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Evolution of Incompatibility Systems in Plants: Complementarity and the Mating Locus in Flowering Plants and Fungi

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Summary. The restriction of sexual pairing by a specificity gene is considered to be an ancient development in the plant kingdom. The diversity and general parallelism of incompatibility systems seen amongst the phyla at the present time can be rationalized in terms of the association of various derived forms of the ancestral specificity unit with differing spectra of accessory factors controlling sexual physiology in the different phyla. Sexual morphogenesis has become divided into distinct phases under the control of complementary genes. These phases are initiated by a regulatory system of "Co-ordinator genes" which control the order in which groups of morphogenetic genes are expressed during development. The entire sexual cycle will be completed only if all the complementary groups are activated in the appropriate sequence. The present article discusses essential features of the evolution of the breeding locus in different phyla. These features are consistent in themselves with the present data and are not dependent on the proposed ancient origin of the specificity gene.

The above hypothesis throws light on the (1) evolution of the complex mating loci in flowering plants and fungi; (2) evolution of complementary incompatibility and heteromorphic incompatibility in flowering plants; (3) anomalous cross-compatibility behaviour of mutants in the fungus *Schizophyllum commune;* (4) nature of homothallism in higher fungi; (5) mode of origin of new functional self-incompatibility alleles; and (6) "homogenic" and "heterogenic" incompatibility.

Key words: Incompatibility - Evolution - Flowering Plants - Fungi - Complementarity

Introduction

There are two basic components to the sexual cycle of eukaryotes: meiosis, giving rise to haploid cells; and karyogamy, recreating the diploid state by fusion of haploid sexual nuclei. To achieve karyogamy the haploid participants must be brought into effective contact, and this has been facilitated in evolution by the development of "Donor" and "Recipient" physiologies, usually leading to sexual morphogenesis. The completion of the sexual process including pre- and post-conjugation phases is based on physiological complementarity of the donor and recipient. This complementarity involves not only the two combining haploid gametes but also all cells of the vegetative phase whose function is connected with the sexual process. For example, in higher plants there is a physiological interaction between the diploid somatic cells of the style and the haploid male gametophyte bearing the actual gametes. The requirement for vegetative as well as sexual complementarity is even more marked in the fungi where vegetative fusion occurs in addition to union of gametes.

The widely occurring "homogenic" incompatibility (Esser 1959) prevents the fusion of gametes carrying identical alleles at a particular genetic locus, such as the S locus in higher plants and the A and B factors in higher fungi. This system has evolved to prevent unsuitably matched individuals from completing the sexual cycle, and has become thoroughly integrated with the genes controlling other aspects of donor and recipient sexuality.

The following discussion will develop the theme that the evolution of a specificity gene to restrict sexual pairing is an ancient development in the plant kingdom. The diversity of incompatibility systems seen amongst the phyla of the present day can be rationalised in terms of primary, secondary or tertiary developments based ultimately on the association of one ancestral specificity gene with differing spectra of accessory factors governing the various aspects of sexual reproduction in the different phyla. The second major theme of the discussion, not necessarily dependent upon the first, will be the concept of complementation - genetic, physiological and morphological - necessitated by the harmonizing requirements of the

Table 1. Comparative incompatibility systems in angiosperms and fungi

Genetic systems	Angiosperms	Fungi
(1) One locus with two alleles	*Primula, Linum, Pulmonaria, Fagopyrum, Forsythia.*	Ustilaginales and Uredinales ("Coniomycetes" - Whitehouse 1951).
(2) One locus with multiple alleles	A large number of gametophytic and sporophytic species.	Hymenomycetes (Raper 1960).
(3) One locus with multiple alleles and second locus with two alleles	*Solanum pinnatisectum, S.ehrenbergii.*	*Ustilago zeae* (Ustilaginales) (Rowell and De Vay 1954; Rowell 1955).
(4) Two loci with two alleles each	*Lythrum, Oxalis.*	---------- (Considered unlikely on theoretical grounds - Mather 1942; Whitehouse 1949, 1951).
(5) Two loci each with multiple alleles	*Physalis ixocarpa.*	----------
(6) Two loci each with multiple alleles and interlocus complementation	*Gramineae.*	Basidiomycetes (Esser 1967).
(7) Three or more loci each with multiple alleles and interlocus complementation	*Ranunculus acris, Beta vulgaris.*	*Psathyrella coprobia* (Basidiomycetes) (Jurand and Kemp 1973).

Donor and Recipient components of the system. Sexual morphogenesis has become divided into distinct phases under the control of complementary genes. The entire sexual cycle can only be completed if all of these complementary groups are activated in the appropriate sequence. It will be shown that the specificity elements themselves may have a part to play in the regulation of this complementary activation process.

