutation rate for VI. Consequently, the total number of VCGs over all these populations may be very large.

This study clearly shows that population size N and mutation frequency u will be very important parameters in any theory on the evolution of VI. However, nothing is known about their values in nature. In these microorganisms population size may be very high, so $Nu > 1$ might very well be true. Assuming that VI is neutral, Nu can be estimated by counting the numbers of VCGs in a sample. Theory on the number of alleles found in a sample (Ewens, 1972, 1979) can be applied, as expected number of VCGs k in a sample of size r is:

$$E(k) = \sum_{i=1}^{r} \frac{2Nu}{2Nu + i - 1} \qquad (3)$$

But as this is only valid in the neutral case, this does not allow to test for selection. Only when independent estimates of N and u can be made, the occurrence of selection for VCGs in nature can be tested. Possibilities to do this may lie in independent estimates using other neutral traits (like RAPDs or RFLPs). Also methods using the frequency distribution of VCGs or the spatial and/or temporal variation in these frequencies may be used, as these can be expected to be different for neutral and non-neutral traits (Ewens, 1979).

A study on the evolution of VI in populations with sexual reproduction is far more difficult then in clonal populations. The frequency of sex, the frequency of outbreeding, seasonal fluctuations, local interactions, mating structure: all these things may be important, but data are scarce. The simulations of a population with sexual reproduction are therefore very preliminary, and only offer some first indications on the influence of sex. As expected, sex increases the number of VCGs in a population, due to recombination. A low frequency of sex is sufficient to achieve this, although the maximum number of VCGs possible (1024 in this study) is never approached.

A comparison of empirical data on VI in sexual and asexual ascomycete species (like in table 1 in chapter 4), might also show higher numbers of VCGs in equally sized samples of species with sexual reproduction. Unfortunately, such a comparison is not easy, as the available data are generally not collected in the same way. Furthermore, it is very hard to study the genetics of VI in imperfect species, because incompatible strains cannot be crossed. Therefore the numbers of loci and

the genetic mechanism cannot be compared. If differences in numbers of VCGs between sexual and asexual species do exist, they will be difficult to detect.

In our previous study (chapter 4) it was concluded that selection alone cannot explain the evolution of large numbers of VCGs. This conclusion is supported by the present study, which shows that only very strong ('compatibility'-) selection yields a significantly increased number of VCGs. The major difference between the two studies is the introduction of effects of population size and mutation rate. As stated above these effects are very great, and it has clearly been shown now that the quantitative results of the first study are not realistic. However, the qualitative conclusion, that selection by cytoplasmic element is more efficient than that by a nuclear parasitic gene, still holds.

It seems that a satisfactory explanation for the high number of VCGs is not yet found. Empirical population studies on ascomycetes are necessary and theoretical studies including population structure will then be a next step to find convincing explanations.

Appendix

I Selection in a population with infinite size

In a previous study (chapter 4) the following system of recurrence relations for a population suffering 'cytoplasmic selection' with several VCGs i was derived, with x_i standing for the frequency of the infected and y_i standing for the frequency of the uninfected individuals of VCG i.

$$Wx_i' = \alpha\,[x_i(1-c) + y_i(1-(1-(1-p)x_i)^n)]$$
$$Wy_i' = (1-\alpha)(y_i + x_i(1-c)) + \alpha\, y_i(1-(1-p)x_i)^n \qquad (4)$$

with

$$W = 1 - c\sum_i x_i$$

Here c is the fitness disadvantage of the carriers of the cytoplasmic element (the infection), α the fraction of spores carrying the element, n the number of neighbouring spots, and p the fraction of those spots not occupied by a conspecific. ($n = 1$ and $p = 0$ in the present study) From these equations it was derived that the expected number of VCGs selected for in an infinite population is the smallest integer N that satisfies

$$N \geq \frac{\alpha n(1-p)}{1-\alpha(1-c)} \qquad (5)$$

If the situation with 'compatibility selection' is treated similarly, in case of an infinite population, the system of recurrence relations becomes:

$$Wx_i' = x_i(1 - s(1-(1-(1-p)x_i)^n)) \qquad (6)$$

with

$$W = 1 - s(1 - \Sigma x_i(1-(1-p)x_i)^n)$$

where x_i is the frequency of VCG i, s is the fitness disadvantage of meeting a compatible conspecific and n and p are defined as above. As normally $n = 1$ and

$p = 0$ in this study, (6) becomes

$$Wx_i' = x_i(1 - sx_i)$$

$$with \qquad (7)$$

$$W = 1 - s\Sigma x_i^2$$

It is easy to see that a new VCG will always invade, so an infinite number of VCGs will evolve in an infinite population.

II Analytically derived predictions for VCG numbers

Because recombination between VCGs in imperfect fungi is impossible, population genetic theory on the number of alleles at one locus that can be maintained in a population, can be applied to the number of VCGs.

Diffusion equation methods can be used to derive 'Wright's formula' for the distribution of gene frequency at equilibrium (Wright, 1938). The probability density of the equilibrium distribution can be given by $\phi(x)$,

$$\phi(x) = \frac{C}{V_{\delta x}} \exp\left[2\int \frac{M_{\delta x}}{V_{\delta x}}\, dx\right] \qquad (8)$$

where C is a constant, $M_{\delta x}$ is the mean change in gene frequency x in one generation (by mutation and selection) and $V_{\delta x}$ is its variance.

In a model with K different alleles at one locus in a haploid population of size N, it can be derived (Kimura, 1968) that the average number of alleles in the population is

$$n = K\int_{\frac{1}{N}}^{1} \phi(x)\, dx \qquad (9)$$

In our study, in the situation without selection and a mutation frequency u to new VCGs, $K \rightarrow \infty$ and $M_{\delta x} = -ux$ and $V_{\delta x} = x(1-x)/N$. This means that the average number of VCGs in a population equals

Except for haploidy, this is the same as Ewens (1964) and Kimura (1968) found for the average number of alleles for neutral alleles at one locus.

$$n = 2Nu \int_{N^{-1}}^{1} \frac{(1-x)^{2Nu-1}}{x}\, dx \qquad (10)$$

The situation with 'compatibility selection' is comparable to the model with mutually heterotic alleles, as described by Kimura and Crow (1964). Here Wright's formula (8) is interpreted as the frequency distribution, such that $\phi(x)\ dx$ represents the number of alleles whose frequency is in the range x to $x + dx$. In that case

$$\int_{0}^{1} x\phi(x)\, dx = 1 \qquad (11)$$

and the average number of alleles (or VCGs) present in the population can be estimated by

$$\int_{\frac{1}{N}}^{1} \phi(x)\, dx \qquad (12)$$

Now, for 'compatibility selection', one finds that
$M_{\delta x} = s\ x(\Sigma x^2 - x)/(1 - s\ \Sigma x^2) - ux$
and $V_{\delta x} = x(1-x)/N$, so from (8)

$$\phi(x) = CNe^{\frac{2Nsx}{1-s\Sigma x^2}}(1-x)^{\frac{2N(s+u-s\Sigma x^2(1+u))}{1-s\Sigma x^2}-1}x^{-1} \qquad (13)$$

As the sum of squares of allele (or VCG) frequencies (which is the degree of homozygosity in diploids) is approximately (Kimura and Crow, 1964; Ewens, 1979):

$$\sum x_i^2 = \int_{0}^{1} x^2\phi(x)\, dx \qquad (14)$$

Σx^2 and C can be calculated by (11) and (14), and so the number of VCGs can be determined with (12).

It can be shown that $C = 2u$ as follows.

By putting

$$a = CN$$

$$b = \frac{2sN}{1-s\Sigma x^2} \tag{15}$$

$$c = \frac{2sN(1+(1+u)\Sigma x^2)+2Nu}{1-s\Sigma x^2}$$

so

$$\phi(x) = ae^{bx}(1-x)^{c-1}x^{-1} \tag{16}$$

(11) can be rewritten as

$$a\frac{\gamma(c,b)}{e^{-b}b^c} = 1 \tag{17}$$

with γ(c,b) representing the alternative incomplete gamma function

$$\gamma(c,b) = \int_0^b t^{c-1}e^{-t}dt \tag{18}$$

(14) can be rewritten as

$$\frac{a}{c}(1+\gamma(1+c,b)\frac{b-c}{e^{-b}b^{c+1}}) = \Sigma x^2 \tag{19}$$

As it is known that (Abramowitz and Stegun, 1964)

$$\gamma(1+c,b) = c\gamma(c,b)-b^c e^{-b} \tag{20}$$

it follows from (17), (19) and (20) that

$$a = 2Nu \tag{21}$$

like in the neutral situation (10).

CHAPTER 6

Evolutionary dynamics of Spore killers

with R.F. Hoekstra
Genetics 135 (1993) : in press

Summary

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 chapter 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.

Introduction

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 and 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 et al., 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 to 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 et al., 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 et al., 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.

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 thesis. 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 et al., 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. ($x_1 + x_2 + x_3 = 1$)

Table 1. The fitnesses of the different crosses in a general model. Fitness values apply to the types at the left, when crossed to the types above. All $w_{ij} > 0$.

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

Fitness differences in the vegetative stage of the life cycle:

In this model a Sensitive strain is assumed to have fitness 1, and 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:

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

with

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

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

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

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

$$w_1 > w_3 > 1 \quad \vee \quad \left(w_3 < 1 \ \wedge \ w_1 > \frac{1}{c(1-r)} \right) \qquad (4)$$

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 $w_1 c(1-r) < 1$ 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

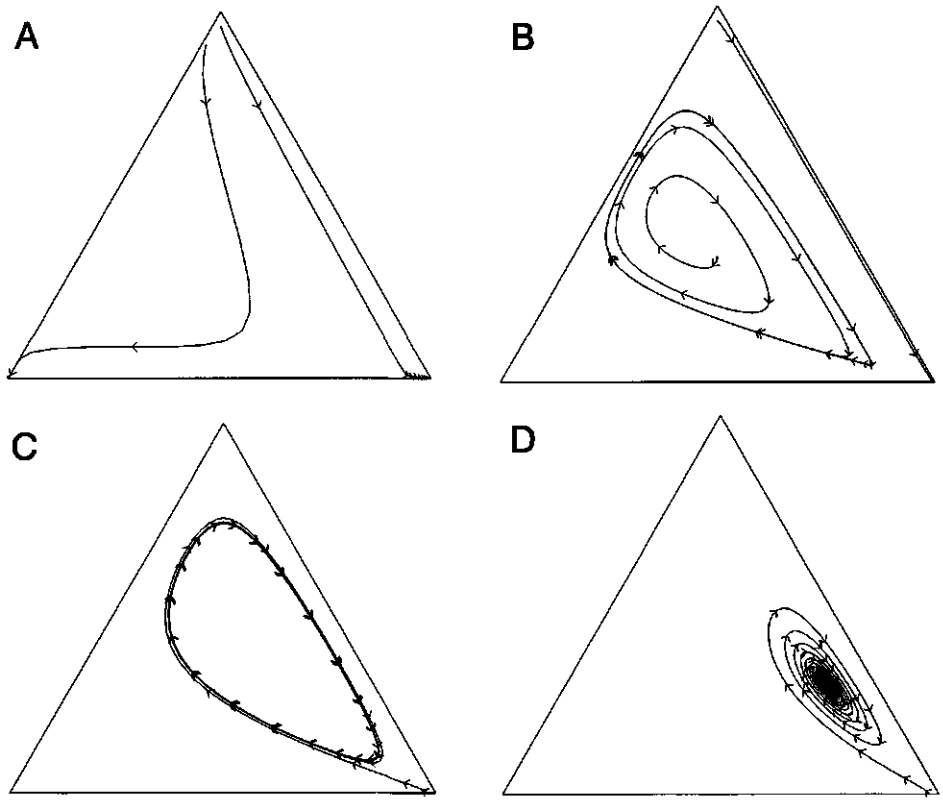

Figure 1. Some examples of the dynamics in the first model. The frequencies of Killers (left), Resistants (above) and Sensitives (right) are given in a de Finetti diagram. Successive arrows mark ten-generation intervals. In all diagrams $r = 0.1$ and $c = 2$. **A:** $w_1 = 0.53$, $w_3 = 0.4$: Killers 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 solution 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.

