PERSOONIA
Published by the Rijksherbarium, Leiden
Volume 7, Part 2, pp. 249–260 (1973)

ISOLATING MECHANISMS IN FUNGI—PREZYGOTIC, POSTZYGOTIC, AND AZYGOTIC[1]

PAUL A. LEMKE

Department of Biological Sciences
Carnegie-Mellon University
Pittsburgh, Pennsylvania 15213, *U.S.A.*

(With two Text-figures)

What then are the biological processes that influence fungal speciation? Three aspects of fungal biology discussed herein deserved to be reemphasized. First, the great majority of fungi are haploid organisms. Secondly, fungi have expended a considerable amount of genetic energy to control the formation and maintenance of specific heterokaryons. Thirdly, the fungi taken as a group are paradoxical; against a background of extravagant and varied sexual cycles, fungi have a tendency to restrict gene flow and recombination.

The prevalence of haploidy, the strict control of heterokaryosis, and an irregular but definite trend toward apomixis are attributes of fungal biology. These phenomena undoubtedly influence the operational details of fungal evolution and must be accommodated by any model which intends to explain the origin of fungal species.

Virtually nothing is known about rates of evolution in fungal populations. However, at least three patterns are now evident among fungi with regard to their potential for change in gene frequency through time. The first pattern is that exhibited by imperfect fungi. Evolution in these fungi would be dependent largely upon mutation and selection with, at best, some parasexual activity but generally impaired gene flow. The second pattern is typified by *Schizophyllum commune*. This pattern includes sexuality; gene flow is continuous and controlled through homogenic incompatibility. The third pattern is recognized in *Podospora anserina* as well as in several basidiomycetes, *Sistotrema brinkmannii*, *Mycocalia denudata*, and *Fomes pinicola*. These species combine opposite effects for recombination, and gene flow within the species is discontinuous. Evolution in species of the third pattern should occur in quantum jumps rather than as a continuum. Sewall Wright (1931, 1932) in considering rates of evolution among populations has suggested that most rapid evolution might progress through subpopulations partially isolated which only occasionally or indirectly exchange genetic material. We simply do not now have sufficient data from fungal populations to integrate the fungi into such evolutionary theory.

[1] Commentary and summary presented at the symposium on "Speciation Phenomena in Fungi" during the First International Mycological Congress, September 8, 1971 in Exeter, England.

Nature abhors categories. Of all categories, the one most difficult to reconcile with nature is the biological species. The fungal species is no exception, and in certain respects the fungi pose special problems for species delimitation. These problems are the subject of this symposium.

The most objective and widely accepted definition of the species is based on genetic homology. Simply defined, the species is an integral system for genetic recombination, and members of a given species are expected to share in a common gene pool.

It is apparent from what has been said here today that not all species of fungi conform with this definition. Gene flow within a fungal species may be discontinuous or even negligible. Moreover, related species of fungi may hybridize and thereby lose their specific identity. Sterility barriers *within* species and hybridization *between* species undermine the concept of the species as a well-integrated, reproductively isolated system for gene flow.

Nonconformity with the common-gene-pool concept of the species has long been recognized in nonfungal life-forms, principally in the higher plants (Stebbins, 1950), and is considered to be indicative evidence that speciation is the result of divergent and convergent evolution of existing populations through change in gene frequency. Clearly in fungi, as in other organisms, the population, not the species, is the unit of evolution. Populations that constitute a species embody change, and that change is brought about principally through three phenomena: mutation, selection, and recombination.

Mutation induction and selective pressures are largely extrinsic phenomena—influences of the environment. The characteristic intrinsic feature of a species is its potential for recombination of genetic material. In this regard, fungi display considerable diversity ranging from sexual dimorphism to apomixis. Recombination in most fungi is under strict genetic control through systems of incompatibility. Systems of incompatibility determine the breeding potential of a species in the absence of sexual dimorphism. Two functional types of incompatibility are now recognized in fungi, and these should not be confused, since one promotes outbreeding and the other promotes inbreeding. The first type is common-factor or homogenic incompatibility, commonly referred to as heterothallism (Blakeslee, 1904a, 1904b; Whitehouse, 1949a, 1949b). Homogenic incompatibility favors recombination through association of dissimilar alleles. The mating-type factors of bipolar and tetrapolar fungi are the elements of homogenic incompatibility. Homogenic incompatibility is analogous to sexuality only insofar as it confers self-sterility, cross-fertility upon individuals in a population. *Schizophyllum commune* demonstrates homogenic incompatibility at its best (Raper, 1966). This fungus with a two-factor, multiple-allelic system of incompatibility enjoys considerable potential for outbreeding. *Schizophyllum* fits well the concept of the species as an integral system for gene flow, since allopatric isolates of *S. commune* are panmictic, and incompatibility in that fungus is predictable on the basis of common mating-type factors. *Schizophyllum commune*, the most extensively studied of fungi, may not be typical of fungi with regard to their potential for recombination.