Comparative Incompatibility Systems

There is a general parallelism among homogenic incompatibility systems wherever they are found in the plant kingdom. For example, many similar systems occur among the angiosperms and fungi (Table 1). This general parallelism has led to the suggestion that all such systems are manifestations of a single primeval specificity element which has grown in complexity and has become integrated with a great variety of genes controlling different aspects of sexual physiology but which retains certain basic properties by which it can be identified in the various phyla (Pandey 1969c). In addition to this parallelism in gross organisation, flowering plants and fungi show certain other parallel features which may represent the intrinsic properties of a common ancestral specificity gene. For example, mutations from one functional allele to another have not been observed in either group, and artificial mutagenesis results only in loss of function. In addition certain of these systems, such as the homomorphic single locus gametophytic system in higher plants and the bifactorial system of the higher basidiomycetes, show a similarity in their potential diversity of incompatibility phenotypes. In both phyla the specificity units are believed to act as "switches" for linked groups of genes controlling aspects of sexual physiology. Also in both groups specificity units within a complex factor are always complementary in function.

In contrast to the above parallels the higher plants and fungi show a number of divergent properties which are thought to result from the combination of the ancestral specificity unit with differing spectra of auxiliary sexual factors, and the subjection of the resulting gene complexes to different evolutionary restructuring and selection pressures. In the fungi, for example, primitive incompatibility is believed to be represented by a single factor with two alleles as seen in the lower fungi (Whitehouse 1951) (Table 1; 1). In the higher plants, however, the homomorphic gametophytic system with a single multiallelic locus is considered primitive. Also, relationships between separate factors are always complementary in the fungi but may be complementary or independent in

higher plants. This property and the greater plasticity of fungal incompatibility systems, i.e. the greater relative ease with which one-factor and two-factor systems appear to be interconvertible (Koltin, Stamberg and Lemke 1972), are thought to result from a very early introduction of interunit functional complementarity in the higher fungi. In the evolution of gametophytic incompatibility in flowering plants, where the reaction occurs between haploid pollen and diploid style, the required selection for independent action of alleles in the style meant that complementarity between separate factors was only introduced late as a secondary development to restore self-incompatibility under special conditions (Pandey 1976b). In the fungi, however, where haploid-haploid interaction determines incompatibility, selection for independent action of alleles has not been a basic requirement, and therefore complementary systems could have evolved early. Differing spectra of auxiliary genes associated with the basic specificity units may also account for differences in observed mutation rates and types of mutation associated with incompatibility factors.

As a unifying hypothesis for the derivation of complex incompatibility systems, the basic evolutionary changes in architecture of the primeval system are visualised as:

(1) Convergence (increasingly tight linkage) with other genes controlling physiological and morphological aspects of sex expression to give a complex locus (Mather and De Winton 1941; Mather 1950; Esser and Straub 1956, 1958; Raper and Flexer 1970).

(2) Partial or full duplication of the complex elsewhere in the genome often leading to functional breakdown of the system.

(3) Restructuring of duplicated complexes to restore efficient breeding behaviour; e.g. by selection for independent action and subsequently for co-operative production of a single functional specificity by the duplicated loci.

(4) Further cycles of convergence, duplication and restructuring.

Figure 1 illustrates the concepts embodied in the above broad evolutionary hypothesis. Convergence of complementary factors would, in effect, produce a multi-unit, potentially multiallelic, complex locus with a single functional specificity. This locus in turn would be affected by further combinations of evolutionary changes similar to those acting on the primeval gene. Splitting of such a locus into two or three groups of linked complementary factors would produce a multifactorial system of the type seen in higher Basidiomycetes. Similar basic processes are believed to be operating for both the higher plants and the fungi.

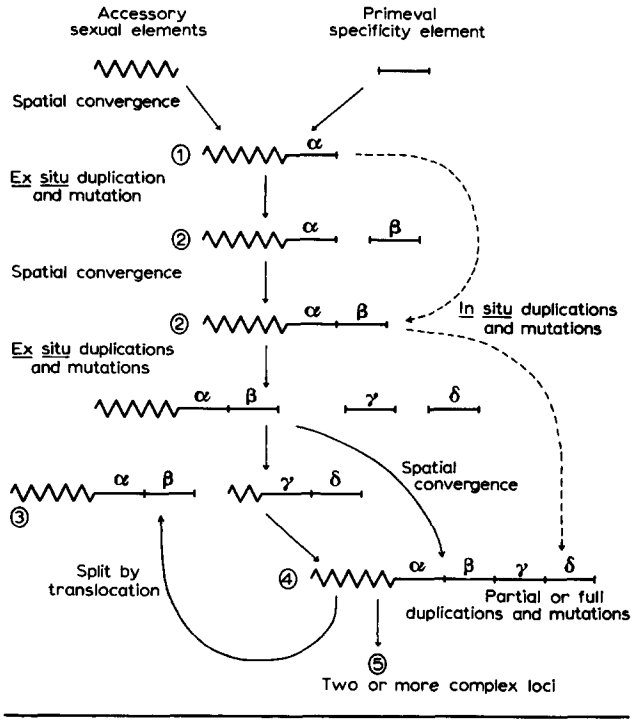

Fig.1. Diagrammatic representation of the conceptual processes involved in evolution of complex incompatibility systems (1) Breeding system gene complex with two or a few specificity alleles. (2) Duplication breaks down incompatibility and is followed by selection for independent action and finally for co-operation of α and β to give a single specificity. Spatial convergence of separate co-operating units will be favoured. (3) Two-factor multiallelic complementary system. (4) One-factor multiallelic system with subunits co-operating to produce a single specificity. (5) Two or more complex loci