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

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

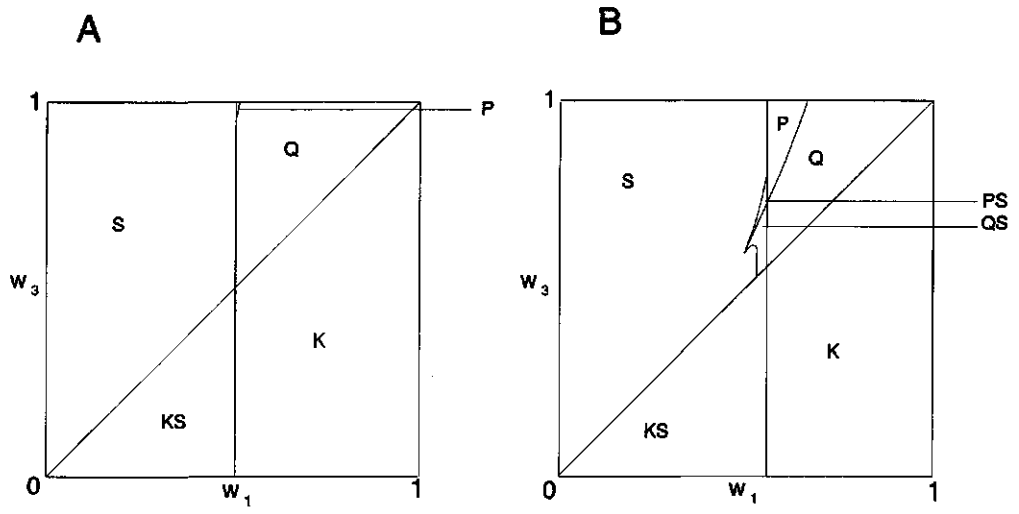

figure 2. Overview of the results of stability analysis in the first model.
S : $x_2 = 1$ (only Sensitives) is stable; K : $x_1 = 1$ (only Killers) is stable; KS : Both $x_1 = 1$ and $x_2 = 1$ are stable, polymorphism is unstable; Q: Only a quasi periodic orbit is stable; P: A polymorphic point with 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 $w_3 > w_1$.

only found if (5) is true, but can also be found in addition to the stable state x_2=1, if $w_1\, c(1\text{-}r) < 1$. 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 x 500 different values of w_1 and w_3 upto 10000 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, 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* x *-s*) be w_1, and for unnecessary resistance (in a cross *ks* x *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:

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

with

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

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

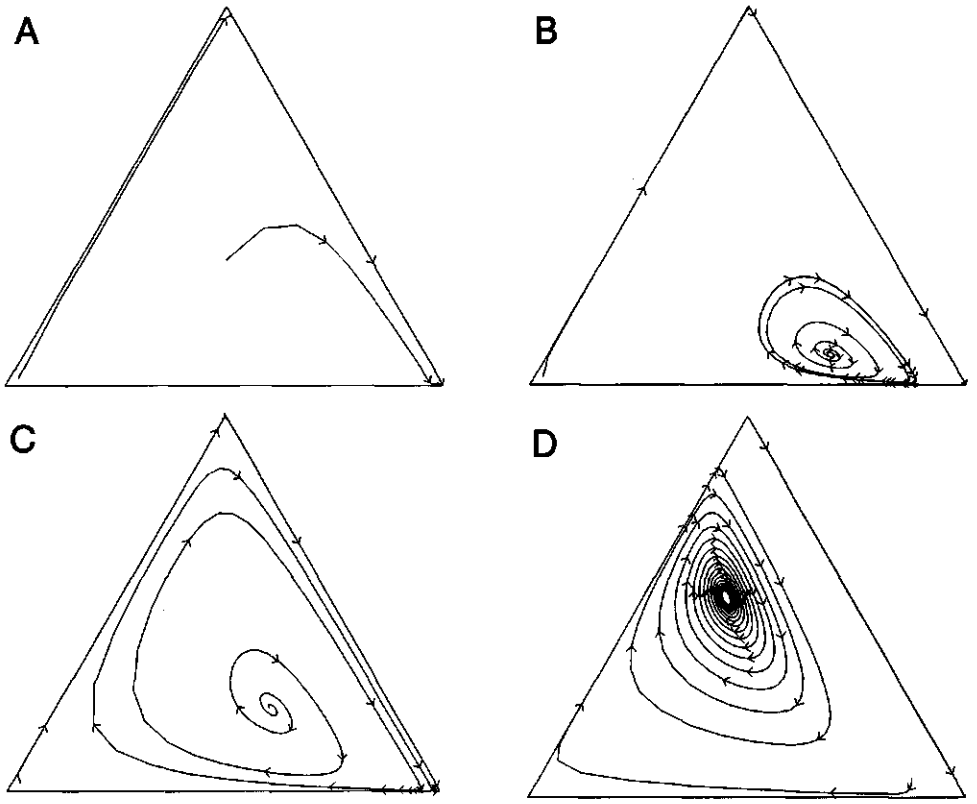

Figure 3. Some examples of the dynamics in the second model with $r = 0.01$, $c = 1$ and $w_3 = 0.6$. **A:** $w_1 = 0.05$: $x_2 = 1$ (Sensitives) is the only stable state. **B:** $w_1 = 0.25$: $x_2 = 1$ (Sensitives) is a stable state, but a quasi periodic solution is also a stable solution. **C:** $w_1 = 0.45$: $x_2 = 1$ (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.

$$w_1 > w_3 > 1 \quad \vee \quad c(1-r) > 1 > w_3 \tag{8}$$

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

$$1 > w_1 > w_3 > 2 - \frac{1}{w_1} \tag{9}$$

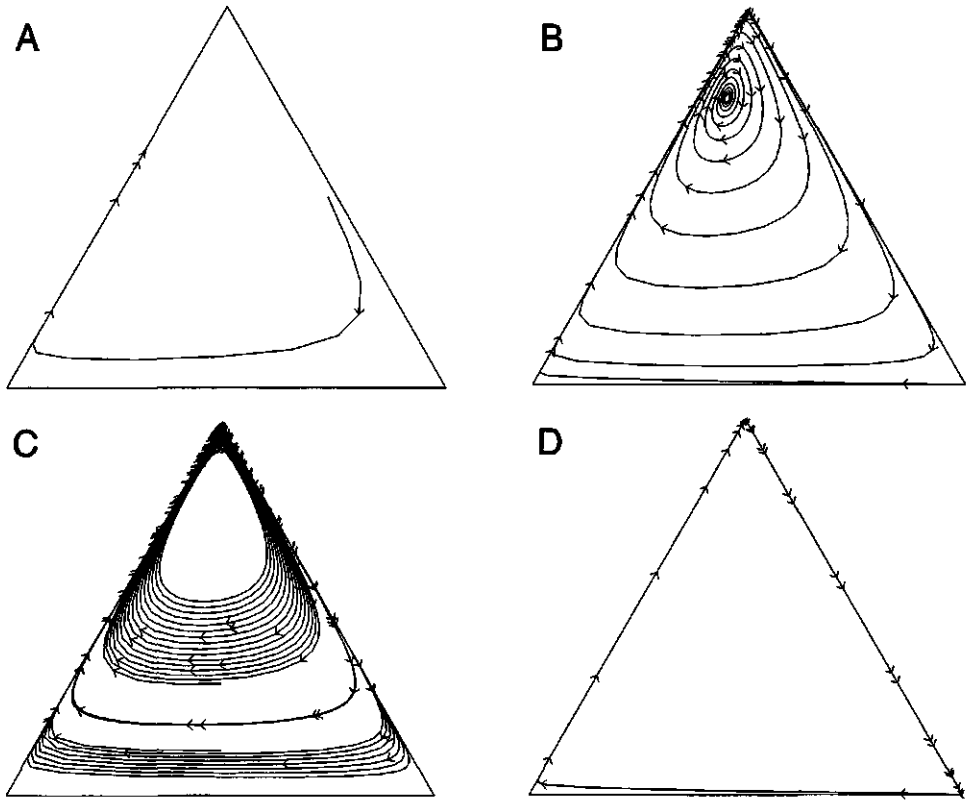

Figure 4. Some examples of the dynamics in the second model with $r = 0.01$, $c = 2$ and $w_1 = 0.75$. **A:** $w_3 = 0.6$: A polymorphism with Killers and Resistants at (12) is the only stable state. **B:** $w_3 = 0.8$: One polymorphic stable point. **C:** $w_3 = 0.85$: An unstable quasi periodic orbit 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 (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

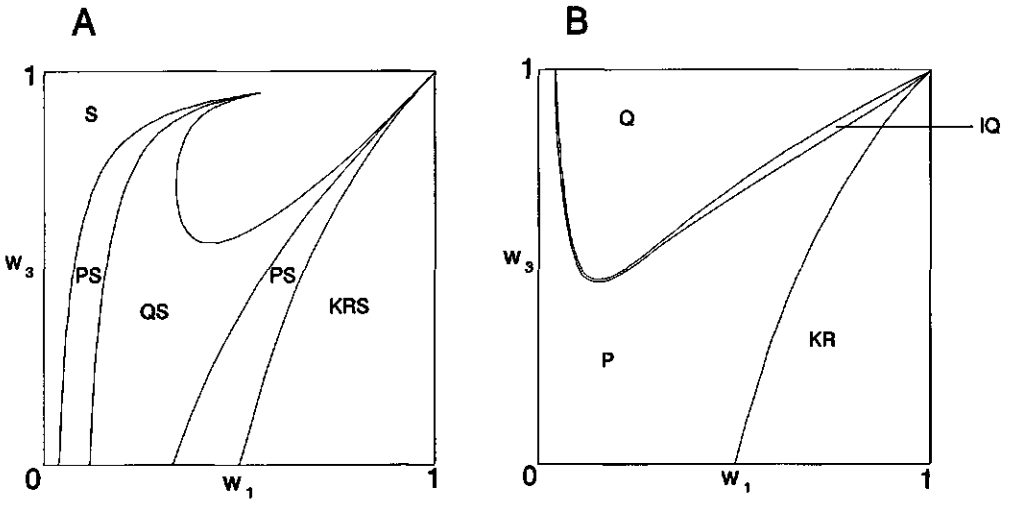

Figure 5. Overview of the results of stability analysis in the second model.
S : $x_2 = 1$ (only Sensitives) is stable; Q: Only a quasi periodic orbit is stable; P: A polymorphic point with Killers, Sensitives and Resistants is stable. QS : $x_2 = 1$ (only Sensitives) is stable, but a quasi periodoc 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 $0 < w_1, w_3 < 1$. 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 $w_3 > w_1$.

given in figure 5. Note that, unlike in the first model, also for $c = 1$ and $r > 0$ a polymorphism of the three 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 $w_1 c(1-r) > 1$ 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 x Sensitive and Killer x 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 et al., 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 intermedi-

ate 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 x 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 results) 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 chapter 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.