Some fungi are inbreeders and are not preoccupied with recombination. Ecological barriers and/or genetic factors operate in these fungi as isolating mechanisms to delimit gene flow. Although fungal populations have not been studied extensively in this regard, there is already sufficient evidence to indicate that isolating mechanisms act not only between species of fungi but *within* species of fungi as well.

This brings us to a second functional type of incompatibility now evident in fungi (Biggs, 1937; Burnett & Boulter, 1963; Esser, 1965, 1971; Grindle, 1963; Lemke, 1969; Mounce & Macrae, 1938). This type of incompatibility is heterogenic (Esser, 1965). Heterogenic incompatibility, in contrast with homogenic incompatibility restricts recombination when alleles differ. It therefore promotes inbreeding and homozygosity. Many of the sterility barriers recognized in species of fungi apparently have a genetic basis in heterogenic incompatibility. Heterogenic incompatibility may be superimposed on homogenic incompatibility as it is in *Podospora anserina* (Bernet, 1963; Bernet & *al.*, 1960; Rizet & Esser, 1953). As Bernet has pointed out, allelic differences at several loci are involved in producing partial or complete intersterility among isolates of *P. anserina*. Heterogenic incompatibility operates at different stages of the life cycle. It may influence heterokaryon formation or any of several sexual events leading to the formation of a zygote. Heterogenic incompatibility may function in the absence of homogenic incompatibility, as it does in certain homothallic and imperfect species of *Aspergillus* (Grindle, 1963; Jinks & *al.*, 1963; Caten, 1971).

Reduced potential for gene flow in fungi can be influenced by genetic factors other than those underlying heterogenic incompatibility. Homothallism in its various forms restricts recombination. Secondary or heterokaryotic homothallism promotes inbreeding in heterothallic fungi. Primary or homokaryotic homothallism is *de facto* loss of sexual competence, since no recombination is effected through meiosis from a homozygote. The formal absence of sexuality in the imperfect fungi represents considerable, if not complete loss of potential for recombination. Parasexuality in natural populations of these fungi doubtfully compensates for this loss (Caten, 1971).

Cytogenetic mechanisms, principally inversions and translocations, are known to control genetic recombination in higher plants and animals (for review see Stebbins, 1950; Ehrlich & Holm, 1965). Fungal populations are poorly understood in this regard. Since chromosomal abberations operate as underlying mechanisms for postzygotic isolation in other organisms, such mechanisms conceivably could also fractionate natural populations of fungal species.[2]

Prezygotic isolation, however, appears to be more characteristic of fungi and is

[1] Recently, Perkins (1972) has shown that a translocation involving the mating-type locus of *Neurospora crassa* can lead to inviable progeny or to progeny inhibited for growth. The latter progeny are heterozygous for mating-type and contain duplications of the translocated region. The former progeny represent corresponding lethal deficiencies of the translocated region.

directed frequently toward the formation and maintenance of heterokaryons. Regulation of heterokaryosis provides fungi with a unique opportunity to experiment with genetic isolation, much as behavioral patterns in animals enforce species recognition and prevent gene wastage (Mayr, 1970). Heterogenic controls of heterokaryosis and of sexual events subsequent to heterokaryosis clearly operate not only *between* species but also *within* certain species of fungi. In *Podospora anserina* such controls are polygenic. It is reasonable to assume that incompatibility *between* species of fungi is also heterogenic and determined through numerous genetic loci. Fungi, relative to higher plants, have a limited potential for interspecific hybridization. As a rule, restrictions to heterokaryon formation between fungal species are stringent. Confirmed examples of interspecific hybridization in the fungi, although indeed rare, are available for all major taxonomic groups of the true fungi. Undoubted instances of such interactions have been reported in phycomycetes, principally *Allomyces* (Emerson & Wilson, 1954); in ascomycetes, both in yeasts (Winge & Roberts, 1949) and filamentous genera, *Neurospora* (Dodge, 1927; Howe & Haysman, 1966) and *Cochliobolus* (Nelson, 1963); and finally in basidiomycetes, notably in smuts (Holton, 1931; Holton & Fischer, 1941; Holton & Kendric, 1956) and recently in *Sistotrema* of the homobasidiomycetes (Lemke, 1966, 1969).

