INCOMPATIBILITY IN BASIDIOMYCETES: THE HETEROGENIC PENTAX

R. F. O. KEMP*

A heterogenic system of incompatibility is described in *Coprinus bisporus* which involves two alleles at two loci, in addition to the unifactorial homogenic incompatibility locus already described for this two-spored species. The patterns of non-allelic heterogenic incompatibility found in *C. bisporus* are used to predict those expected in species with bifactorial homogenic incompatibility. This type of heterogenic incompatibility could lead to speciation.

INTRODUCTION

In basidiomycete species with bipolar or tetrapolar homogenic incompatibility it is possible to work out the system of incompatibility by mating the progeny of a single fruit-body. All matings between the basidiospore progeny of different fruit-bodies are expected to be compatible if there are no homogenic incompatibility alleles in common. If there are homogenic alleles in common the modification in the mating pattern is consistent with the homogenic mating-type loci having multiple alleles. However, studies in *Coprinus bisporus* have shown that this is not always true. Unexpected negative results are sometimes obtained which cannot be explained by assuming that the two isolates have a homogenic incompatibility allele in common. In other matings between the spore progeny of different fruit-bodies there are no compatible matings at all. Taken in isolation this latter result would suggest that two biological species were involved – but these results are expected if a species has non-allelic heterogenic incompatibility. In *Coprinus bisporus* and possibly in other basidiomycete species, the traditional homogenic incompatibility system (Raper, 1966) has a heterogenic incompatibility system (Burnett, 1975) superimposed on it. This heterogenic system may form the basis of reproductive isolation which could lead to speciation in basidiomycetes. The existence of several different types of incompatibility in a basidiomycete species will only become apparent if rigorous procedures are followed in determining both the mating reactions of the progeny of a single isolate and also those between the progeny of different isolates.

In a basidiomycete species having homogenic incompatibility a dikaryon can only be formed if the mated strains carry different alleles at one or more incompatibility loci. In a species with bipolar incompatibility the mating A1 × A2 is compatible but A1 × A1 is incompatible. In a tetrapolar species the mating A1 B1 × A2 B2 is compatible but the matings A1 B1 × A1 B1, A1 B1 × A1 B2 and A1 B1 × A2 B1 are incompatible. Homogenic incompatibility occurs when a mating involves two strains which

* 2 Mortonhall Road, Edinburgh EH9 2HW, UK.

have an allele in common (Raper, 1966). Although the homogenic incompatibility system of a species can be worked out by mating the progeny from a single fruit-body, for the detection of heterogenic incompatibility it is necessary to mate the progeny of different dikaryotic isolates. Non-allelic heterogenic incompatibility involves two loci each with two alleles and these will be designated C1/C2 and D1/D2. A mating between two monokaryons which brings together the C2 allele in one monokaryon and the D2 allele of a second strain is designated as being incompatible. A mating between the strains A1 C2 D1 × A1 C1 D1 would be incompatible because of the common homogenic allele, but the mating A1 C2 D1 × A2 C1 D2 would be incompatible because of a reaction between the C2 and D2 heterogenic alleles. Heterogenic incompatibility can only be detected by mating the progeny of two or even three fruit-bodies formed by different dikaryotic isolates, depending on the genotypes involved. The heterogenic system overlies the homogenic one.

In addition, two monokaryons may fail to form a dikaryon if they are homoallelic for an allele which blocks nuclear migration (Kemp, unpublished). With three possible systems of incompatibility in operation it is essential to sample the progeny of each dikaryotic isolate in a precise manner and to follow a rigorous procedure when doing the matings.