Acknowledgements: The authors thank an anonymous referee for helpful comments.

Appendix

Below the criteria for invasion of a Killer into a population and the stabiliy 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, (5) is true and polymorphism is expected.

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

$$\hat{x}_1 = \frac{\{\ 2+w_1(1-2c(1-r))-w_3(1+r)-w_1w_3(1-c) \quad \pm \sqrt{(1-cr)^2w_3^2-2w_1w_3(1-cr)(1-w_3(1-c+2cr))+w_1^2((1-(1-c)w_3)^2-4c^2w_3r(1-r))}\ \}}{2(1+w_1(1-c+cr)-crw_3)} \tag{10a}$$

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 (10a) is negative; also we require $0 \le \hat{x}_1, \hat{x}_2, \hat{x}_3 \le 1$. 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 (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 (11) is expected if (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:

$$\hat{x}_1 = \frac{\{\ (w_3-1)(2(1-c+cr)+w_1) - w_1cr - 1 + c \quad \pm \sqrt{(1-c)^2 + 2w_1((w_3-1)(1-c+2c^2r(1-r)) + cr(1-c)) + w_1^2((1-w_3+cr)^2}\ \}}{2((w_3-2)(1-c+w_1)+(w_3-1)cr)} \qquad \textbf{(13a)}$$

and

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

The function within the square root of (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 (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 thesis.

If it is assumed that $w_{ij} = w_i . w_j$, 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 x

Sensitive yields a double heterozygote (*KkSs*) and crosses of Killer x Resistant (*KkSS*) and Sensitive x 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.

CHAPTER 7

Sexual incompatibility in *Podospora anserina*: An anti meiotic drive device?

Summary

In the ascomycete fungus *Podospora anserina* a non-allelic heterogenic system of sexual incompatibility (S.I.) is found. The genes causing S.I. have been thoroughly investigated, but a functional explanation for the evolution of S.I. has not been proposed yet. In a population genetic model we show that selection acts against the evolution of S.I. It is proposed that S.I. might be a suppressor of spore killing, a form of segregation distortion (or meiotic drive) found in *P. anserina*. In a model incorporating S.I. and spore killing, it is shown that S.I. can invade a population where spore killing occurs. An essential assumption in this model is that the gene conferring resistance to spore killing, should also be one of the genes involved in S.I.. Experimental evidence for the latter is still missing.

Introduction

In many species there exist superimposed upon the basic sexual differentiation various mechanisms to restrict (intraspecific) exchange of genetic material. The fungi in particular show a great variety of these so-called incompatibility systems. Within the fungi the ascomycete *Podospora anserina* is serving as one of the model organisms for studies on incompatibility (Esser, 1965). This close relative of *Neurospora crassa* can be found on herbivore dung and is hermaphroditic and pseudohomothallic, with a life cycle as shown in figure 1.

Roughly, there are two types of incompatibility (Esser, 1971):

1) Homogenic incompatibility : The exchange of genetic material between individuals is prevented, when their nuclei carry *identical* incompatibility alleles.

In *P. anserina*, as in many other species, the mating types (+ and -) cause homo-

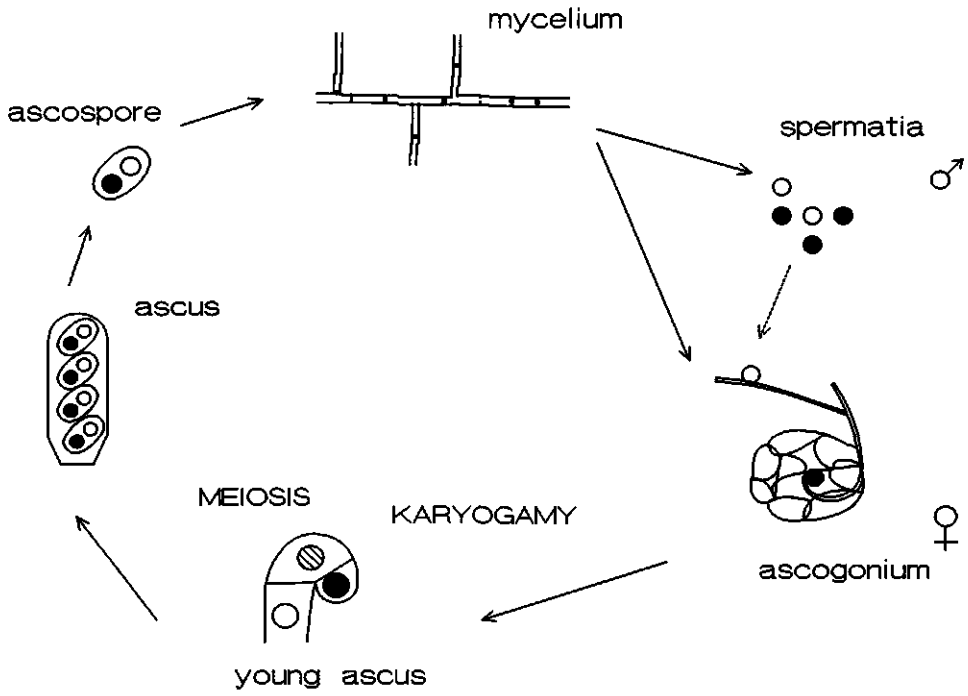

Figure 1. Life cycle of *P. anserina*. The mycelium contains two different types of haploid nuclei, one with mating type + (black dots) and the other with mating type - (open dots). Both male and female gametes are formed, the spermatia and ascogonia. (These spermatia cannot survive as asexual propagules, like the conidia in *Neurospora*.) Spermatia can fertilize ascogonia of the opposite mating type, via the trichogyn that grows towards it. After a very short diploid phase meiosis occurs, followed by ascospore formation. Four ascospores are formed, enclosing two nuclei each. These can grow out to a new mycelium.

genic incompatibility: Sexual fusion does not occur between gametes with identical mating types:

2) Heterogenic incompatibility : The exchange of genetic material between individuals is prevented, when their nuclei carry *non-identical* incompatibility alleles.

In *P. anserina* two types of heterogenic incompatibility are known:

i) Allelic incompatibility: This type prevents heterokaryon formation (somatic fusion) and is regulated by several genes with two alleles each. It has been found in many different fungi (Carlile, 1987). An allelic difference in one of these genes

is sufficient to cause incompatibility in the vegetative stage, but sexual fusion is not prevented.

ii) Non-allelic incompatibility: This type prevents both sexual fusion and heterokaryon formation. It is caused by the antagonistic interaction of a pair of genes. In total five pairs of genes causing this type of sexual incompatibility (S.I.) are found (Delettre and Bernet, 1976). An example is the unlinked gene pair *c* and *e*[1]. In this pair the combination of the alleles *CE* causes an incompatibility reaction, whereas the combinations *Ce*, *ce* and *cE* are compatible. The rather complicated course of events after a cross of a *Ce* strain with a *cE* strain is explained in the model below and in Appendix I.

Although S.I. in *P. anserina* has been thoroughly studied, the meaning of the phenomenon is not clear yet. Esser (1971) has studied the incompatibility reactions between 19 geographic races and found that 77.7% of all possible combinations did show some S.I.-reaction. He concludes that S.I., like pseudohomothallism, is an isolating mechanism, promoting inbreeding and stimulating speciation.

Bernet (1992) found that the S.I. genes are involved in (proto-) perithecium formation, converting vegetative cells from quiescence to a source of nutrient available to the female developmental cycle until spore formation. So S.I. might be a deviant expression of genes regulating cell death. The different alleles of the S.I. loci are regarded as a consequence of independent 'neutral' mutations.

Although the latter idea, that S.I. is a byproduct of a combination of genes which have suffered from (otherwise) neutral mutations may be true, the abundance of S.I. (-genes) and the enormous effects of the S.I.-reaction seem to imply differently. In this chapter we will therefore try to find a selective explanation for the evolution of S.I. by population genetic modelling. In the first model the evolution of S.I. will be studied on its own, and in the second spore killing will be considered as a potential selective force behind the evolution of S.I. Spore killing has been found in *P. anserina* by Padieu and Bernet (1967) and by us (Nauta et al., 1993). It is a special form of segregation distortion, or meiotic drive (Turner and Perkins, 1991; this thesis, chapter 6). In the model S.I. will be considered as a potential defense system against meiotic drive, the existence of which is suggested by Hurst and Pomiankowski (1991) and Turner (1993).

[1] Unfortunately Esser (1956) and Bernet(1965) have used different names for identical genes in their studies. In this paper we will use the terminology of Bernet(1992). His alleles C/c correspond to a_1/a and E/e to b/b_1 of Esser(1956)

The evolutionary dynamics of sexual incompatibility

The genetics of S.I. has been studied by Esser (1956, 1959) after a cross of two strains of genotype *Ce* and *cE*. It was found that a 'semi-incompatibility' (or 'unilateral incompatibility') reaction occurs. This semi-incompatibility means that a cross ♂ *Ce* x ♀ *cE* is possible and gives a 'normal' production of perithecia, whereas the reciprocal cross ♂ *cE* x ♀ *Ce* is prevented. After meiosis following a fertile cross ♂ *Ce* x ♀ *cE* all *CE* nuclei disintegrate, whereas the parental types *Ce* and *cE* and the recombinant *ce* are normally viable. (The precise results of such a cross and the consequences of pseudohomothallism are elaborated in Appendix I.).

To understand the evolution of S.I. this system has been modelled as given in Appendix I. The following characteristics are essential for understanding the dynamics:

i) The genes *C* and *E* are unlinked, showing 50% recombination as found by Esser (1956).

ii) The rate of selfing (α) in *P. anserina* is probably very high, and outcrossing will be rare. Because the spermatia (the male gametes) are not air-borne (Bernet, 1992) outcrossing will only occur between two neighbouring strains, in the mycelial zone where the two strains meet (Esser, 1956). As the value of α is only important for the speed of the evolutionary process and has no influence on the stable states, it has been given lower values in some computations.

iii) The ascogonia that are not (cross-) fertilized due to the S.I. (after a cross ♂ *cE* x ♀ *Ce*) will be selfed instead. This is based on the notion that new side-arms grow from the trichogyne after an incompatibility reaction (Esser, 1959). Because the incompatibility reaction might nevertheless lower the fitness of the ascogonium, this selfing is given a fitness value w ($0 \leq w \leq 1$). (Here it is relevant to know that protoperithecia do not develop unless fertilization has succeeded (Alexopoulos, 1962)). In the model this 'second chance' for ♀ *Ce* results in an advantage of *Ce* relative to *cE*.