Interspecific hybridization, when it does occur in fungi, does not occur with impunity. Hybrid progeny show reduced viability, an indication that postzygotic isolation may operate as well in fungi to maintain separate species.

The resupinate basidiomycete *Sistotrema brinkmannii* embodies several of the speciation phenomena discussed in this symposium. Biggs (1937) recognized six intersterile groups among fourteen isolates of this fungus. Two groups were bipolar, three groups were tetrapolar, and the remaining group was homothallic. All six groups were sympatric and morphologically similar on the basis of hymenial structures. Biggs in 1937 suggested that *S. brinkmannii* represented a minimum of three cryptic species, a homothallic species, a bipolar species, and a tetrapolar species; and that sterility barriers were present in the two heterothallic species. Between 1963–1966 I reinvestigated this system and essentially confirmed Biggs' earlier observations (Lemke, 1966, 1969). Sterility barriers for heterokaryon formation exist in all three component species, the homothallic as well as both heterothallic species. The homothallic species exhibits primary or homokaryotic homothallism, and homokaryons are phenotypically dikaryotic with clamp connections. Dikaryosis in heterothallic species is heterokaryotic and controlled through multiple-allelic incompatibility.

Homothallic strains were paired with heterothallic strains, and genetic evidence for heterokaryosis (allothallism) was obtained in one instance (Fig. 1). This nutritionally forced heterokaryon between a homothallic (I) strain and bipolar (II) strain proved to be dikaryotic in phenotype and was brought to sporulation after eleven weeks' incubation. From the specific cross a sample of 109 germinating basidiospores was isolated. From this sample only eight mycelia developed, and an analysis of these sparse progeny revealed a number of interesting points: (a) seven of the eight progeny were recombinant, (b) only one of eight progeny was homo-

CROSS

I *inos* X II A1 *meth*

PROGENY

4 II A1 *inos meth*
II A3 *inos meth*
II A1 *meth* (parental)
II A1 *inos*
I *meth*

[a] I = homothallic homokaryon; II = bipolar homokaryon; *inos* = *inositol*-less; *meth* = methionine-less (for details see Lemke, 1969).

Fig. 1. Hybridization in *Sistotrema brinkmannii*[a]

thallic (phenotypically dikaryotic) and this strain was recombinant for both nutritional markers, it sporulated and appeared normal in all respects, (c) all seven bipolar (nondikaryotic) progeny were compatible with a nonparental bipolar strain of *A*2 mating-type, (d) only six of these bipolar progeny were incompatible with the parental bipolar strain, *A*1, (e) the aberrant strain was compatible with *both the parental, A*1, *and nonparental, A*2, *strains*. This atypical strain, designated *A*3, apparently obtained a new mating-type specificity through recombination with its homothallic parent. This result is subject to further testing but provides evidence for latent genetic structure for incompatibility in the homothallic strain.

In higher basidiomycetes the homothallic and heterothallic conditions may not be phylogenetically as distinct as they outwardly appear to be. Raper and coworkers (1965) and Parag (1962) have demonstrated that the *A* and *B* incompatibility factors in the tetrapolar *Schizophyllum commune* are subject to mutational impairment. The homokaryon of *Schizophyllum* carrying mutations for both factors, *A mut B mut*, is phenotypically dikaryotic and fertile (Koltin, 1970). This doubly mutant homokaryon provides experimental evidence for derivation of a homothallic condition from a heterothallic one. The idea that homothallism evolved from heterothallism in higher basidiomycetes through specific mutations is at least mechanistically feasible.