The evolution of sex in eukaryotes also involved the development of a process of nuclear migration. Three major evolutionary phases can be distinguished: (1) cell fusion accompanied by nuclear fusion (eukaryotic algae and lower fungi); (2) cell fusion accompa-

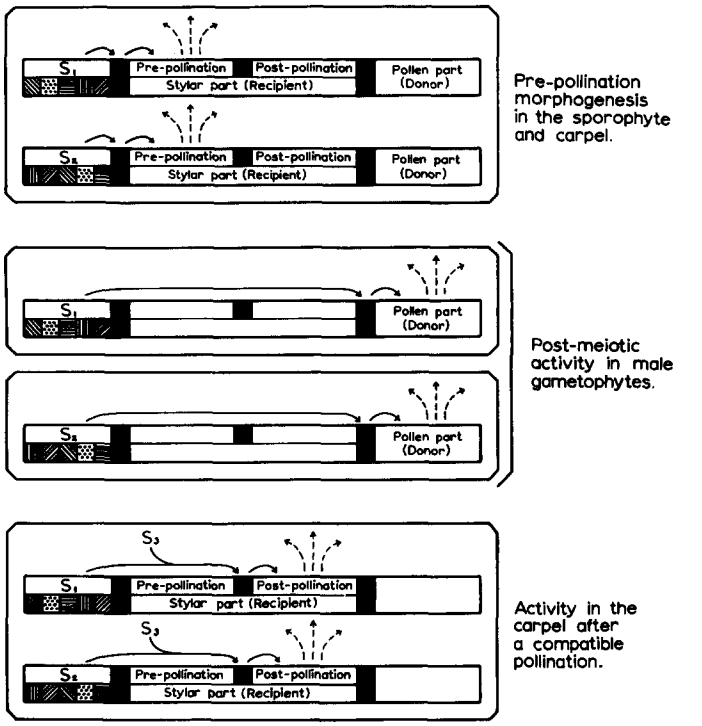

Fig. 2. Diagrammatic representation of structural organisation and alternative activation at the complex S locus in higher plants with homomorphic gametophytic incompatibility. In the pre-pollination morphogenesis of the sporophyte and carpel the S specificity proteins combine with the female adaptor protein to produce the female incompatibility determining molecules. Similarly in the pollen the S specificity protein combines with the male adaptor protein to produce the male incompatibility determining molecule. In the compatible pollination where the S specificity components of the two reacting molecules are different activation of the post-pollination physiology facilitates pollen tube growth. Also see Fig. 3.

nied by reciprocal nuclear migration (higher fungi); and (3) cell fusion accompanied by unilateral nuclear migration, true donor/recipient physiology (flowering plants).

The S Locus of Higher Plants: A Working Model

In the homomorphic gametophytic systems of higher plants there is a single S locus with a series of up to several hundred alleles which act autonomously in the pollen and independently in the style. Specificity is thought to be controlled by a number of intra-locus, complementary, specificity elements which act together to produce a unique specificity. Different combinations or orders of these elements determine different alleles (Pandey 1968). All diploid plants are morphologically alike, regardless of their S genotype. The locus comprises two major functional segments: a "pollen part" and a "stylar part" which can be individually affected by spontaneous or artificial mutagenesis (Lewis 1954; Pandey 1956a). These two functions have been respectively integrated into the donor and recipient physiologies and as a consequence have been subjected to somewhat different selection pressures, as revealed, for example, by the presence or absence of the capability for allelic interaction (Lewis 1947, 1954; Pandey 1956b). The pollen and stylar functions are thought to be alternately activated during the haploid and diploid phases of the life cycle respectively as illustrated diagrammatically in Fig. 2.

The homomorphic sporophytic system is derived from the gametophytic system principally by a change in the time and site of action of the pollen part of the S locus, so that the incompatibility specificity of the haploid pollen is no longer determined autonomously by the single allele which it carries, but is controlled by the S genotype of the diploid somatic floral tissue of the male plant (Lewis 1949, 1956; Pandey 1960, 1970a, 1974a).

The heteromorphic system (Table 1; 1) is also thought to be secondarily derived after a temporary intervening period of self-compatibility. The latter led to erosion of the S gene specificity complex, eventually to render it capable of producing only two specificity alleles (Pandey 1969b, c, 1973). However, subsequent to the recurrence of strong selection for outbreeding, these two alleles have become tightly linked to factors producing floral dimorphism. Thus in the heteromorphic system there is morphological as well as physiological complementarity in the donor and recipient physiologies.

An alternative evolutionary development from the original one-locus, multiallelic system is the appearance of "complementary incompatibility" based on genetic complementarity between any two alleles of two (or more) separate loci (Table 1; 5 and 6) (Lundqvist 1964; Lundqvist et al. 1973). The types of hybridization and polyploidy events, and selection against S allelic interaction, which are believed to have led to the formation of complementary incompatibility, have been discussed in another paper (Pandey 1977b).