An example of the dynamics is given in figure 2.

It is clear that in a population which initially exists of *Ce* and *cE* strains, therecombinant *ce* will always invade. This type is compatible to the other two and shows no fitness disadvantage (Esser, 1959). As a cross between *Ce* and *cE* will reduce the frequency of the parental types and increase the frequency of the recombinant *ce*, whereas selection is absent in all other crosses, the final stable state in each population will always be one with only *Ce* + *ce* or *cE* + *ce* types.

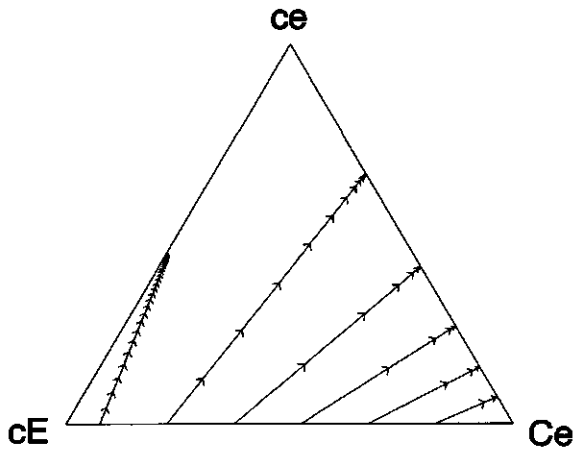

Figure 2. Dynamics of a population which initially exists of individuals with the incompatible genotypes *cE* and *Ce*. The frequencies of *cE*, *ce* and *Ce* are given in a de Finetti diagram for a selfing rate $\alpha = 0.75$, $w = 0.667$, fitness of mononucleate mycelia $v = 0.75$, % SDS of $c = p = 0.18$, % SDS of $e = q = 0.04$ (See Appendix I). Arrows indicate every tenth generation. Each population ends up without S.I.

Neither of them shows S.I..

This means that it is predicted that selection will operate against S.I.. As a new S.I. type can never invade a population, the evolution of S.I. cannot be explained.

Sexual incompatibility as a suppressor of spore killing

Spore killing is a form of meiotic drive that has been described in *Neurospora* (Turner and Perkins, 1979, 1991), *Gibberella fujikuroi* (Kathariou and Spieth, 1982; Sidhu, 1984) and *P. anserina* (Padieu and Bernet, 1967; Nauta et al., 1993). After crossing a Spore killer strain with a sensitive strain, only half of the ascospores survive, all surviving spores having the genotype of the killer. Population studies (Turner and Perkins, 1979; Turner, 1993) revealed the existence of several different Spore killers in *Neurospora*. Also resistant strains were found, that neither kill, nor are killed.

The first description of an instance of spore killing in fungi, was in *P. anserina* (Padieu and Bernet, 1967). They investigated a cross between two natural isolates in which two Spore killers were involved. Recently we sampled some fresh natural isolates of *P. anserina* from rabbit dung in The Netherlands and found another Spore killer (Nauta et al., 1993). This suggests that Spore killing may also be

frequent in *Podospora*. (An indication that spore killing not only occurs in *P. anserina* comes from Moreau (1953) who found abortion of spores in *Podospora curvula*.) Resistant types have never been described in *P. anserina*.

The genetics of spore killing in *Neurospora* has been studied by Campbell and Turner (1987) who found that at least two genes are involved, one causing the killing *(K)* and one conferring resistance to killing *(R)*. Both appeared to be very closely linked by a recombination block, associated with the occurrence of killing.

A Killer will thus have genotype *KR* (killing and resistant), a Resistant genotype *kR* (not killing and resistant) and a Sensitive genotype *kr* (not killing and not resistant).

In a previous model (chapter 6) we showed that a polymorphic population with only Killers and Sensitives can never be stable. If Spore killers are able to invade a population, they will easily increase in frequency, until the Sensitives have disappeared. However, if Resistants arise by (rare) recombination and if Killers have an additional advantage during the process of Spore killing, stable polymorphism may result.

As the model above shows that selection acts against the evolution of S.I., it seems that another function of S.I. must be found, which might offer an explanation for its existence. Here we want to suggest as its possible function the suppression of spore killing. Although there is as yet no empirical evidence for this, we do think some arguments can support this hypothesis.

Next to the apparent high frequency of occurrence, S.I. and spore killing have in common that both act during the reproductive phase and are involved in eliminating sister nuclei. Possibly S.I. can be an example of an anti-meiotic drive device, as suggested by Hurst and Pomiankowksi (1991): Because S.I. prevents certain crosses, it may also prevent spore killing.

We therefore propose the following model for the evolution of S.I.:

In a population where spore killing occurs, a S.I. mutant arises. This S.I. acts by an *I*-gene that affects the resistance-gene *R* of the spore killer. We then have two gene couples, *K* and *R* that operate in the killing system and *I* and (again) *R* that act like the genes *c* and *e* of the S.I. system. So we assume a double function of *R*, which is completely hypothetical but essential to the model. All genes have two alleles: *K/k* for killing/not killing, *R/r* for resistance/ no resistance and equivalents of *E/e* in the S.I. systems, and *I/i* for being incompatible/not incompatible with *R*, equivalent to *C/c*.

In this model only four viable genotypes can be formed, because *Kr* (suicidal)

and *RI* (self-incompatible) are inviable. These four viable types are the Killer (*KRi*), the Resistant (*kRi*), the Sensitive (*kri*) and the Incompatible (*krI*). Without the first type Spore killing is missing, and without the last S.I. will be absent. It is assumed that *R* and *I* recombine freely and *K* and *R* are tightly linked (as in *Neurospora*), without recombination.

Incorporating the specific characteristics of *P. anserina* into the model, the following system of recurrence relations can be derived, x_i' denoting the frequency of type t in the next generation (see Appendix II):

$$
\begin{aligned}
Wx_k' &= x_k(1-(1-\alpha)x_i(1-\epsilon_1)) \\
Wx_r' &= x_r(1-(1-\alpha)x_i(1-\epsilon_1)) \\
Wx_s' &= x_s(1-(1-\alpha)x_k) + (1-\alpha)x_i(x_k\epsilon_{2k} + x_r\epsilon_{2r}) \\
Wx_i' &= x_i(1-(1-\alpha)(x_k(1-\epsilon_{3k}-w) + x_r(1-\epsilon_{3r}-w)))
\end{aligned}
\qquad (1)
$$

with

$$
W = 1-(1-\alpha)(x_k x_s + x_r x_i(2-\epsilon_1-\epsilon_{2r}-\epsilon_{3r}-w) + x_k x_i(2-\epsilon_1-\epsilon_{2k}-\epsilon_{3k}-w)) \qquad (2)
$$

In (1) x_k , x_r , x_s and x_i stand for the relative frequency of the Killer, Resistant, Sensitive and Incompatible type, α is the frequency of self-fertilization and the ϵ_i are the frequencies of the different types as they are produced in crosses suffering S.I. ($\Sigma\epsilon_i < 1$, see equation (6) in Appendix II).

Analysis of this system shows that only populations consisting of Killers and Resistants or Sensitives and Incompatibles can be stable. In both cases no killing and no S.I. are observed.

Incompatibles may invade in a population where Killers have invaded a population of Sensitives, ultimately leading to the disappearance of the Killers. This is illustrated in figure 3. It means that S.I. can be explained as an adaptive trait against spore killing if w has a value close to 1 (Appendix II), so if the ascogonia which did not get cross-fertilized due to incompatibility are selfed instead, without a considerable loss of fitness. A polymorphism of *Ce* and *cE* strains, the equivalents of Incompatibles and Resistants, remains unstable. An independent sampling of such strains can however be explained, if they are representatives from two subpopulations. In one of them the Resistant will have invaded after recombination

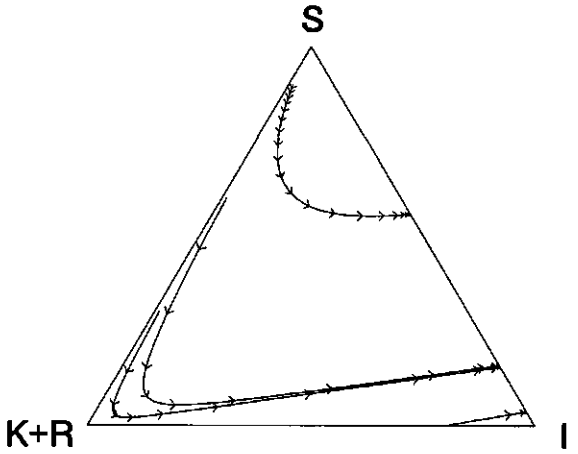

Figure 3. Dynamics of a population where S.I. invades in a population polymorphic for spore killing. The frequencies of Killers + Resistants (*cE*), Sensitives (*ce)* and Incompatibles (*Ce*) are given in a de Finetti diagram. The ratio Killer : Resistant is constant and 1:1. Parameter values equal those in figure 2.

(chapter 6), in the other the Incompatible will have invaded as given above.

Discussion

In a population genetic analysis we showed that the evolution of sexual incompatibility in *P. anserina* is difficult to explain, because selection will always act against new mutant S.I. genes. A possible explanation for the existence of S.I. might however be the suppression of spore killing.

The model presented in this chapter is adapted to the natural life cycle of *P. anserina*. Crosses only occur between strains in direct contact, because a random dispersal of male gametes (as assumed in other models for the spread of pollen (Charlesworth and Charlesworth, 1978b) and conidia (this thesis, chapter 2)) is not realistic (Bernet, 1992). Because there is no other male partner around, the ascogonia that were not fertilized after a S.I. reaction will get selfed instead.

An essential assumption in the model explaining S.I. as a suppressor of spore killing, is that S.I. seizes upon the resistance locus of the spore killing system. This assumption is purely hypothetical but necessary, as without it the suppression would not be effective. If seizing upon the killer locus would be assumed, a system with S.I. as found by Esser (1959) and Bernet (1965) would not be possible without the presence of a spore killer.

Another assumption is the equivalence of gene *r* to *e*. At first sight equivalence of *r* to *c* might be assumed instead. However, slightly modifying the system given in Appendix II in this way, showed that then the Incompatible type could never compete with the Killer. This result can be understood by noting that if *i* were equivalent to *e*, the Killer would function as *cE* in the S.I. system. Its ascogonia would be selfed after crossing, giving it an advantage over the Incompatible type.

In the model 50% recombination is assumed between the two S.I. loci, and no recombination between *K* and *R*. This agrees with the data of S.I. loci in *P. anserina* (Marcou et al., 1990) and the Killer complex in *Neurospora* (Campbell and Turner, 1987). (In *P. anserina* no Resistant strains have been found, but this is probably merely the result of a lack of screening of natural isolates.) The appearance of some Resistants in the model is therefore explained by mutation or a rare recombination between *K* and *R*. A tight linkage of *c* and *e* would favour the maintenance of the S.I. system, because this would result in a lower increase in frequency of recombinant *ce* types. Therefore the unlinked condition of *c* and *e* cannot be explained if S.I. would have had a selective advantage on its own, e.g. to promote isolation (Esser, 1971) .