The homothallic (homodikaryotic) species of *Sistotrema brinkmannii* has been investigated further (Lemke, 1966). Mutations that lead to self-sterility in homokaryons were obtained readily and these often disrupted dikaryosis. Four distinct phenotypes were observed among self-sterile homokaryons. Hyphae were either (a) simple-septate, (b) irregularly clamped with scattered pseudoclamp-connections, (c) regularly clamped and dikaryotic or (d) dikaryotic with aborted or immature basidia. Forty of the self-sterile mutations were analyzed through complementation

analysis. In this analysis for cross-fertility, only one of the forty mutations proved to be dominant, and all crosses involving it exhibited heterokaryon incompatibility. Otherwise, the mutations to self-sterility were recessive and crosses resulted in normal dikaryosis and sporulation in practically all cases. Six of the self-sterility mutations were genetically mapped and are distributed on three linkage groups.

These results with a homothallic strain of *Sistotrema* are comparable to those from studies conducted with homothallic ascomycetes by other investigators (El Ani & Olive, 1962; Olive 1958; Wheeler, 1954). Studies with both groups of homothallic fungi indicate that sexual progression in a homothallic homokaryon comprises a large number of distinct stages subject to mutational impairment. In *Sistotrema* the loci for forty such mutations are scattered and their number, although uncertain, is a minimum of six and a maximum of thirty-six, probably closer to the latter number. It should be emphasized that none of the mutations to self-sterility in *Sistotrema* formed a pattern of heterothallism comparable to bipolarity or tetrapolarity of higher basidiomycetes. These mutations rather constitute a *separate* order of phenomena leading to self-sterility-cross-fertility. They are unrelated to multiple-allelic heterothallism and most likely represent mutations that modify any of the many structural genes that encode for dikaryosis and sporulation in a basidiomycete.

The relatively simple biallelic form of heterothallism present in the ascomycetes may have been derived from homothallic ancestry through complementary mutations to self-sterility. This hypothesis has been proposed independently by Olive (1958) and Wheeler (1954) and is supported principally by studies with *Sordaria fimicola* (El Ani & Olive, 1962). In that homothallic fungus, two very closely linked mutations to selfsterility have been obtained which exhibit complementation for crossfertility. Two complementary, nonrecombinable mutations for self-sterility would, in essence, constitute the biallelism characteristic of bipolarity in ascomycetes. The suggestion that heterothallism in ascomycetes evolved repeatedly from homothallic forms through intragenic self-sterility mutations is at least plausible.

Isolation among fungi can be brought about by ecological factors-microecological as well as macroecological (Kukkonen, 1971). The physiological races of rust fungi demonstrate intraspecific isolation imposed through host specialization (Stakman & Harrar, 1957). Host and parasite are genetically balanced with respect to resistance and virulence. This balance is restrictive for outbreeding and maintains the physiological race, (Flor, 1956; Person, 1966). Although host-parasite associations are not, strictly speaking, heterokaryotic, they do involve a highly specialized form of genetic "complementation".

Ecological barriers presumably operate but are not always apparent in saprobic species. Intersterile races have been recognized among North American isolates of *Fomes pinicola*, a wood-rotting basidiomycete. Isolates from the same tree may belong to separate races (Mounce & Macrae, 1938). An extreme case for the presence of a sterility barrier within a single ecotype of a given species involves *Mycocalia denudata*, a bipolar gasteromycete (Burnett & Boulter, 1963). Two genetically isolated races of this fungus were obtained from a single fructification. In this instance, the one

apparent fructification encompassed two confluent but reproductively isolated basidiocarps. *Mycocalia denudata* illustrates well a problem inherent in the study of fungal populations. A fungus in nature may represent a genetic mosaic. The intermingling of genetically distinct heterokaryons complicates resolution of fungal populations into individual phenotypes. Any statistical analysis of gene frequencies within fungal populations must take into consideration.

Little has been said during this symposium about a rather large group of fungi, the imperfect or azygotic fungi, and of their potential for speciation. The deuteromycetes are considered to be the derived or relic species of perfect ancestors, and there is ample taxonomic evidence to support this conclusion. The imperfect fungi have yet retained specific identity in the formal absence of sexuality. The large number of imperfect species and their diversity provide convincing testimony for successful exploitation of prezygotic, or more correctly azygotic, isolation by fungi to maintain the integrity of species.