The present model is also relevant to an understanding of incompatibility determination. Lewis (1960) proposed two basic hypotheses concerning S gene determination in flowering plants. In both an allele codes for proteins in two different sites. In the first, the allele codes for two distinct proteins, one for the pollen tube and one for the style. The proteins S_p and S_{st} have complementary configurations analogous to antigen-antibody or enzyme-substrate systems. In the second hypothesis, favoured by Lewis, the allele codes for (a) a specific protein pattern common to both sites; (b) an activator for protein production in the pollen tube, and (c) an activator for protein production in the style. These hypotheses do not resolve the problem of how a single allele can produce two

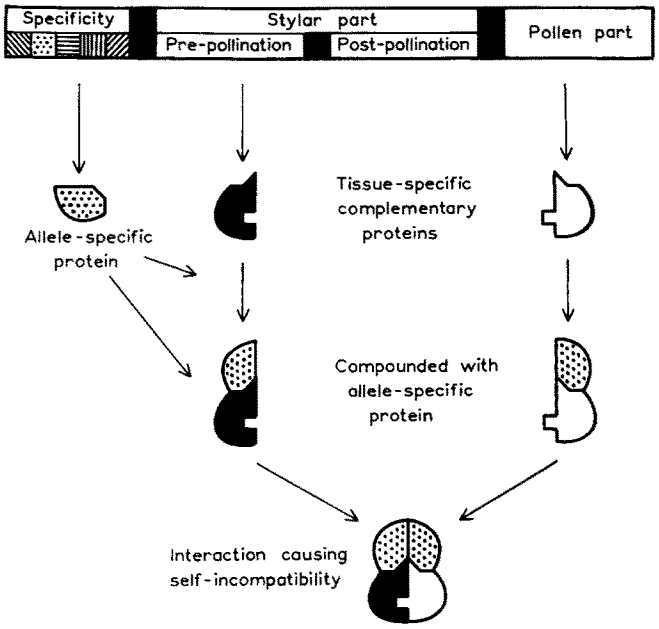

Fig.3. Incompatibility determination: The use of pollen and stylar "adaptor" proteins in the specificity determination and interaction of the two sexual components

mutually reactive proteins in the two relevant sites (Burnet 1971).

The present model (Figs.2 and 3) is an extension of the second hypothesis of Lewis and involves compounding of the specific identical proteins in the pollen and style with their respective pre-pollination complementary products. Such a protein retains the original specificity but has gained the complementary configuration for the necessary pollen-style interaction. On contact the pollen and style proteins are thought to unite to function as an inhibitor whose action has the final effect of repressing genes involved in the production of pollen growth promoting substances.

Evolution of the Mating Locus in Fungi: A Hypothesis

1. Phycomycetes

The lower forms of fungi, the Phycomycetes, are predominantly homothallic (self-fertile), each plant possessing dual donor and recipient physiology (hermaphrodite). However, self-fertility resulting from this genetic totipotency can be naturally or ex-

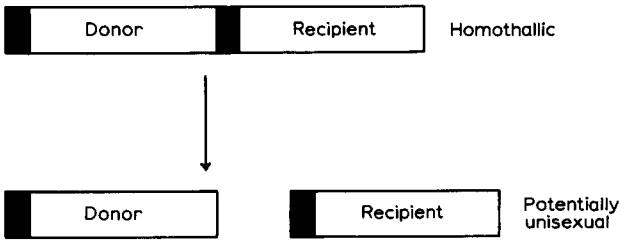

Fig. 4. Separation of the "donor" and "recipient" functions of the mating locus in the Phycomycetes

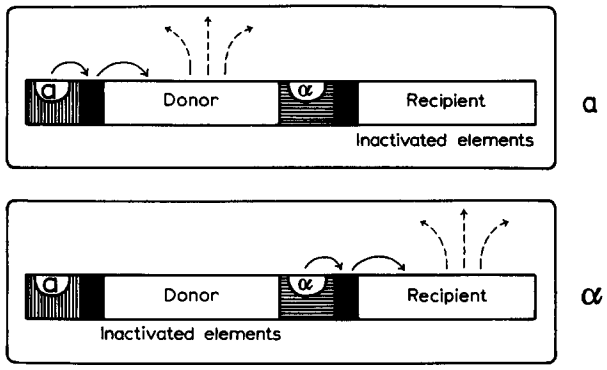

Fig. 5. Pseudoallelism caused by alternative activation at the mating locus in *Saccharomyces*

perimentally disrupted, with a consequent approach to heterothallic self-sterility.

It is proposed that in these fungi the genes for donor and recipient physiology, each linked in a discrete genetic segment, are usually combined into one linked block. Under certain conditions the block may be split up into the two component segments which may become separated in special circumstances to give rise to forms comparable to different sexes (Fig. 4). Such breeding forms may be associated with some degree of sexual dimorphism. For example, in the aquatic Phycomycete *Allomyces*, interspecific hybridization results in the segregation of aneuploid lines, some of which are almost unisexual (Emerson and Wilson 1954; Machlis 1958; Raper 1960).