In a model on the evolution of Spore killing (chapter 6) we considered several lower fitness values of Killers and Resistants during different stages of the life cycle and an additional advantage of Killers after spore killing, for example due to some effects of local sib competition. This was necessary to explain a polymorphism of Killers, Resistants and Sensitives. These assumptions have not been incorporated in the present model, as given in the main text. We found that introducing these assumptions does not change the qualitative results significantly, so a relatively simple model seems sufficient. A description of the more complex model, however, is given in Appendix II.

The genes of the non-allelic incompatibility system have been studied extensively. In all interacting gene couples two different genes can be found. The product of the first (*c*) is diffusable in the hyphae and the product of the other (*e*) is non diffusable and probably associated with the plasma membrane (Bernet et al., 1973; Assilineau et al., 1981; Bernet 1992). Esser (1959, 1965) found that the nuclei in the trichogyne are degenerating after fertilization. He explaines the semi-incompatibility by the activity of the *E* gene in the spermatium, which stops plasmogamy and thereby establishes incompatibility with a *C* trichogyne. In the reciprocal cross the *E* allele is not activated, due to the degeneration of the maternal nucleus.

In the model presented here this means that the *i*-gene product is a diffusable m

molecule and that the *r*-gene product is associated with the plasma membrane. The latter seems a proper place for the product of a gene conferring resistance to killing, as in *Neurospora* the degeneration of the sensitive ascospores begins after the second postmeiotic mitosis, when the ascospore walls are formed (Raju, 1979).

Next to the *e*-gene another gene (*d*) is known, which also gives a S.I. reaction with *c* (Bernet, 1965). Of all these three genes several alleles have been found (Esser, 1969). Translating this to the hypothesis of our model, this means that the *r* and the *i* locus must be multiple allelic. The fact that the *i*-gene product seizes upon two different resistant (*r*) genes (*d* and *e*) seems very well possible.

From the results of Bernet (1967) it can be deduced that there is no *e* or *d*-gene incompatible to the *c*-allele, the normally compatible *i*-allele in our model. In connection with this it is remarkable that Esser (1959) found that ascospores with only *CE* nuclei, formed after a cross of *cE* and *Ce*, germinated badly, but usually mutated to *cE* after 3-4 days of growth and became normally viable. A mutation from *CE* to *Ce* never occurred. A specially regulated mutability can be more easily expected in the gene regulating incompatibility (*I*), than in the target gene (*R*).

Until now nine genes (five gene couples) have been found that show the typical S.I. reaction (Delettre and Bernet, 1976). If our hypothesis holds, this high number is an indication that S.I. has repeatedly evolved, acting against different Spore killers. So also different Spore killers will occur in the species, which is somehow confirmed by the fact that Padieu and Bernet (1967) found two Spore Killers in one cross, and we (Nauta et al., 1993) found a different one in our first sample of five isolates. Unfortunately, it also means that it is probable that a newly discovered Spore killer complex interacts with a specific S.I. gene couple, that has not been found yet.

In his recent studies Bernet (1988, 1992) found that the genes of the non-allelic S.I. system play an important role in the female developmental cycle. These findings are in no contrast with the hypothesis presented in this chapter, because the incompatibility reaction may very well be a pleiotropic effect of genes already functioning in regulating cell death preceding (proto-) perithecium formation. However, Bernet's (1992) explanation for the polymorphic state of the loci involved cannot account for the occurrence and the specificity of the S.I. reaction. If the polymorphism of the S.I. loci is only a consequence of independent, neutral mutations, the specific non-allelic S.I. reactions and there wide occurrence are still not convincingly explained. Moreover, as shown by our first model, S.I. is not neutral, but selection acts against it.

A very important assumption of Bernet (1992) is the absence of outcrossing in *P. anserina*, which, if complete, would make any selective explanation for S.I. (or any other heterogenic incompatibility system) pointless. Without outcrossing however the presence of spore killing genes is inexplicable as well, unless a pleiotropic effect is found for these genes too. The assumption of pure inbreeding is probably mainly based on the fact that the progeny of more than 20 (genetically different) isolates did not show any incompatibility reaction after self-fertilization (Bernet et al., 1960; Bernet, 1967). It is however in contrast with the finding that many of these isolates appear as recombinants of others (Bernet, 1965). Another indication for outbreeding is that the well studied strains *S* and *s* are isolated from a group of fruiting bodies growing side by side in one sample of herbivore dung (Rizet, 1952). They show several genetic differences (Turcq et al., 1991), like for example two different alleles at the incompatibility locus *c* (Bernet, 1965).

New field studies on *P. anserina* are needed to get more data on this subject and are in fact presently carried out. Our first results show that incompatibility reactions and spore killing occur between isolates from rabbit dung pellets collected in each others proximity (M.J. Nauta, M. van der Gaag, A.J.M. Debets and R.F. Hoekstra, unpublished results). The model presented is this chapter shows that rare outcrossing may be sufficient for selection to operate.

Until now *P. anserina* is the only species where heterogenic sexual incompatibility has been described (Glass and Kuldau, 1992). It is uncertain however how common this kind of incompatibility is for ascomycetes. In *Cryphonectria parasitica* the existence of non-allelic incompatibility is suggested, but apparently it is not effective in the sexual stage (Anagnostakis, 1984, 1988). Incompatible (or nearly incompatible) crosses between conspecifics are found in several ascomycetes (Olive, 1956; Brasier, 1984; Puhalla and Spieth, 1985), but here the genetics are not known. An obvious reason for this fact is that crossings between incompatible strains are impossible, so no progeny can be studied. Also the term 'conspecifics' for individuals that cannot interbreed is rather confusing. The fact that two individuals from different species cannot be crossed is normally not regarded as 'incompatibility'.

Because both spore killing and S.I. occur frequently in *P. anserina*, the question may arise whether this species is specially prone to spore killing and S.I.. Possibly pseudohomothallism, the dikaryotic vegetative phase and the non-random occurrence of crossing over and recombination (Marcou et al., 1979; Marcou et al., 1990) can give a clue. In a mycelium with two different nuclei, competition

between these two is possible, competitive elements will be favored and an arms race can be expected. With intratetrad selfing (Zakharov, 1968; Kirby, 1984) a preferential homo- or heterokaryotic state of a gene can be regulated. Recombination blocks, probably necessary for the killer system to operate (Campbell and Turner, 1987; chapter 6, this thesis), will occur frequently (Marcou et al., 1990).

The problem of explaining the evolution of S.I. in *P. anserina* remains unsolved. The hypothesis proposed in this chapter needs experimental support before it can be accepted. New field isolates must be sampled to make estimates of variation within and between strains and to assess the amount of outcrossing, by using suitable molecular techniques. If the model is valid it must be possible to find a population with an interacting system of spore killing and S.I.. Also, cloning of essential genes of *P. anserina*, which has already been achieved for some incompatibility genes (Turcq et al., 1991; Bernet, 1992) and the mating types (Picard et al., 1991), might allow a comparison of the killer complex and the genes involved in S.I..

Appendix

I. The evolutionary dynamics of sexual incompatibility

During ascospore formation normally two diffent nuclei are enclosed in one ascospore in *P. anserina* (Franke, 1957). Because these two nuclei usually have different mating types, self-fertilization is possible: The species is pseudo-homothallic, like e.g. *Neurospora tetrasperma.*

When crossing the strains *Ce* and *cE*, only the cross ♂ *Ce* x ♀ *cE* appears to be compatible. Esser (1959) found that in the ascospores produced in the F1 of this cross, the four types of nuclei produced (*Ce*, *cE*, *ce* and *CE*) can end up in pairs of all possible combinations. The frequency of each type of ascospore depends on the percentages of second division segregation (SDS) of the two loci involved, as the percentage of SDS is the percentage in which a gene ends up heterokaryotic in the ascospore. (The mating type for example has nearly 100% SDS, leading to nearly 100% ascospores heterokaryotic for mating type).

As the alleles *C* and *E* are incompatible, all *CE* nuclei are inviable. So dikaryotic ascospores with one such nucleus become monokaryotic for the other nucleus and ascospores with two *CE* nuclei are assumed to die. (The fact that the *CE* nuclei in such a *CE* + *CE* ascospore mutate to *cE* after a few days (Esser, 1959) is not incorporated into the model, because these spores germinate badly and will probably not survive in nature.) If the parental types *Ce* and *cE* end up in the same ascospore, the *Ce* nucleus is eliminated, due to the *C-E* incompatibility reaction (Esser, 1959). In all other combinations both nuclei are normally viable.

Knowing this, with x_1, x_2 and x_3 denoting the frequencies of *cE*, *ce* and *Ce* ($x_1 + x_2 + x_3 = 1$), the following system of recurrence relations can be derived for a population with these genotypes:

$$
\begin{aligned}
Wx_1' &= x_1(1-(1-\alpha)x_3) + (1-\alpha)x_1x_3\epsilon_1 \\
Wx_2' &= x_2 + (1-\alpha)x_1x_3\epsilon_2 \\
Wx_3' &= x_3(1-(1-\alpha)x_1) + (1-\alpha)x_1x_3(\epsilon_3 + w)
\end{aligned}
\qquad (3)
$$

with

$$W = 1 - x_1 x_3 (1-\alpha)(2-\epsilon_1-\epsilon_2-\epsilon_3-w) \quad (4)$$

and α is the frequency of self fertilization, w is the fitness of selfing after an incompatible attempt to fertilize and ϵ_i are the frequencies of the three types i as they are produced after the cross ♂ *Ce* x ♀ *cE*:

$$\epsilon_1 = 0.5pv + 0.25(1-p)$$

$$\epsilon_2 = 0.5pqv + 0.25(1-pq) \quad (5)$$

$$\epsilon_3 = 0.5(1-p)qv + 0.25(1-q)$$

where v is the fitness of mononucleate, self-sterile spores ($0 \leq v \leq 1$), and p and q are the fractions SDS of the genes *c* and *e*. (For the 'real' genes *c* and *e* these values are $p = 0.18$ and $q = 0.04$ (Marcou et al., 1990)). Because these values are different for different couples of non-allelic S.I. genes, p and q are introduced as parameters.)

Because *CE* + *CE* spores are considered inviable, $\epsilon_1 + \epsilon_2 + \epsilon_3 < 1$. Heterokaryotic non-incompatible ascospores (like *Ce* + *ce*) are supposed to split up in two homokaryotic types after several generations of selfing.

From system (3) it can easily be seen that the recombinant *ce* will always increase in frequency as long as *Ce* and *cE* are present. *Ce* will have an advantage compared to *cE* because usually $\epsilon_3 + w > \epsilon_1$. An example of the dynamics is given in figure 2.