Parasexual or somatic recombination was discovered twenty years ago in *Aspergillus nidulans* (Pontecorvo & *al.*, 1953) and has been recognized experimentally in several fungi. Parasexuality offers a recourse for some recombination in the absence of meiosis, and, in view of this, the parasexual process should have special significance among populations of imperfect fungi. This, however, does not appear to be the case in *Aspergillus* (Caten, 1971; Grindle, 1963; Jinks & *al.*, 1966). Conspecific isolates of *Aspergillus* have been examined in considerable detail for competence to form heterokaryons. Heterokaryon incompatibility has proven to be rampant among wild-type isolates of a given species. For example, Caten (1971) reported that among 126 combinations involving 21 isolates of *A. versicolor* only three combinations or about 2 percent of the sample formed heterokaryons. Heterokaryon incompatibility effectively precludes parasexual recombination. Thus, parasexuality may simply be an incidental derangement of mitosis with no real significance for genetic recombination in natural populations.

The trend to restrict recombination is not exclusively that of imperfect fungi. Many fungi with known perfect states are essentially asexual species in nature. The incidence of sexual reproduction in the Mucorales is recognized to be low because of the poor frequency for germination of zygotes and the common occurrence within the order of sexually neutral strains (Blakeslee & *al.*, 1927). Even in higher fungi, sex is often vestigial. Witness such species as *Emericella* (*Aspergillus*) *nidulans*, *Neurospora* (*Monilia*) *sitophila*, or *Thanatephorus cucumeris* (= *Rhizoctonia solani*). Although these fungi exhibit metagenesis, they are for all intents and purposes deuteromycetes.

The following figure (Fig. 2) is an attempt to provide a synoptic outline of speciation phenomena in fungi. Three basic phenomena underlie speciation in fungi, as in other biological systems. These are mutation, selection, and recombination. Although the induction of mutations and the pressures of selection are acknowledged as significant factors in fungal speciation, these phenomena have not been discussed extensively here today. This symposium has been concerned rather with the competence of fungal populations to exchange genetic material.

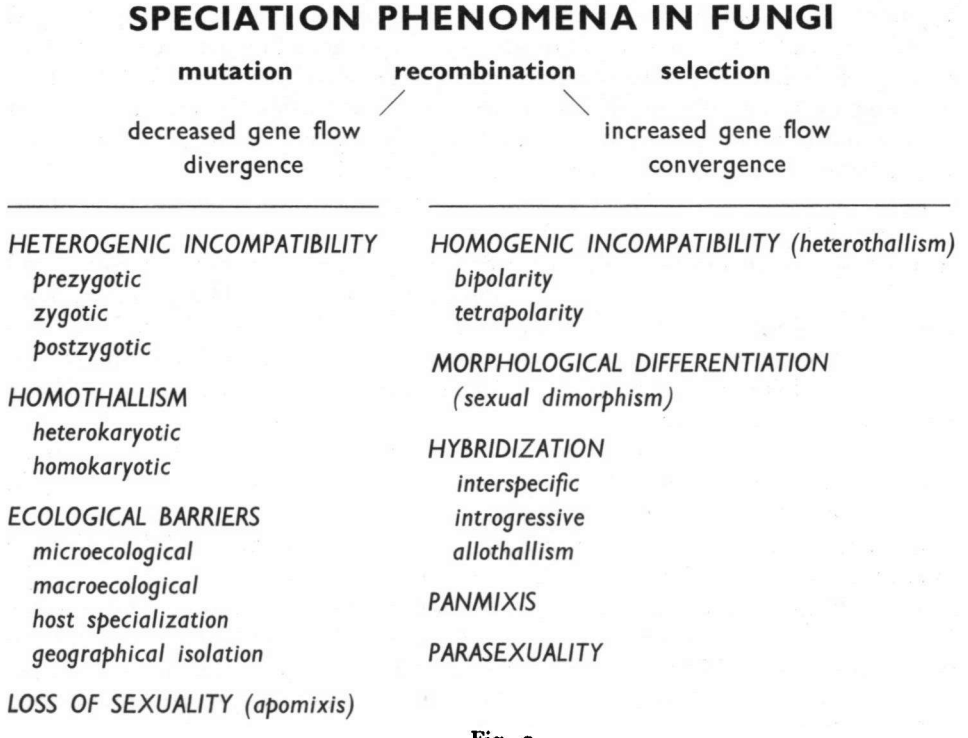

Fig. 2.