METHODS

The homogenic incompatibility system in a species is found by mating monokaryons derived from a single fruit-body. The monokaryons are mated by placing the two inocula 3–5mm apart on the surface of agar medium. After 1–2 days' incubation the plates are examined before the inocula have made contact to check that none is already dikaryotic. After 5–7 days' incubation, small inocula are taken, from the centre and from both sides of the mating, and transferred onto fresh plates which after further incubation can be examined microscopically to distinguish between the characteristic growth forms of the monokaryotic and dikaryotic mycelia. Examination will also show if nuclear migration has occurred. It is essential to do this in a tetrapolar species to distinguish between common-B heterokaryons and true dikaryons which may be found only at the junction-line in certain matings (Kemp, 1980). Blocker monokaryons into which nuclei cannot migrate are not uncommon in many species of *Coprinus* (Kemp, 1974) and *Psathyrella* (Jurand, 1975). A compatible mating between a blocker and a normal strain obtained from a different fruit-body usually results in unilateral nuclear migration, but a mating between two blocker monokaryons, derived from the same fruit-body, may be fully incompatible in *Coprinus bisporus* and *C. congregatus* (Kemp, unpublished) or give a junction-line dikaryon as in *C. sassii* (Kemp, 1974). Several loci of each type could be present in a species. Blocker monokaryons are genetically determined at loci which are distinct from the homogenic and heterogenic incompatibility loci. They should, if possible, be excluded when mating the progeny of different isolates, as their presence

can make the interpretation of mating patterns more difficult. If unilateral nuclear migration is under heterogenic control, as suggested for *Cyathus stercoreus* (Fulton, 1950), then it is even more important to test for nuclear migration, both in sib matings and in matings between different isolates.

Matings between monokaryons derived from two different dikaryotic isolates are made in the same way as described above. Assuming that blocker monokaryons have been excluded a mating table would be expected to consist entirely of compatible matings if the two strains have no homogenic alleles in common. Any common alleles would result in a characteristic pattern of incompatible matings. Matings between monokaryons derived from two dikaryotic isolates of a tetrapolar species are usually done by selecting one monokaryon of each of the four mating-type combinations determined initially in sib matings of 8–10 monokaryons of each dikaryotic isolate. The four monokaryotic strains (A1 B1, A2 B2, A1 B2, A2 B1) from one isolate are then mated with the four representative genotypes from a second isolate. If all 16 matings form dikaryons then the strains have no allele in common. In a bipolar species it is usually safer to isolate two monokaryons of each homogenic mating type. The four monokaryons from each dikaryotic isolate are then mated in all combinations with the four strains from a second isolate.

PROBLEMS OF INTERPRETATION

Table 1a shows the pattern expected, in a bipolar species, when two dikaryotic strains have one allele in common. Table 1b shows that the same result could be formed if heterogenic incompatibility was present. Table 1c has a pattern which cannot be interpreted as homogenic bipolar incompatibility and could be considered an error. By selecting another strain having the A2 allele all matings could be compatible. Table 1d shows that the same result could be formed if heterogenic incompatibility was present.

Table 2 shows mating tables for a tetrapolar species. Although one of each of the four homogenic mating-type allele combinations is selected these four strains would carry the heterogenic alleles at random. If strains carrying the C2 or D2 alleles were under-represented the resulting incompatible matings could be considered as errors. The examples in Tables 1 and 2 show that heterogenic incompatibility could be misclassified as being due to a common homogenic allele, or an apparent error in the expected result could be 'corrected' by choosing another strain with the same homogenic mating-type allele. To interpret these anomalous results with certainty it is necessary to be aware of the basic patterns of heterogenic incompatibility which can occur when matings are done between the various genotypes which are formed by a non-allelic heterogenic system having two alleles at two loci.

To summarize, the term non-allelic heterogenic incompatibility refers to a reaction of a specific allele of one locus with a specific allele of a second locus. For example if the two loci are designated C and D each having two alleles, C1/C2 and D1/D2,

TABLE 1. The interpretation of various bipolar mating tables.