II. The evolutionary dynamics of S.I. and spore killing

With spore killing in *P. anserina* the fate of Sensitive nuclei in dikaryotic spores of this pseudohomothallic species has to be taken into account. It has been shown that Sensitive nuclei are not killed if they are enclosed in one ascospore together with a Killer nucleus (Padieu and Bernet, 1967; Turner and Perkins, 1991). So only homokaryotic spores, that contain two Sensitive nuclei, will get killed, when a Killer is present in some of the other spores. (It is remarkable that this is exactly the opposite of the action of S.I.. In a heterokaryotic ascospore with a *Ce* and a *cE* nucleus, *Ce* is eliminated. However, all ascospores survive if they are homokaryotic for these different nuclei.)

However, in our model the exact events in dikaryotic spores is assumed to be

irrelevant in a cross of Killer x Sensitive, as after several generations of self-fertilization the heterokaryotic types will strongly decrease in frequency and all Sensitives will get killed. It is therefore assumed that the Sensitives in a Killer + Sensitive ascospore are inviable. In a cross of Killer x Incompatible however, these processes are important in calculating the frequencies of the genotypes that survive this cross (the ϵ_i). It should be noticed here that the S.I. reaction (which occurs after ascospore formation (Esser, 1959)) takes place after the killing reaction (which happens during this proces (Turner and Perkins, 1991)).

So if spore killing is incorporated in the model derived in Appendix I, with the assumptions above and those given in the main text, equations (1) can be derived, with the following values of the ϵ_i:

$$\begin{aligned}
\epsilon_1 &= 0.5pv + 0.25(1-p) \\
\epsilon_{2k} &= 0.5pqv \\
\epsilon_{2r} &= 0.5pqv + 0.25(1-pq) \\
\epsilon_{3k} &= 0.5(1-p)qv \\
\epsilon_{3r} &= 0.5(1-p)qv + 0.25(1-q)
\end{aligned} \tag{6}$$

The dynamics of system (1) is first examined for a population without the Resistant type. An unstable equilibrium point can then be found for

$$\begin{aligned}
\hat{x}_i &= \frac{(\epsilon_3+w)(1-\epsilon_3-w)}{(\epsilon_3+w)(2-\epsilon_1-\epsilon_2-\epsilon_3-w)+\epsilon_2} \\
\hat{x}_s &= \frac{\epsilon_2}{\epsilon_3+w}\hat{x}_i \\
\hat{x}_k &= \frac{1-\epsilon_1}{1-\epsilon_3-w}\hat{x}_i
\end{aligned} \tag{7}$$

The Killer type will be driven out of the population if

$$x_i > \frac{1-\epsilon_3-w}{1-\epsilon_1} x_k \tag{8}$$

so it will never be stable if

$$w + \epsilon_{3k} > 1 \tag{9}$$

which is always true if $w = 1$.

An example of these dynamics is given in figure 4.

The system of equations (1) can be regarded as a special case of a more general model, that incorporates fitness differences in the haploid, vegetative stage of the life cycle, like in our model on the dynamics of Spore killing (chapter 6). Here it is assumed that each type t, has a fitness f_t, relative to the fitness $f_s = 1$ of the Sensitive type. Probably these $f_t \leq 1$, as the Sensitive type may be considered as the ancient and fittest type. Also, it is assumed that a cross Killer x Sensitive yields a higher fitness for the surviving ascospores, due to the effects of local sib competition or some sort of heterozygotic advantage. This is expressed in the factor c ($1 \leq c \leq 2$). Recombination between the loci K and R is neglected. Then, more generally, system (1) can be written as:

$$\begin{aligned}
Wx_k' &= f_k x_k (1-(1-\alpha)[x_i(1-\epsilon_1)-(c-1)x_s]) \\
Wx_r' &= f_r x_r (1-(1-\alpha)x_i(1-\epsilon_1)) \\
Wx_s' &= x_s(1-(1-\alpha)x_k) + (1-\alpha)x_i(x_k\epsilon_{2k} + x_r\epsilon_{2r}) \\
Wx_i' &= f_i x_i (1-(1-\alpha)[x_k(1-\epsilon_{3k}-w) + x_r(1-\epsilon_{3r}-w)])
\end{aligned} \tag{10}$$

with

$$\begin{aligned}
W = {} & f_k x_k + f_r x_r + x_s + f_i x_i - \\
& (1-\alpha)[x_s x_k (1-(c-1)f_k) + \\
& \quad x_i x_k (f_k(1-\epsilon_1) - \epsilon_{2_k} + f_i(1-\epsilon_{3k}-w)) + \\
& \quad x_i x_r (f_r(1-\epsilon_1) - \epsilon_{2r} + f_i(1-\epsilon_{3r}-w))]
\end{aligned} \tag{11}$$

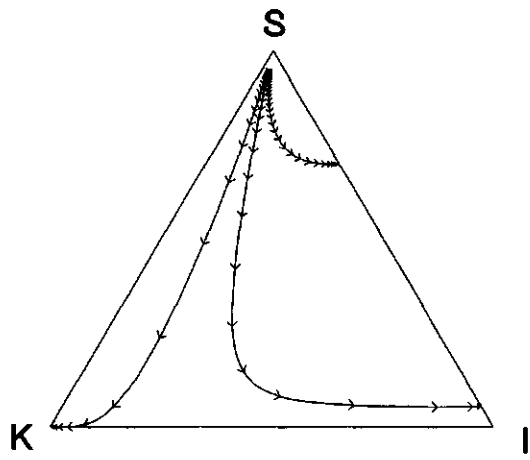

Figure 4. Dynamics of a population with an unstable equilibrium. The final state (depending on initial frequencies) is only Killers or no Killers at all. Parameter values equal those in figure 2.

which is identical to (1) and (2) if all $f_t = 1$ and $c = 1$.

Adding the parameters f_t and c raises the complexity of the system too much for algebraic analysis. Numerical analysis showed that, as was to be expected, $c > 1$ is of benefit to the Killer. However, introducing these parameters did not change the qualitative results from the more simple system (1).

The most important issue of this study is to find conditions that allow an invasion of the Incompatible type, and thus the evolution of S.I. From the complete system (10) it can be derived that the Incompatible type will invade if $x_i' > x_i$, so if (with $x_i \approx 0$)

$$f_i - [f_k x_k + f_r x_r + x_s] > (1-\alpha)[x_k(1-e_{3k}-w) + x_r(1-e_{3r}-w) - x_k x_s(1-(c-1)f_k)] \quad \textbf{(12)}$$

This is always (but not only) true if $w = 1$ and $f_k, f_r \leq f_i \leq 1$. So the evolution of S.I. can be explained by this model.

CHAPTER 8

General discussion

In the preceding chapters a number of different aspects of the genetic systems of filamentous ascomycetes have been studied by the use of theoretical population genetic models. As a result, quantitative conditions for the evolution of different systems have been formulated. Random mating, random dispersal and discrete non overlapping generations are assumed and, except for the stochastic models with finite population sizes in chapter 5, all models are deterministic, assuming infinite population sizes. Although these assumptions clearly don't correspond to the real, complex, situation in nature, they are approximations that allow a relatively easy mathematical analysis. The value of such models has been proved in population genetic models on higher organisms (Crow and Kimura, 1970).

Throughout this thesis, a model is regarded as satisfactory, if it can explain an observed phenomenon, and if it can be supported by rational scientific arguments. If it did not succeed in this respect, extensions to a higher degree of complexity were necessary. By this procedure it has become clearer, which aspects of the evolution of genetic systems in filamentous ascomycetes can be understood, and which are as yet unexplained. Some important conclusions could be drawn, and some new research questions are raised.

In this chapter the main results of the research described in the preceding chapters and the questions that arose, will be summarized. After that, three topics that deserve more extensive consideration are discussed: mating types, the selective advantage of incompatibility and homothallic sex. Overall, some suggestions for further research are put forward.

Reproductive systems

In chapters 2 and 3 the evolutionary relationships between several widely occurring reproductive systems have been studied. The three major modes of

reproduction (asexual, homothallic and heterothallic) are compared. It is concluded that heterothallism is probably the original system, and that a stable polymorphism of homo- and heterothallism may be possible. The existence of such a polymorphism has not yet been proved by genetical analysis, but there are indications that it may exist (Milgroom et al., 1993). Hermaphroditism in heterothallic species (like *Neurospora crassa*) and the formation of both sexual and asexual spores in homothallic species (like *Aspergillus nidulans*) are explained by assuming a variable fitness of ascospore production. Evolution of asexual species can easily be explained, if, for some reason, sexual spores do not show a substantially higher fitness than asexual spores.

In principle some of these results can be tested by experiments in the laboratory and in the field. Experiments measuring fitness differences between the progeny of asexual spores and of selfed and outcrossed sexual spores, can be performed in a homothallic species like *Aspergillus nidulans*. Such experiments will have to deal with some problems, like finding a way to properly measure fitness and detecting the right growth conditions. However, comparable experiments on *Aspergillus niger* (V. Perrot, unpublished results) and the green alga *Chlamydomonas* (A. De Visser, unpublished results) are in progress and show that such research is possible. Possibly they can be supplemented by competition experiments between the progeny of the different kinds of spores. Also, data should be collected on the frequencies of asexual reproduction, selfing and outcrossing in nature.

Vegetative incompatibility

In chapters 4 and 5 the evolution of vegetative incompatibility (VI) has been studied, in an attempt to explain the large amounts of Vegetative Compatibility Groups (VCGs) found in nature. A comparison of selective mechanisms shows that harmful cytoplasmic elements offer a better explanation for the evolution of VI than a parasitic nuclear gene. However, to explain large numbers of VCGs, population size and mutation rate appear to be important parameters. It is found that conditions, that are necessary to explain the existence of a high number of VCGs in a population with strong selection for VI, are also sufficient to get nearly as many VCGs in a population without selection.

With these models the occurrence and the frequency of VI in nature are not yet convincingly explained. Probably the assumption of random dispersal of spores is not adequate, because in practice hyphal fusion and interaction will take place on a

local scale (Brasier, 1987). Population structure, in particular the range of local interactions, may therefore be essential and will have to be studied both experimentally and theoretically.

Spore Killing

In chapter 6 the evolutionary dynamics of spore killing is studied, in order to find conditions for a stable polymorphism of Killers and Sensitives, as apparently found in nature. It appears that Killers need an additional advantage during the process of spore killing, to invade a population of Sensitives. This advantage may be the result of local interactions after sporulation, or some heterozygotic advantage. If, next to that, Resistant types have a higher fitness than the Killers in the vegetative, haploid, stage of the life cycle, polymorphism can be explained.

The data on the occurrence of Spore killers in nature, like those collected by Turner (1993) for several species of *Neurospora*, need to be extended on a more local and on a temporal scale. Possibly cycling behaviour of the genotype frequencies, as predicted in some of the models, can then be found. Experiments in the laboratory can be performed to test fitness differences between the different genotypes. As proposed in the paragraph on reproductive systems above, this can possibly be done both by measuring individual fitnesses differences and by competition experiments.

Podospora anserina

Finally, in chapter 7 it is shown that the evolution of the system of non-allelic sexual incompatibility, as found in *Podospora anserina*, is difficult to understand. A hypothesis is proposed that explains sexual incompatibility as an anti meiotic drive device, that is as a weapon against spore killing. A theoretical model shows that this may be possible, although experimental evidence is lacking.