It has been pointed out that fungal species vary considerably in this regard. Certain fungi are inbreeders and limit gene flow through any of several mechanisms—i.e., heterogenic incompatibility, homothallism, apomixis, or restrictive ecological adaptations. On the other side of this ledger are fungi that typically outbreed and recombine efficiently through homogenic incompatibility and panmixis. A few fungal species exhibit morphological differentiation *as per* sexual dimorphism, and even fewer fungal species are known to converge through hybridization. The best documented case of interspecific hybridization in fungi involves formation of the natural hybrid, *Allomyces javanicus*, in a cross between related species of different ploidy (Emerson & Wilson, 1954). Among viable hybrids from the cross were forms intermediate between the two parents, *A. arbuscula* x *A. macrogynus*, and karyological data confirmed the hybrid nature of these progeny.

In another study of hybridization, Nelson (1963, 1964) crossed sixteen species of *Cochliobolus* (*Helminthosporium*) in all combinations. The majority of crosses were either completely infertile or produced only immature or sterile asci. However, thirteen out of 120 crosses yielded progeny, but the viability of ascospores was, in all instances, low. A few of the surviving hybrid progeny were subsequently back-

crossed or outcrossed, and in some instances, second generation progeny from these crosses exhibited increased viability (Nelson, 1964). Improved viability of hybrid progeny through backcrossing or outcrossing has been observed in higher plants and is known as introgressive hybridization (Stebbins, 1950).

Some fungi are clearly paradoxical with regard to their potential for gene flow, as populations often combine genetic systems that have opposite effects on recombination. Secondary or heterokaryotic homothallism is often superimposed on homogenic incompatibility. In the bipolar *Mycocalia denudata* secondary homothallism is determined by a dominant allele (*Pd*) for precocious mitotic division of the four meiotic products in the basidium (Burnett & Boulter, 1963). The resultant eight nuclei are distributed at random into four basidiospores. Thus, 50 percent of spores are heterokaryotic with respect to mating-type factors. In the absence of the dominant allele for precocious division (*pd*), basidia of *M. denudata* regularly contain four nuclei and basidiospores are uninucleate upon their inception. Basidiospores at maturity are binucleate but homokaryotic. In *Coprinus bisporus* secondary homothallism is brought about in yet another way—by reduction of spore number per basidium. The selective pressures for secondary homothallism in this species may be related to genetic restriction upon hyphal anastomosis and nuclear migration (Kemp, 1971).

As mentioned earlier, heterogenic incompatibility and homogenic incompatibility can coexist in the same species. *Podospora anserina* demonstrates this paradoxical association, and gene flow in this species is further complicated by secondary homothallism.

In view of the diversity among fungi for the control of recombination, it is indeed difficult to generalize as to the significance of gene flow in fungal speciation. Much has been said in the past about the importance of sexuality and of recombination in fungal evolution (Kniep, 1928; Hartman, 1943; Whitehouse, 1949a, 1949b; Raper, 1966), but there has been relatively little discussion concerning selective pressures for asexuality and for nonrecombination in fungi. Sex and recombination are clearly dispensable commodities in a great many fungi, and the selective advantage for their dispensation is not now apparent.

In the absence of recombination, speciation should be brought about principally through the interplay of mutation and selection. Fungi are predominantly haploid organisms, and mutant genotypes in haploid populations can be conserved or eliminated directly through selection. Thus, fungi, relative to higher diploid organisms, should be more readily susceptible to the affects of mutation and selection (Raper, 1968).

Forty years ago, H. J. Muller (1932) suggested that there was no basic biological reason why evolution, especially in haploid forms, could not go on indefinitely without sexuality. In his opinion, "Sex is not an absolute necessity, it is a luxury. It is necessary only in a relativistic sense, for sexless beings, although often at a temporary advantage, cannot keep up the pace of evolution set by sexual beings. In an evolutionary race between competitive species, the sexless must eventually

lose out." Stebbins (1950) extends this dialectic with two further generalizations. First, "in rapidly reproducing organisms the genetic system that operates is usually one which favors fitness at the expense of flexibility." Secondly, "that genetic system most strongly promoting immediate fitness at the expense of flexibility is one in which sex is absent." Organisms committed to immediate fitness are prone to compromise recombination for the safety of numbers and resort to proliferous asexual multiplication.