2. Ascomycetes and lower Basidiomycetes

In the Ascomycetes and lower Basidiomycetes restriction of self-fertility is usually achieved through incompatibility, which generates two or more morphologically indistinguishable classes that are self-sterile and cross-fertile, and between which sexual interaction is typically reciprocal. In self-incompatibility it is proposed that the donor-recipient genetic block, with complementarity within and between the two segments, is maintained but the ability to split the block into viable component segments is lost. Furthermore, incompatibility specificity elements are thought to be functionally associated with the regulatory elements which in turn control the linked block of morphogenetic elements. All individuals have dual sex physiology and there are no differences in reciprocal unions.

In the specialisted Ascomycete genus, *Saccharomyces*, heterothallism is controlled by an "allelic" pair of factors a and α which are genetically labile. "Mutations" of one "allele" to the other or to a non-functional state have been described in numerous species (Lindegren and Lindegren 1944; Leupold 1950; Ahmad 1953). In the present hypothesis a and α are presented as pseudoalleles comprising the two main segments of linked genetic elements and acting in a complementary fashion to complete the sexual process. Each segment has its own attached regulatory and specificity elements (Fig. 5).

Early steps in the evolution of sexuality, including the stage typified by *Saccharomyces*, are better understood with reference to the concept of complementation rather than in terms of true donor/recipient sexuality which evolved later. Hence, in *Saccharomyces* the situation should be viewed as "potentially donor" and "potentially recipient", later evolution chanelling these complementary interactions into true sexuality.

A critical feature of the present hypothesis is that in heterothallic species of *Saccharomyces* the two breeding forms are considered to be produced not by mutation but by regular differential inactivation of one or other genetic segment, as suggested previously for the two functional segments of the S locus in higher plants. In both the *Saccharomyces* and higher plant systems genetic elements controlling incompatibility specificities and acting as "switches" are associated with a gene complex containing mutationally distinct regulatory elements governing donor and re-

cipient physiologies. Sexual differentiation is presumably due to expression of one segment (donor or recipient) while the other is inactivated. A mutation (including loss) in a regulatory element may allow activation of the associated genetic elements independently of control by the specificity "switch". This may lead to self- and universal cross-compatibility. Such mutations have apparently been recovered both in flowering plants and fungi (Lewis 1954; Pandey 1956a; J.R. Raper and C.A. Raper 1973). In *Saccharomyces* inactivation, occurring during or soon after meiosis, leads to production of the two forms in which either a or α is active but not both. After conjugation both a and α are active in the zygote and the full sexual cycle is completed. Inactivation is influenced by the presence of two specific independent regulatory genes, HO and HM_a, but other independent genes modifying the expression of sexuality are also known (Takahashi 1958; Winge and Roberts 1949; Harashima et al. 1974; Gerlach 1974).

Mutational or recombinational changes may break down heterothallism by dislocating the machinery for systematic activation and inactivation of the dual segments of the complex. Such derived secondarily homothallic strains may include an array of conditions depending on the genetic changes involved and may possess concealed alternative mating capabilities. Hybrids between "mutants" or between "mutant" and "normal" may segregate new forms not represented among the parents (Takahashi 1961). The four types of life cycle described for *Saccharomyces cerevisiae* and related species (Harashima et al. 1974) may be viewed in this light. The possibilities for variant sexual forms are increased by polyploidy (euploidy) and aneuploidy (Takahashi 1961; Pandey 1965, 1969a).

Regulatory alterations imposed on a genetic background of the *Saccharomyces* type may account for the breeding behaviour of the yeast-like Basidiomycete *Ustilago violacea* described by Cummins and Day (1973). The genetic system in this species underlines the cell cycle phase dependence of the control of activation and inactivation of genetic elements in the mating complex.

In most Ascomycetes other than *Saccharomyces*, and in the Basidiomycete rusts, heterothallism is characterised by: (1) regular segregation of an allelic pair of incompatibility factors, A and a; (2) equivalent reciprocal mating competence of the two classes of individuals A and a, which are bisexual but self-sterile; and (3) genetic stability of the incompatibility alleles. These features are consistent with the proposed mating locus structure shown in Fig.6.

In contrast to *Saccharomyces* the two forms A and a are separately-occurring true alleles rather than linked pseudoalleles. Each allele contains only one

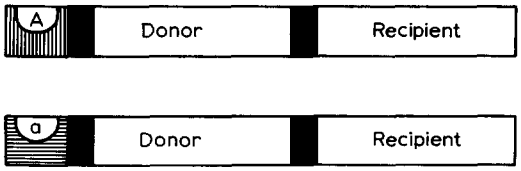

Fig.6. Mono-factorial biallelic systems as found in most Ascomycetes and in Basidiomycetous rusts

switching unit but two separate regulatory elements for the donor and recipient physiologies. Since alternative activation is not involved, switching may possibly not be open to relatively easy modification by independent genes as it is in *Saccharomyces*. This accounts for the stability of incompatibility alleles demonstrated by extensive studies, particularly in *Neurospora crassa*.