New field studies on *Podospora anserina*, as we have recently started (Nauta et al., 1993), will probably shed some light on the occurrence of spore killing and sexual incompatibility in natural populations. An extensive study over time, on a local scale, will be necessary to understand more of the ecology and the population dynamics of *P. anserina*. Also, the levels of outbreeding in this apparently preferentially inbreeding species, will have to be measured. Possibly this can be done by using molecular markers. The secondarily homothallic reproductive

system, with the dikaryotic ascospores, causes a slow decline of heterozygosity (or actually heterokaryosis) in these spores, over several generations of inbreeding (Zakharov, 1968; Perkins, 1991). This can probably be measured by comparing the variation in molecular markers within and between natural isolates. Unfortunately, some preliminary molecular studies are not particulary promising, as they show amazingly little variation between different strains. (M. Van der Gaag, unpublished results).

Experimental work on *P. anserina* is mainly focused on senescence (Osiewacz and Esser, 1984; Belcour and Vierny, 1986; Griffiths, 1992) and on the analysis of the mitochondrial genome (Cummings et al., 1990), incompatibility genes (Turcq et al., 1990; Bernet, 1992) and the mating type genes (Debuchy and Coppin, 1992). The ecology and evolutionary biology of the species have largely been neglected, although they certainly seem to be promising fields of research too. The aberrant reproductive system of secondary homothallism, the strange interference phenomena on the chromosomes (Marcou et al., 1990), the non-allelic system of sexual incompatibility and the occurrence of spore killing deserve more attention from evolutionary biologists.

Mating types

The genetic system with two mating types, i.e. the one-locus two-allele determination of sexual compatibility, is the characteristic mating system of ascomycetes (Glass and Kuldau, 1992). The mating types *A* and *a* of *Neurospora crassa* have been cloned and characterized (Glass et al., 1988). It has been shown that the two mating types are highly dissimilar, and apparently not related by structure or common descent (Metzenberg and Glass, 1990). The mating types of the related species *Podospora anserina* show much similarities with these two different 'idiomorphs' (Debuchy and Coppin, 1992).

Equivalents of the mating type genes have also been searched for in homothallic species. It has been found that most homothallic *Neurospora* species carry homologous sequences of both mating types (Glass et al., 1990). A thorough research on the mating types in some other species may be recommended. In the heterothallic chestnut blight fungus *Cryphonectria parasitica*, the regulation of self-fertilization, which is possible once outcrossing has occurred, calls for clarification (Milgroom et al., 1993). In species like *Chromocrea spinulosa* and *Glomerella cingulata*, that do not seem to fit into one of the classes 'homothallic' or 'hetero-

thallic' (see chapter 1), the genetics of mating type differentiation deserves further research, which may supply information about the determination of the homo- and/or heterothallic character of the strains. Also, this may shed some light on the hypotheses that explain the genetic systems by high frequencies of mutation (Mathieson, 1952), or by mating type switching, like in yeast (Perkins, 1987; Anderson et al., 1992).

In this context the frequent 'mutation' of the non-allelic sexual incompatibility allele *C* to *c* in *P. anserina* (chapter 7) is very interesting. Esser (1959) found that a badly growing strain, originating from a germinating ascospore with the incompatible genotype *CE*, always changes to the compatible type *cE* after a few days and never to *Ce*. He calls this change a mutation. It is doubtful, however, whether this is the right term, as the change is both predictable and non-random. If switching, or some kind of regulated gene modification, can occur at the mating type locus, it can probably also occur at other loci. The process of 'mutation' of the *C*-allele is a one-way change and therefore it cannot be similar to mating type switching. But nonetheless, this incompatibility locus could be a candidate for the study of regulated gene modifications, or directed mutations, in non-mating type genes.

The selective advantage of incompatibility

In the models on vegetative and sexual incompatibility, potential selective pressures have been explored, that may cause the evolution of incompatibility systems. However, in all cases the hypothesis that incompatibility is selectively neutral, could not be rejected. In chapter 5, it has been shown that selective neutrality of incompatibility could almost equally well explain the existence of high numbers of VCGs as selection. Also, for the sexual incompatibility system in *Podospora anserina*, Bernet (1992) claims that it is a selectively neutral trait, a side effect of genes with a different function.

Therefore the question whether incompatibility is functional or not, has not yet been convincingly answered. However, the arguments that have led to the idea that selection must be important still stand. The wide occurrence, the complexity and the specificity of the incompatibility systems make it hard to believe that they are neutral traits or accidental side effects.

To test for the significance of selection, incompatibility polymorphisms could be compared to other polymorphisms, which are known to be neutral. The model

presented in chapter 5 has shown that information about population sizes and mutation frequencies in natural populations is essential. A theoretical framework will have to be developed, to estimate these parameters in natural, haploid populations. It may be possible to do this by using gene frequency distributions or frequency changes over time.

It may be that some pleiotropic effects of the incompatibility genes are overlooked. The action of the genes has only been studied intraspecifically, whereas interspecific interactions may be equally, or perhaps more important. In nature the fungi will certainly be confronted with many other species, with which they have to compete (Wicklow, 1992; Boddy, 1992). During evolution this must have inevitably led to a large defense system against parasites and competitors, which will be genetically determined. The incompatibility systems may be part of this large defense system. However, this cannot explain the precise intraspecific interactions between VI genes.

Homothallic sex

A topic that is especially interesting in the context of the evolution of sex, is the existence of homothallic (selfing) sex. This type of sexual reproduction does not result in recombination, which is often regarded as the function of sex (Maynard Smith, 1978; Stearns, 1987). The explanation suggested in chapter 3 of this thesis, that a variable fitness of ascospore production can explain the coexistence of (genetically identical) homothallic sexual spores and asexual spores, does not make clear why sex is necessary. Two different types of asexual spores could be equally sufficient in this case.

An explanation for homothallic sex may be found in occasional events of outcrossing, which do result in recombination. Alternatively, it may be possible that sex functions as some sort of purifying device. Different deleterious mutations or DNA damage may occur in different nuclei in the mycelium. Fusion of these nuclei followed by meiosis and recombination may lead to elimination of such mutations or DNA repair, which may offer a selective advantage (Bernstein et al., 1981). This scenario, however, can only operate if the fusing nuclei carry different mutations, so if they originate from different parts of the mycelium. In general, this appears not to be the case (K. Swart, pers comm.).

If *secondary* homothallism is compared to homothallism in this respect, secondary homothallism is remarkably effective. In a secondarily homothallic

species, the two nuclei that fuse to form the diploid nucleus, which will be going through meiosis, are both the product of one meiosis, the generation before. These two fusing nuclei, which have different mating types (chapter 1), will always have had a different history during mycelial growth: they will have suffered non-identical mutations and DNA damage. So recombination of these mutations and DNA repair may operate effectively in selfing secondarily homothallic species. This can only be hampered by parasexual recombination between nuclei with different mating types, which may result in nuclei with non-identical mating types and identical mutations. So, if this purifying effect is selectively significant, it can be predicted that selection will act against the occurrence of parasexual recombination between nuclei with different mating types in a species like *Podospora anserina*.

Homothallic sex is also relevant with respect to theories on the evolution of the number of sexes, as discussed by Hoekstra (1987) and Hurst and Hamilton (1992). Hurst and Hamilton (1992) present an overview, showing that living organisms can be divided in two categories, with only a few exceptions: Organisms with two sexes and no incompatibility types (like most organisms) and organisms with no sexes and many (sexual) incompatibility types (like most ciliates (Grell, 1973) and Basidiomycetes (Raper, 1966a; Burnett, 1976)). They explain these categories as the two basic means that prevent cytoplasmic conflicts by uniparental inheritance of cytoplasm: If cells fuse during mating, there are two sexes, and the cytoplasm of only one of them will be inherited; if cells do not fuse, only nuclei will pass between mating partners, and only the recipients cytoplasm is inherited. Clearly, homothallic ascomycetes do not fit into this scheme, and there are not 'possibly a few' (Hurst and Hamilton, 1992) of them, but certainly many (Raper, 1966b; Perkins, 1991). As Hurst and Hamilton (1992) argue, the homothallic mating system can only fit into their theory if outcrossing is rare (and the probability of conflict is small) or if uniparental inheritance is achieved, despite the absence of mating types regulating it. This issue is presently investigated experimentally in *A. nidulans* (A. Coenen and K. Swart, pers. comm.).

Future research

Research on the evolutionary biology and the population genetics of fungi should be continued in several ways. Theoretical studies, like those described in this thesis, can be performed on the fascinating genetic systems of lower fungi, such as the

true slime moulds, like *Didymium iridis* (Collins, 1987) and the cellular slime moulds, like *Dictyostelium discoideum* (Loomis, 1982). Also the genetic systems of the other subdivision of higher fungi, the Basidiomycetes, where sexual reproduction is preceded by vegetative fusion and a neatly regulated prolonged dikaryotic state (Raper, 1966a; Burnett, 1976), have not yet been thoroughly studied by the use of theoretical population genetic models.

Furthermore, the theoretical studies in this thesis have made clear that more experimental research is required to answer some important questions. Essential information about modes and frequencies of sex and reproduction, estimates of population sizes and their variation in space and time will have to be collected from nature. Also more information on the detailed (molecular) genetics of different fungal genetic systems (like the incompatibility systems and the spore killing system) is badly needed. More fundamental research on the population genetics and evolutionary biology of fungi will enlarge our insights in explanations for the diversity of fungal life styles, and the process of evolution as a whole.

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Samenvatting

Evolutie van genetische systemen in hyphenvormende zakjeszwammen

Bij de meeste (hogere) organismen is de overdracht van erfelijke eigenschappen, de genen, eenvoudig geregeld: In principe wordt tijdens de sexuele voortplanting de helft van de genen van de vader gevoegd bij de helft van de genen van de moeder. De nakomeling heeft dan weer evenveel genen als zijn (of haar) ouders. Bij de mens bijvoorbeeld, is sexuele voortplanting de enige mogelijkheid om genen door te geven.

Er zijn echter ook grote groepen organismen waar de gang van zaken wat ingewikkelder ligt. Dit is bijvoorbeeld het geval bij een grote groep van de schimmels, de hyphenvormende zakjeszwammen. Dit zijn schimmels die uitgroeien in de vorm van draadachtige structuren, de hyphen, die een netwerk vormen dat mycelium wordt genoemd. Ze vormen hun sporen in zakvormige structuren, de asci. Genen kunnen bij deze organismen op vele manieren worden overgedragen. Naast sexuele voortplanting komt ook asexuele voortplanting voor. Dit komt er meestal op neer dat een stukje weefsel wordt afgesnoerd, dat weer zelfstandig kan uitgroeien. Maar het is ook mogelijk dat er genen uitgewisseld worden zonder dat er sprake is van voortplanting. Soms kunnen twee schimmels die met elkaar in aanraking komen namelijk vergroeien tot één geheel. De genen komen dan gemengd voor in één (stukje) mycelium, een zogenaamd heterokaryon.