Spores and vegetative propagules of several types are formed by fungi in great profusion. These cells represent a vast collection of haploid genotypes which, subject to mutation and selection, could be channelled into a wide variety of specialized ecological situations. Divergent speciation could thus occur in haploid organisms without recombination. However, species generated in this fashion would probably be highly specialized, isolated entities, filling extremely narrow ecological niches. The physiological races of parasitic fungi and the heterogenic races of imperfect and perfect fungi conceivably have arisen through such divergence and may represent incipient species.

BIBLIOGRAPHY

BERNET, J. (1963). Sur les modalités d'expression de gènes pouvant conduire à une incompatibilité cytoplasmique chez le champignon *Podospora anserina*. *In* C.r. hebd. Séanc. Acad. Sci., Paris **256**: 771–773.

BERNET, J., K. ESSER, D. MARCOU & J. SCHECROUN (1960). Sur la structure génétique de l'espèce *Podospora anserina* et sur l'interêt de cette structure pour certain recherches de génétique. *In* C.r. hebd. Séanc. Acad. Sci., Paris **250**: 2053–2055.

BIGGS, R. (1937). The species concept in *Corticium coronilla*. *In* Mycologia **29**: 686–706.

BLAKESLEE, A. F. (1904a). Sexual reproduction in the Mucorineae. *In* Proc. Am. Acad. Arts Sci. **40**: 205–319.

—— (1904b). Zygospore formation a sexual process. *In* Science **19**: 864–866.

BLAKESLEE, A. F., J. L. CARTLEDGE, D. S. WELCH & A. D. BERGNER (1927). Sexual dimorphism in Mucorales. I. Intraspecific reactions. *In* Bot. Gaz. **84**: 27–50.

BURNETT, J. H. & M. E. BOULTER (1963). The mating systems of fungi. II. Mating systems of the gasteromycetes *Mycocalia denudata* and *M. duriaeana*. *In* New Phytol. **62**: 217–236.

CATEN, C. E. (1917). Heterokaryon incompatibility in imperfect species of *Aspergillus*. *In* Heredity **26**: 299–312.

DODGE, B. O. (1928). Production of fertile hybrids in the ascomycete, *Neurospora*. *In* J. agr. Res. **36**: 1–14.

EL ANI, A. S. & L. S. OLIVE (1962). The induction of balanced heterothallism in *Sordaria fimicola*. *In* Proc. natn. Acad. Sci. U.S.A. **48**: 17–19.

EMERSON, R. & C. M. WILSON (1954). Interspecific hybrids and the cytogenetics and cytotaxonomy of *Euallomyces*. *In* Mycologia **46**: 393–434.

EHRLICH, P. & R. W. HOLM (1963). The Process of evolution. New York.

ESSER, K. (1965). Heterogenic incompatibility. *In* K. ESSER & J. R. RAPER (Eds), Incompatibility in Fungi: 6–13. New York.

—— (1971). Breeding systems in fungi and their significance for genetic recombination. *In* Molec. Gen. Genetics **110**: 86–100.

FLOR, H. H. (1956). The complementary genic systems in flax and flax rust. *In* Adv. Genet. **8**: 29–54.