Although most filamentous Ascomycetes exhibit this basic 1-locus, 2-allele incompatibility system, numerous variants occur in nature. Certain of these appear to bridge the gap between homothallism and heterothallism (Raper 1960), while others seem to show relic traces of a *Saccharomyces*-type system in their possession of independent genes affecting sexual expression (Esser 1959; Nelson 1960). In *Chromocoea spinulosa* (Mathieson 1952) and probably in *Ceratostomella fimbriata* (Olson 1949) the heterozygosity at a single (incompatibility?) locus, which is a prerequisite for mating, has been suggested to be achieved de novo by mutation at each sexual generation. As proposed for *Saccharomyces* this may be a case of alternative inactivation within the mating locus rather than mutation. Similarly in *Glomerella cingulata* the conversion of the wild self-fertile form to a series of classes differing in degree of self-fertility may involve selective inactivation, rather than mutation as previously suggested by Wheeler (1950). Thus some degree of selective inactivation, possibly a relic of a more primitive system of the *Saccharomyces* type, may still influence the breeding system in certain filamentous Ascomycetes.

3. Higher Basidiomycetes

The most important feature of breeding behaviour in the Hymenomycetes and Gasteromycetes is the extensive series of alternative and apparently equivalent alleles at each incompatibility factor. Systems are typically controlled by one or two separate factors (Whitehouse 1949; Raper 1960; Day 1960; Parag 1962; Raper et al. 1965; Koltin 1968). In a majority of the higher Basidiomycetes (Table 1; 6) compatibility between haploid partners is controlled by 4 loci linked in two independently-assorting pairs designated

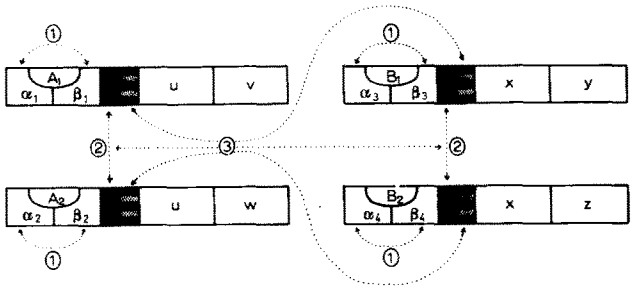

Fig.7. Levels of complementation in bifactorial incompatibility systems in the fungi: (1) Cis complementation between linked "allelic" cistrons within the A and B factors. The α β combinations are naturally selected for complementation. (2) Trans complementation between allelic factors allowing transcription of those linked elements which are largely similar or identical in the different forms of each factor (u in factor A; x in factor B). (3) Trans complementation between non-allelic factors allowing transcription of the remaining dissimilar genetic elements (v, w, y, z), thus allowing completion of the sexual process

the A and B factors. Within each factor the two linked loci, α and β, each possess a series of alleles, and any given combination of α and β alleles determines a unique factor phenotype (Raper and Flexer 1970). Both factor phenotypes must be different for full compatibility, and both must be matched for full incompatibility.

The basic structure of the mating locus in these multiallelic systems is suggested to be similar to that in Fig.6, except that the specificity component comprises a number of genetic elements which together determine the individual specificity. Bifactorial systems have 3 levels of functional complementation shown diagrammatically in Fig.7. Firstly, the α and β specificity cistrons within each factor have been selected for intra-factor complementation, possibly because not all non-identical products of α and β are functionally viable. This is supported by the results of recombination studies involving different α and β alleles of the B factor derived from geographically diverse sources (Stamberg and Koltin 1971). Certain combinations of specific alleles could not be obtained, presumably because they were non-functional. Secondly, if the specificity products of one factor (A or B) are dissimilar in the two conjugating cells (e.g. A_1, A_2 or B_1, B_2) a feedback reaction results in transcription of those linked genetic elements which are largely similar or identical in the different forms of the particular factor (A or B). This independent control of feedback at the A and B factors has led to identification of distinct phases in sexual morphogenesis (Raper and Flexer 1970). Finally, when both factors are different in the conjugating cells (e.g. A_1, A_2 and B_1, B_2), complementation occurs between different factors (or factor products) permitting transcription of elements which are dissimilar between factors, and allowing completion of the entire sexual process. Identity at either or both of the two factors in conjugating cells leads to lack of complementation disrupting sexual morphogenesis. Thus sexual compatibility is based mainly on functional complementation.

In the bifactorial system, as in *S. commune*, each factor is thought to have at least two regulatory elements concerned with the second and third levels of complementation. Regulatory elements of the first level of complementation between α and β, as stated earlier, are usually naturally selected to be complementary. Such selection is possibly also responsible at least in part for the morphogenetic developments typical of all normal incompatible reactions. Similarly in the trifactorial system, as in *Psathyrella* (Jurand and Kemp 1973), there is presumably a minimum of three regulatory elements in each factor.

Regulation of the Incompatibility Complex in the Higher Fungi

The results of mutation studies in *Schizophyllum commune* throw some light on the nature of regulatory elements and their functional relationship with the other genes of the complex. Mutations in the B factor have revealed two general types of mutation. (1) Primary Mutations. These lack specificity but regulation is permanently switched "on" giving the physiology and phenotype of a normal B compatible reaction (the "B-on" phenotype). The primary B_β mutant strains are universally compatible, i.e. with self, with the progenitor B_β allele and with all other alleles. They are thus constitutive for the B-sequence. (2) Secondary Mutations. These arose from the primary mutants but were many times more frequent. In these the "B-on" phenotype of the primary mutants is suppressed, but they have varying "B-off" phenotypes (J.R. Raper and C.A. Raper 1973).