Het feit dat het doorgeven van erfelijke informatie door veel verschillende mechanismen en processen geregeld kan zijn, betekent dat er een grote variatie aan genetische systemen bestaat. Sommige soorten kunnen zich alleen asexueel voortplanten, anderen alleen sexueel en weer anderen kunnen het allebei. Sommige soorten die zich sexueel voortplanten kunnen zichzelf bevruchten, anderen niet. Het vormen van een heterokaryon wordt vaak voorkomen door de werking van genen die 'vegetatieve incompatibiliteit' veroorzaken.

In dit proefschrift is een poging gedaan iets te begrijpen van het bestaan van al deze verschillende genetische systemen. Een goede manier om dit te doen, is je af te vragen hoe ze geevolueerd kunnen zijn. Het bestaan van levensvormen valt namelijk alleen te begrijpen, als je kunt begrijpen hoe ze ontstaan kunnen zijn en vervolgens het proces van natuurlijke selectie hebben kunnen overleven. En omdat

het niet goed mogelijk is dit proces met experimenten na te bootsen, zijn er theoretische, wiskundige modellen opgesteld, die het evolutieproces beschrijven. Daarbij wordt gebruik gemaakt van de regels die gelden bij de overdracht van genen.

In het eerste hoofdstuk wordt een inleiding gegeven met een overzicht van de genetische systemen, zoals die voorkomen by de hyphenvormende zakjeszwammen.

Hoofdstuk 2 behandelt een model, waarin de evolutie van zelfbevruchtende (homothallische) naar niet-zelfbevruchtende (heterothallische) schimmels, en vice versa, wordt beschreven. Hieruit blijkt dat homothallische soorten waarschijnlijk ontstaan zijn uit de heterothallische. Tevens blijkt dat het heel goed mogelijk is dat er soorten bestaan, waarin zowel heterothallische als homothallische individuen voorkomen. Er zijn aanwijzingen dat dit laatste ook in de natuur het geval is, maar zeker is dat niet.

Hoofdstuk 3 gaat dieper in op de voortplantingssystemen. Over het algemeen zijn de onderzochte schimmels hermafrodiet: zowel mannelijk als vrouwelijk. Het is de vraag, onder welke omstandigheden dit te verwachten is, en wanneer ze beter 'tweehuizig', dus afzonderlijk mannelijk en vrouwelijk zouden kunnen zijn. Ook wordt er gekeken wanneer het te verwachten is, dat ze zich zowel sexueel als asexueel kunnen voortplanten. Hier blijkt dat de in de natuur gevonden verschijnselen alleen te verklaren zijn, als de overlevingskracht (de fitness) van de sexueel gevormde sporen variabel is. Dit betekent bijvoorbeeld, dat de sexueel gevormde sporen op het ene moment veel beter kiemen dan de asexueel gevormde sporen, terwijl het een andere keer niet uitmaakt. Alleen als dat het geval is kunnen de beide typen sporen naast elkaar blijven bestaan.

Vervolgens wordt in de hoofdstukken 4 en 5 het verschijnsel vegetatieve incompatibiliteit (VI) nader bekeken. Uit de natuur geisoleerde schimmels van één soort blijken namelijk meestal niet in staat te zijn met elkaar een heterokaryon te vormen. Ze zijn te verdelen in een groot aantal groepen, de Vegetative Compatibiliteits Groepen (VCGs). Binnen een VCG is heterokaryonvorming mogelijk, maar tussen VCGs niet. Alle leden van een VCG zijn, voor wat betreft de VI-genen, genetisch gelijk.

Het bestaan van zoveel VCGs wijst erop dat er waarschijnlijk selectiedruk bestaat tegen heterokaryonvorming. In modellen wordt daarom onderzocht welke selectieve krachten hiervoor verantwoordelijk zouden kunnen zijn. Een vergelijking van deze krachten, in hoofdstuk 4, heeft als resultaat dat een kwaadaardig cytoplasmatisch element, of een virus, beter in staat is deze selectiedruk te leveren, dan een

parasitair gen dat 'gewoon' in de kern zit.

In hoofdstuk 5 wordt in de modellen rekening gehouden met populatiegrootte (N) en mutatiefrequentie (u), de frequentie waarin spontaan nieuwe VCGs ontstaan. Dan blijkt dat het product van N en u een bepaalde grootte moet hebben, om het grote aantal VCGs in stand te houden. Maar als dat zo is, is er eigenlijk ook geen selectiedruk nodig: VI kan ook een neutraal kenmerk zijn. De rol van selectie is daarmee nog niet opgehelderd.

Hoofdstuk 6 beschrijft de evolutionaire dynamica van het verschijnsel 'spore killing'. Als een schimmel met een killergen gekruist wordt met een sensitieve schimmel, worden alle nakomelingen gedood, die het killergen niet hebben. Dit lijkt erg nadelig, want het betekent een halvering van het aantal nakomelingen. Toch is het herhaaldelijk en bij verschillende soorten gevonden. In modellen wordt nagegaan hoe een 'Spore killer' in een populatie kan ontstaan, en hoe hij kan blijven bestaan. Dan blijkt dat de 'Spore killer' een voordeel moet hebben bij het doden van sensitieven, bijvoorbeeld omdat hij minder concurrenten heeft bij het opgroeien. Daarnaast moet zo'n 'Spore killer' een fitnessnadeel hebben tijdens het vegetatieve stadium, bijvoorbeeld doordat hij slechter groeit. Dan is het bestaan van 'spore killing' te begrijpen.

In hoofdstuk 7 wordt het optreden van sexuele incompatibiliteit (SI) bij de schimmel *Podospora anserina* bestudeerd. SI is een vorm van incompatibiliteit die bepaalde kruisingen verhindert. Uit modellen blijkt dat de evolutie ervan eigenlijk niet goed te verklaren is. Maar onder de hypothese dat het een middel is dat 'spore killing' tegengaat, lijkt de evolutie ervan wel mogelijk te zijn. Er moeten echter eerst experimentele bewijzen gevonden worden, dat dit ook werkelijk zo is. Eén van de genen die betrokken is bij sexuele incompatibiliteit, moet dan namelijk ook betrokken zijn bij 'spore killing'.

Met de in dit proefschrift beschreven modellen zijn een aantal onderzoeksvragen beantwoord. Er zijn, zoals gebruikelijk, echter ook een groot aantal vragen bijgekomen. Om de evolutie van genetische systemen te begrijpen, is uitgebreider experimenteel en theoretisch onderzoek aan natuurlijke schimmelpopulaties een vereiste. Dit is niet alleen erg boeiend, het is ook nodig om de vaak moeilijk te begrijpen eigenschappen van schimmels te kunnen doorzien.

Abstract

A great variety of genetic systems exist in filamentous ascomycetes. The transmission of genetic material does not only occur by (sexual or asexual) reproduction, but it can also follow vegetative fusion of different strains. In this thesis the evolution of this variability is studied, using theoretical population genetic models.

First the evolution of different reproductive systems is studied. It is found that homothallism (allowing selfing) most probably evolved from heterothallism (with two mating types), and that a polymorphism of homo- and heterothallism can be evolutionary stable. A variable fitness of ascospore production is predicted as an explanation for hermaphroditism in heterothallic species and the formation of both asexual and sexual spores by homothallic species.

Secondly the evolution of vegetative incompatibility (VI) is studied. VI prevents vegetative fusion of different strains, and is very common between different natural isolates. In many species a large number of Vegetative Compatibility Groups (VCGs) is found, that only show vegetative fusion within and not between groups. After a comparison of different selective regimes, it is concluded that a harmful cytoplasmic element offers the most plausible selective explanation for the evolution of VI. However, the effects of genetic drift appear to be important in generating large numbers of VCGs and can override the effects of selection.

Next, attention is focused on spore killing. This is a form of segregation distortion (or meiotic drive), causing the death of half the number of spores in an ascus. In a model it is found that the evolution of spore killing can only be explained if 'Spore killers' have some additional advantage during the process of killing.

Finally, a model is presented for the evolution of sexual incompatibility (SI) in *Podospora anserina*. As the existence of SI cannot be explained on its own, a hypothesis is studied, that explains SI as an anti meiotic drive device. Although the model shows that this hypothesis could be true, experimental evidence is needed to confirm this.

Curriculum Vitae

Maarten Nauta is geboren op 19 september 1962 in Middelburg. Met zijn ouders en broers verhuisde hij eerst naar Vlaardingen en vervolgens naar Meppel, waar hij in 1980 zijn VWO diploma behaalde aan de Rijksscholengemeenschap. Hij ging biologie studeren aan de Rijksuniversiteit Groningen, waar hij in 1983 zijn kandidaats behaalde en in januari 1987 cum laude afstudeerde. Zijn hoofdvak was theoretische biologie, met als bijvakken populatiegenetica en antropogenetica.

Hierop werd zijn carriere hinderlijk onderbroken door de militaire dienstplicht, die hij in 1987 en 1988 vervulde. Een lichtpunt in deze periode was dat hem de Unilever Researchprijs 1987 werd toegekend. Onmiddelijk na zijn diensttijd was hij gedurende een jaar universitair docent aan de R.U. Groningen, waar hij deel had aan het geven van wiskunde-onderwijs aan biologiestudenten.

Vervolgens werd hij in september 1989 door de Nederlandse organisatie voor Wetenschappelijk Onderzoek (NWO) aangesteld als onderzoeker in opleiding aan de vakgroep Erfelijkheidsleer van de Landbouwuniversiteit te Wageningen, om te werken aan het project 'Evolutie van sexuele incompatibiliteit' van de BION werkgemeenschap Theoretische Biologie. Dit heeft uiteindelijk geresulteerd in dit proefschrift.

STELLINGEN

1. Spore killing is niet de drijvende kracht achter de evolutie van secundaire homothallie.
   Turner, B.C. and Perkins, D.D., American Naturalist 137 (1991): 416-429.
   Lyttle, T.W., Annual Review of Genetics 25 (1991): 511-557.

2. Om de essentie van hun bipolaire en antagonistische karakter te benadrukken, zouden de mating type allelen van ascomyceten algemeen de benaming '+' en '-' moeten hebben. Dit verdient de voorkeur boven een weergave met letters of constructies als 'MAT1-1' en 'MAT1-2'.
   Yoder O.C. et al., Phytopathology 76 (1986): 383-385.

3. *Podospora anserina* dankt zijn naam niet aan de ganzehalsachtige vorm van de top van het perithecium, maar aan hij feit dat de soort het eerst gevonden is op een ganzekeutel.
   Moreau, C., Encyclopédie Mycologique 25 (1953): 239.
   Esser, K., *Kryptogamen,* Berlin (1986): 354.

4. Zonder sex valt goed te leven.
   dit proefschrift

5. In heterokaryotische schimmels ligt een kernwapenwedloop voor de hand.

6. Zoeken naar buitenaards leven is geldverspilling.

7. Conclusies uit wetenschappelijk onderzoek die de voorpagina van de krant halen, zijn met grote kans onjuist.

8. Vanaf het derde kind dient de kinderbijslag te worden vervangen door milieubelasting.

9. Alleen met een beperkte blik kan men alles overzien.

10. De computer voert ons terug naar het tijdperk der hiërogliefen.

Stellingen behorend bij het proefschrift 'Evolution of genetic systems in filamentous ascomycetes' van Maarten Nauta, Wageningen, 12 januari 1994.