GRINDLE, M. (1963). Heterokaryon compatibility of unrelated strains in the *Aspergillus nidulans* group. *In* Heredity **18**: 191–204.
HARTMAN, M. (1943). Die Sexualität. Jena.
HOLTON, C. S. (1931). Hybridization and segretation in the oat smuts. *In* Phytopathlogy **21**: 835–842.
HOLTON, C. S. & G. W. FISCHER (1941). Hybridization between *Ustilago avenae* and *U. perennans*. *In* J. agr. Res. **62**: 121–128.
HOLTON, C. S. & E. L. KENDRIC (1956). Problems of delimitation of species of *Tilletia* occurring on wheat. *In* Res. Stud. State Coll. Wash. **24**: 318–325.
HOWE, H. B. & P. HAYSMAN (1966). Linkage group establishment in *Neurospora tetrasperma* by interspecific hybridization with *N. crassa*. *In* Genetics **54**: 293–302.
JINKS, J. L., C. E. CATEN, G. SIMCHEN & J. C. CROFT (1966). Heterokaryon incompatibility and variation in wild populations of *Aspergillus nidulans*. *In* Heredity **21**: 227–239.
KEMP, R. F. U. (1971). Breeding systems, speciation and taxonomy of the genus *Coprinus* (abstract). *In* G. C. AINSWORTH & J. WEBSTER (Eds), First International Mycological Congress. Exeter, September 1971: 50. Surrey.
KNIEP, H. (1928). Die Sexualität der niederen Pflanzen. Jena.
KOLTIN, Y. (1970). Development of the *A mut B mut* strain of *Schizophyllum commune*. *In* Arch. Microbiol. **74**: 123–128
KUKKONEN, I. (1971). Micro- and macroecological factors in the speciation of obligate parasites (abstract). *In* G. C. AINSWORTH & J. WEBSTER (Eds), First International Mycological Congress, Exeter, September 1971: 54. Surrey.
LEMKE, P. A. (1966). The genetics of dikaryosis in a homothallic basidiomycete, *Sistotrema brinkmanni*. Ph. D. Thesis. Harvard University.
—— (1969). A reevaluation of homothallism, heterothallism, and the species concept in *Sistotrema brinkmanni*. *In* Mycologia **61**: 57–76.
MAYR, E. (1970). Populations, species, and evolution: An abridgment of animal species and evolution. Cambridge (U.S.A.).
MOUNCE, I. & R. MACRAE (1938). Interfertility phenomena in *Fomes pinicola*. *In* Can. J. Res. (Bot. Sct.) **16**: 364–376.
MULLER, H. J. (1932). Some genetic aspects of sex. *In* Am. Nat. **66**: 118–138.
NELSON, R. R. (1963). Interspecific hybridization in the fungi. *In* Mycologia **55**: 104–123.
—— (1964). Bridging interspecific incompatibility in the ascomycetous genus *Cochliobolus*. *In* Evolution **18**: 700–704.
OLIVE, L. S. (1958). On the evolution of heterothallism in fungi. *In* Am. Nat. **92**: 233–251.
PARAG, Y. (1962). Mutation in the *B* incompatibility factor of *Schizophyllum commune*. *In* Proc. natn. Acad. Sci. U.S.A. **48**: 743–750.
PERKINS, D. D. (1972). An insertional translocation in *Neurospora* that generates duplications heterozygous for mating type. *In* Genetics **71**: 25–51.
PERSON, C. (1966). Genetic polymorphism in parasitic systems. *In* Nature, Lond. **212**: 266–267.
PONTECORVO, G., J. A. ROPER, L. M. HEMMONS, K. D. MACDONALD & A. W. J. BUFTON (1953). The genetics of *Aspergillus nidulans*. *In* Adv. Genet. **5**: 141–237.
RAPER, J. R. (1966). Genetics of sexuality in higher fungi. New York.
—— (1968). On the evolution of fungi. *In* G. C. AINSWORTH & A. S. SUSSMAN (Eds), The fungi **3**: 677–693.
RAPER, J. R., D. H. BOYD & C. A. RAPER (1965). Primary and secondary mutations at the incompatibility loci in *Schizophyllum*. *In* Prov. natn. Acad. Sci. U.S.A. **53**: 1324–1332.
RIZET, G. & K. ESSER (1953). Sur des phénomènes d' incompatibilité entre souches d'origines différentes chez *Podospora anserina*. *In* C. r. hebd. Séanc. Acad. Sci., Paris **237**: 760–761.
STAKMAN, E. C. & J. G. HARRAR (1957). Principles of plant pathology. New York.
STEBBINS, G. L. (1950). Variation and evolution in plants. New York.

WHEELER, H. E. (1954). Genetics and evolution of heterothallism in *Glomerella*. *In* Phytopathology **44**: 342-345.
WHITEHOUSE, H. L. K. (1949a). Multiple allelomorph heterothallism in the fuugi. *In* New Phytol. **48**: 212-244.
—— (1949b). Heterothallism and sex in the fungi. *In* Biol. Rev. **24**: 411-447.
WINGE, Ø & C. ROBERTS. (1949). A gene for diploidization in yeasts. *In* C.r. Trav. Lab. Carlsberg (Physiol.) **24**: 341–346.
WRIGHT, S. (1931). Evolution in mendelian populations. *In* Genetics **16**: 97–159.
—— (1932). The roles of mutation, inbreeding, crossbreeding and selection in evolution. *In* Proc. sixth intern. Congr. Genet. **1**: 356–366.