(a) Progeny of A1/A2 × A1/A3

	A	1	1	2	2
A					
1		–	–	+	+
1		–	–	+	+
3		+	+	+	+
3		+	+	+	+

(b) Progeny of A1 C1 D1/A2 C2 D1 × A3 C1 D1/A4 C1 D2

			A	1	1	2	2
			C	2	2	1	1
			D	1	1	1	1
A	C	D					
3	1	2		–	–	+	+
3	1	2		–	–	+	+
4	1	1		+	+	+	+
4	1	1		+	+	+	+

(c) Progeny of A1/A2 × A3/A4

	A	1	1	2	2	2 replacement
A						
3		+	+	+	+	+
3		+	+	–	+	+
4		+	+	–	+	+
4		+	+	+	+	+

(d) Progeny of A1 C1 D1/A2 C2 D1 × A3 C1 D1/A4 C1 D2

			A	1	1	2	2
			C	1	1	2	1
			D	1	1	1	1
A	C	D					
3	1	1		+	+	+	+
3	1	2		+	+	–	+
4	1	2		+	+	–	+
4	1	1		+	+	+	+

then non-allelic heterogenic incompatibility occurs when the opposing monokaryons have the genotypes C2 D1 and C1 D2. Matings between the three viable monokaryotic genotypes (Fig. 1) can form only five dikaryotic heterogenic genotypes (Fig. 2). Matings between the three monokaryotic genotypes and the progeny of the five dikaryons when fruited will give characteristic patterns of incompatibility involving both homogenic and heterogenic incompatibility. It is essential to be able to detect which incompatible reactions might be due to heterogenic incompatibility so that further tests can be made. An overall view of these patterns is essential if heterogenic

TABLE 2. The interpretation of various tetrapolar mating tables.

(a) Progeny of A1 B1/A2 B2 × A3 B1/A4 B3

		A	1	1	2	2
		B	1	2	1	2
A	B					
3	1		−	+	−	+
3	3		+	+	+	+
4	3		+	+	+	+
4	1		−	+	−	+

(b) Progeny of A1 B1 C1 D1/A2 B2 C2 D1 × A3 B3 C1 D1/A4 B4 C1 D2

				A	1	1	2	2
				B	1	2	1	2
				C	2	1	2	1
				D	1	1	1	1
A	B	C	D					
3	3	1	2		−	+	−	+
3	4	1	1		+	+	+	+
4	3	1	1		+	+	+	+
4	4	1	2		−	+	−	+

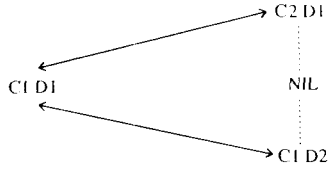


FIG. 1. The mating pattern of the three monokaryotic heterogenic genotypes with the C2 and D2 alleles being incompatible. No homogenic alleles in common.

incompatibility is to be detected when the progenies of just two dikaryotic isolates are mated together.

THE THEORETICAL PATTERN FOR NON-ALLELIC HETEROGENIC INCOMPATIBILITY

In a species with non-allelic heterogenic incompatibility, the C2 × D2 combination of alleles is designated as being incompatible and there are only three viable monokaryotic genotypes: C1 D1, C2 D1 and C1 D2. These will have the mating pattern shown in Fig. 1 if there are no homogenic alleles in common. The matings C1 D1 × C2 D1 and C1 D1 × C1 D2 will behave as expected for strains having just homogenic incompatibility. The mating C2 D1 × C1 D2 will be completely incompatible and if taken in isolation a mating between these two strains would suggest that the isolates belonged to different species. Only if strains of all three genotypes are available for mating tests will there be a reasonable chance of detecting heterogenic

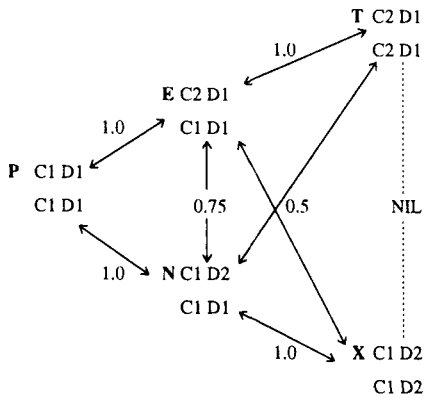


FIG. 2. The mating pattern of the monokaryotic progeny of the five dikaryotic genotypes involving two alleles at the C and D loci. The proportion of compatibility in the matings is at one of the four levels 1.0, 0.75, 0.5 and 0. This assumes that there are no homogenic alleles in common.