According to the present hypothesis the regulatory component comprises physiologically single but mutationally twin elements controlling two-way complementary relationships: (i) with the specificity genes

determining compatibility or incompatibility ("on" or "off" phenotype), and (ii) with the associated morphogenetic genes, controlling their physiologically compatible sequential expression. If the regulatory component acts through the production of a protein molecule the regulatory and catalytic properties of this protein may, in fact, be independent of each other, as has been suggested for the histidine operon in *Salmonella typhimurium* (Kovach et al. 1973; Voll 1972; Goldberger 1974). Any change such as deletion or point mutation occurring in either the specificity or the morphogenetic genes might be expected to alter or destroy the complementarity of that set of elements with the regulatory component. Thus primary mutations presumably affect the specificity genes causing loss of the first relationship (i) enumerated above. This leads to permanent, non-specific, switching-on of the B sequence, which is alwyas carried through to completion because the second relationship, (ii) above, concerning the sequential expression of the morphogenetic genes, remains intact. Secondary mutations presumably affect the morphogenetic genes and cause the loss of the second relationship, producing "B-off" phenotype and giving haphazard morphogenetic expression. With one rare exception, all secondary mutants are self- and inter-incompatible, displaying B-off morphology. A combination of the secondary mutants and the primary mutant, however, leads to complementation producing B-on morphology of the primary mutant (dominance of primary mutation).

A very intriguing feature of secondary B factor mutants concerns their behaviour in reaction with the different wild type alleles (J.R. Raper and C.A. Raper 1973). The same mutant form shows a different type of intercompatibility pattern in terms of morphogenetic expression in interaction with different wild B_β alleles. In view of the fact that all normal alleles are considered equivalent in terms of physiology of incompatibility, this is indeed surprising. It is suggested here that in these combinations the presence of the normal B_β alele allows complementation so that expression of the morphogenetic elements is restored. However, such a restoration is probably partial, resulting in the expression of the morphogenetic elements according to the physical order of linkage relationship in the complex, rather than, as is believed to occur under normal control of the regulatory element, according to the required physiological sequence. The occurrence of such a partial dominance is supported by interaction in a specific combination, that between a B-always-on primary mutation and B-always-off type of secondary mutation. In this case the primary mutation is dominant over the secondary mutation only with respect to fusion of hook-cells (C.A. Raper and J.R. Raper 1973).

In wild type alleles different morphogenetic elements may be variously linked, but since normally the regulatory gene controls their complementary sequential expression, the physical order of linkage is inconsequential, and as such, non-complementary linkage sequences may accumulate in nature. In compatible B combinations where one of the parents is a secondary mutant of the B complex and therefore has its relevant regulations non-functional, the expression of the morphogenetic elements of the wild type allele according to the physical order of their linkage may result in the breakdown of complementation at any one of the different stages. Thus various patterns and degrees of partial cross-compatibility behaviour observed in different combinations of B_β mutants and the B_β wild type alleles reflect the different linkage relationships of the constituent morphogenetic elements in the wild type B_β alleles.

Homothallism

Homothallic forms represent a minority among the higher fungi. Most studies have shown that many homothallic forms are basically heterothallic but apparently display derived homothallism (Whitehouse 1949; Lemke 1966, 1969, 1973). Although some critical studies have been made of "secondary" homothallism, the genetic determination of "primary" homothallism and its relationship to heterothallism have not been adequately explained (Ullrich and Raper 1975). Recent mutation studies appear to throw some light on this problem. For example, the "primary" homothallism in certain forms of the higher fungus *Sistotrema brinkmannii* may have been derived from monofactorial heterothallism through mutations (including loss) in the incompatibility factor involving both regulatory elements. In such mutants mor-

phogenetic elements of both donor and recipient segments of the complex will be permanently switched on (comparable to complete, pollen + stylar part, mutation in the higher plants, Pandey 1956a). Such an interpretation, favouring the constitutive model, is also in agreement with the recent observations of Ullrich and Raper (1975).

Many other reports of "primary" homothallism among higher fungi involve species with incomplete sexual progression (Boidin 1958; Boidin and Lanquetin 1965; Lange 1952; McKenzie et al. 1969; Raper 1959) and probably carry mutations affecting only certain of the regulatory elements (comparable to incomplete, pollen- or stylar-part, mutations of the higher plants, Lewis 1954; Pandey 1956a). A majority of so called "homothallic" Homobasidiomycetes (Biggs 1938) are probably derived simply by forfeit of dikaryosis as a prerequisite to fruiting (comparable to apomixis in higher plants), and may have a similar, 'mutational' origin. Alternatively, some of these conditions may have arisen by mutations or a significant shift in the genes comprising the essential polygenic background of the mating system (Mather 1943, 1955; Pandey 1970c, 1974b; J.R. Raper and C.A. Raper 1973), as evidenced by studies in *Schizophyllum commune* discussed earlier.