incompatibility. It was most fortunate that the first dikaryotic isolates of *Coprinus bisporus* studied in detail were dikaryons having the genotypes C1 D1/C1 D1, C2 D1/C2 D1 and C1 D2/C1 D2 (Kemp, 1980).

Matings between these three monokaryotic genotypes will form five different dikaryotic genotypes with respect to the heterogenic incompatibility alleles. Three of these are homozygous for both the C and D alleles, namely C1 D1/C1 D1, C2 D1/C2 D1 and C1 D2/C1 D2. The remaining two are each heterozygous at one of the two loci, namely C2 D1/C1 D1 and C1 D2/C1 D1. The genotypes of the five dikaryotic strains are shown in Fig. 2 together with the degree of compatibility expected if there is no homogenic mating-type allele in common. For ease of reference these dikaryotic genotypes will be referred to as a 'Pentax' as they are five in number and may form the basis for the evolution of two genetically isolated taxonomic groups. The group letters P, E, N, T and X are assigned to these five genotypes to keep to a minimum the need to list the various dikaryotic genotypes in full. None of these letters overlaps with those used for the homogenic incompatibility loci.

To determine the genotype of each dikaryotic isolate, in the first instance it is necessary to isolate and mate a large enough number of monokaryotic basidiospore progeny to make the detection of heterogenic incompatibility reasonably likely. For example, when the progeny of the P and E group dikaryons are being tested this involves mating samples of the monokaryotic strains formed by the fruiting of the dikaryons with the genotypes C1 D1/C1 D1 and C2 D1/C1 D1. As the monokaryotic isolates also carry homogenic mating-type alleles it is necessary to identify these first. Once this has been done, four (or more) representatives of each homogenic mating type from both fruiting isolates can then be selected for mating together in all combinations. The strains will have the heterogenic alleles represented at random

and it is theoretically possible for the results shown in Figs 6 & 7 and 9 & 10 to contain only compatible matings or only incompatible matings.

Basically there are two methods of detecting heterogenic incompatibility in isolates taken from the wild, and they differ in the way the monokaryotic strains are isolated. The more tedious method involves the isolation of about 30–40 single basidiospore strains from each wild isolate so that 4–5 representatives of each homogenic mating type can be identified by sib matings. In a tetrapolar species this might involve 50 or more matings for each wild isolate so that four strains of each of the four homogenic mating-type classes could be identified by sib matings. Matings between the 16 monokaryotic tester strains of two dikaryotic isolates would therefore involve 256 matings. In a bipolar species fewer matings are required to identify four strains of each homogenic mating type by sib matings and only 64 matings are needed for tests between the progeny of two dikaryotic isolates.

The alternative method involves isolating dikaryotic mycelia from small pieces of the stipe or immature cap. After incubation on a suitable agar medium the monokaryotic components of each dikaryon are then obtained by macerating some of the dikaryotic mycelium and plating the fragments onto agar medium. After incubation, samples from the smallest colonies are transferred onto fresh plates. It is then often possible to identify the two components because of their differing morphologies. No matter how many loci are heterozygous in the wild dikaryon, the two component monokaryons obtained by maceration are bound to contain between them copies of all the alleles present in the original dikaryon. A test for heterogenic incompatibility between the monokaryotic components of two isolates involves only four matings in both bipolar and tetrapolar species. The type of result which might be obtained is shown in Fig. 4. Maceration is perhaps better suited to bipolar species as it is not possible for both components of one dikaryon to be homogenically incompatible with both components of another. In a tetrapolar species it is possible, although unlikely, that the two dikaryotic isolates initially chosen would have the genotypes A1 B1/A2 B2 and A2 B1/A1 B2.

Figure 3 shows the basic pattern of heterogenic incompatibility expected when the monokaryotic components of the five dikaryotic genotypes are mated together in all combinations and there are no common homogenic alleles. An incompatible reaction between the heterogenic alleles C2 and D2 affects only 4 of the 15 possible pairwise matings.

Throughout this paper a mating represented by the notation E × N means a mating between the monokaryotic components or a sample of spore progeny of dikaryon E with the monokaryotic components or a sample of spore progeny of dikaryon N. The four matings which show heterogenic incompatibility are E × N, E × X, N × T and T × X and these include three different patterns. Taken in isolation the T × X mating would appear to involve different biological species. Only the pattern formed by the mating E × N could be formed by the presence of common homogenic alleles. The matings between N × T and E × X show that one monokaryotic component of *one* dikaryon is incompatible with *both* components of a second dikaryon. This

			P		E		N		T		X	
		C	1	1	2	1	1	1	2	2	1	1
		D	1	1	1	1	2	1	1	1	2	2
	C	D										
P	1	1	+	+	+	+	+	+	+	+	+	+
	1	1	+	+	+	+	+	+	+	+	+	+
E	2	1			+	+	−	+	+	+	−	−
	1	1			+	+	+	+	+	+	+	+
N	1	2					+	+	−	−	+	+
	1	1					+	+	+	+	+	+
T	2	1							+	+	−	−
	2	1							+	+	−	−
X	1	2									+	+
	1	2									+	+

FIG. 3. The patterns of incompatibility resulting from mating the monokaryotic components of the five dikaryotic genotypes P, E, N, T and X, with no homogenic mating-type alleles in common.

pattern is not possible with bipolar homogenic incompatibility. In the mating involving the dikaryons T × X there are no compatible matings and this too is not possible in a bipolar species if homogenic incompatibility alone is acting. In a tetrapolar species it might be necessary to check for common-A or common-B heterokaryons in case there were any homogenic alleles in common.

The formation of dikaryons in some matings indicates that the isolates belong to the same biological species. Conversely, a failure to form any dikaryons between monokaryons derived from two different isolates is usually considered to indicate that they belong to different biological species. If isolates belonging to the P, E and N groups are rare compared with those in the T and X groups, none may have been found in the first 50 or so wild isolates tested. Two biological species might be thought to exist because of the exhaustion of the researcher rather than the non-existence of strains in the P, E and N groups whose progeny or components will mate with strains in both the T and X groups. If a representative set of all the biological species in a particular section of a genus is obtained in culture, against which all new isolates are tested, it is essential to mate each new strain with all of the others in case it can form dikaryons with two members of the reference set of isolates which were presumed to belong to distinct biological species.

As matings between the progeny of E × N, E × X and N × T are the only ones which will reveal heterogenic incompatibility, and in all of these the C or D locus is heterozygous, then four or five monokaryotic strains of each homogenic mating type must be used in matings between the basidiospore progeny for there to be a reasonable chance of detecting heterogenic incompatibility. The mating tables in Figs 5–7 show the patterns actually found in *C. bisporus* and those in Figs 8–10 the ones expected in a tetrapolar species. All have been constructed using four monokaryotic representatives of each homogenic mating-type allele. It is theoretically possible, if there are no homogenic alleles in common, for these mating tables to contain only

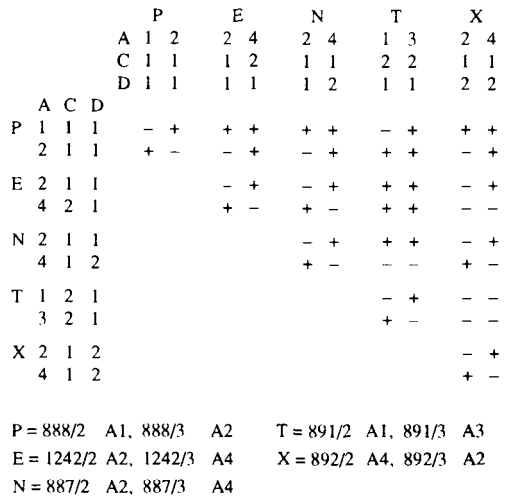


FIG. 4. *Coprinus bisporus*. The patterns of incompatibility resulting from mating the monokaryotic components of representatives of the five dikaryotic genotypes P, E, N, T and X.