Origin of New Self-Incompatibility Alleles

If the above hypothesis to explain the cross-compatibility behaviour between a mutant $\underline{B}_{\beta}$ allele and normal $\underline{B}_{\beta}$ alleles is true, then it also suggests a possibility for the solution of the most intractable problem in the genetics of incompatibility in plants - the mode of origin of new functional $\underline{S}$ alleles. If there are regulatory elements controlling which morphogenetic elements will be expressed at a particular time, and in what precise physiological sequence they will be activated, then the possibility exists that there are similar regulatory elements which control the expression of the structural genes determining allelic specificity. From a relatively small number of structural genes, possibly fewer than ten (Pandey 1967, 1970b), hundreds of different $\underline{S}$ alleles could be generated through regulated sequential expression of a certain specific set of complementary cistrons.

The stability requirements of such a system, and the conditions favouring the spontaneous appearance of new alleles have been discussed elsewhere (Pandey 1977a).

"Homogenic" and "Heterogenic" Incompatibility

In addition to being a basic ingredient in the evolution of primary sexual mechanisms, the phenomenon of complementation also offers an explanation for the occurrence of so-called heterogenic incompatibility as seen in the Ascomycete *Podospora anserina* described by Esser (1959, 1971).

The most common type of incompatibility, homogenic incompatibility, to which belong all the systems described above, is the basis for all primary incompatibility systems throughout the plant kingdom. Here, compatibility is conferred by heterozygosity of incompatibility alleles and has the following relationship to complementation:

Dissimilarity → Complementation → Completion of sexual process → Compatibility

Similarity → Lack of complementation → Sexual process not completed → Incompatibility

In heterogenic incompatibility, on the other hand, compatibility is restricted by dissimilarity of mating genotypes. Complementation requirements are apparently reversed:

Dissimilarity → Lack of complementation → Sexual process not completed → Incompatibility

Similarity → Complementation → Completion of sexual process → Compatibility

Heterogenic incompatibility is generally a secondary development after the primary homogenic incompatibility has broken down to give self-fertility (Homothallism). Such a breakdown may be caused by numerous forms of genetic change which may disrupt complementation at different levels to produce a barrier at any of the many phases in the sexual process. In such cases functional sexuality, but not necessarily self-incompatibility, might be restored by selection for any genes, scattered throughout the genome, with the ability to compensate for specific disturbances, and thus restore essential complementation. These compensatory changes would allow completion of sexual morphogenesis for closely related individuals where these genes would be in the homozygous condition. Crosses between unrelated individuals would produce combinations with non-identical genes at the specific compensatory loci, resulting in failure to complete the sexual sequence. Independent recessive genes restoring fertility or mating competence have been recorded in flowering plants (Edwardson 1970), *Saccharomyces* (Gerlach 1974), *Schizophyllum commune* (C.A. Raper and J.R. Raper 1973) and *Sordaria fimicola* (Carr and Olive 1959).

The Basidiomycete *Sistotrema brinkmannii* exemplifies the present concept of homogenic and heterogenic incompatibility. This species complex exists as an aggregation of morphologically indistinguishable forms possessing different mechanisms controlling sexual morphogenesis. These include homothallism (self-fertility) and bipolar and tetrapolar heterothallism (homogenic incompatibility). Intersterility (heterogenic incompatibility) groups have been delineated within both heterothallic patterns of sexuality (Lemke 1969). Members of any one group are intersterile with members of other groups, but are interfertile with members of the same group provided mates possess differing mating types (Ullrich 1973; Ullrich and Raper 1974). The occurrence of different forms of breeding system in the same species suggests not only derivation of homothallism from heterothallism but also the possible breakdown of homogenic incompatibility and restructuring to give rise to heterogenic incompatibility.

Conclusion

The above hypothesis for the functional organisation and alternative activation in the mating locus of higher plants and fungi is based on the belief that, since all specificity units are derived from a single ancestral unit, it is not surprising, under similar selection pressures and consequent channelling effect (Pandey 1969c), to find parallel properties amongst the incompatibility systems of diverse plants. Logic suggests that the molecular functions of these specificity units should remain analogous and, extrapolating from this concept, that gene complexes controlled by specificity genes are also likely to show parallelism in structural and functional organisation, even though specific auxilary factors may differ.

Underlying this basic theme, but not necessarily dependent upon it, is a particular genetic regulatory mechanism, probably of a wider evolutionary significance (Pandey 1977a), which controls precisely which elements and in what sequence they will be expressed at a specific phase of development. Indeed, the evolution of a regulatory mechanism to control the sequential expression of the complementary elements in the mating complex, irrespective of their physical linkage, is considered fundamental to the evolution of the mating loci in plants. On it depended not only the completion of the complicated sexual process but also the generation of the large number of allelic specificities essential for the efficiency of the breeding system.

The specificity unit itself has become integrated in a "switching" role in the complex, governing, through regulating element(s), the activation of some particular phase in sexual morphogenesis. If specificity requirements are not fulfilled the sexual process halts at a specific point and the remaining complementary processes cannot take place.

The hypothesis is consistent with a large body of data, and throws light on a number of problems concerning incompatibility in both higher plants and fungi.

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