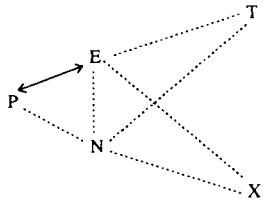
compatible or only incompatible matings if the four representatives of each homogenic allele all contain the same heterogenic allele.

RESULTS WITH *C. BISPORUS*

Figure 4 shows the pattern of incompatibility found in *C. bisporus* using the components of the first representatives of each of the five dikaryotic groups which were identified. The incompatible matings shown in Fig. 4 but not in Fig. 3 are due to there being common homogenic alleles. Only four homogenic alleles have been found in *C. bisporus* in a worldwide sample of 31 dikaryotic isolates (Kemp, unpublished). It is therefore essential to be able to distinguish between the characteristic patterns formed by the two systems of incompatibility.

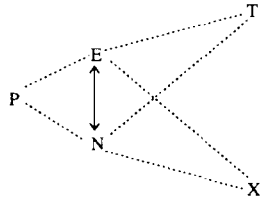
Figure 5 shows the pattern of homogenic incompatibility in *C. bisporus* when monokaryotic strains isolated from basidiospore platings of dikaryons belonging to the P and E groups were mated and when one of the homogenic alleles was common to both strains. Four representatives of each homogenic mating type were selected. The table shows the genotypes for isolates in the P and E groups, and a similar pattern is formed in the following matings depending on the particular homogenic alleles involved: P × T, E × T, P × N, P × X and N × X, as well as in the matings P × P, E × E, and N × N, T × T and X × X. These 11 sets of matings between the progeny of the five 'Pentax' dikaryotic genotypes will identify the homogenic alleles and give no indication that heterogenic incompatibility is present. If there are no homogenic alleles in common all of the matings would be compatible.

Figure 6a shows the pattern of incompatibility when the monokaryotic basidiospore progeny of dikaryotic strains belonging to the groups E and N were mated.



				E Gp	A	2	2	2	2	4	4	4	4
					C	1	1	2	2	1	2	2	2
					D	1	1	1	1	1	1	1	1
P Gp	A	C	D		1	2	3	4	5	6	7	8	
	1	1	1	1	+	+	+	+	+	+	+	+	
	1	1	1	2	+	+	+	+	+	+	+	+	
	1	1	1	3	+	+	+	+	+	+	+	+	
	1	1	1	4	+	+	+	+	+	+	+	+	
	2	1	1	5	–	–	–	–	+	+	+	+	
	2	1	1	6	–	–	–	–	+	+	+	+	
	2	1	1	7	–	–	–	–	+	+	+	+	
	2	1	1	8	–	–	–	–	+	+	+	+	

FIG. 5. *Coprinus bisporus.* The pattern found when mating the progeny of P (888) and E (1242) group isolates, with one homogenic allele in common. Four monokaryons of each homogenic mating type were selected at random. A mating between the progeny of P (888) and E (1268) group isolates with no homogenic alleles in common gives only compatible matings.



(a)

			E Gp	A	2	2	2	2	4	4	4	4
				C	1	1	2	2	1	2	2	2
				D	1	1	1	1	1	1	1	1
N Gp	A	C	D		1	2	3	4	5	6	7	8
	3	1	2	1	+	+	–	–	+	–	–	–
	3	1	1	2	+	+	+	+	+	+	+	+
	3	1	1	3	+	+	+	+	+	+	+	+
	3	1	2	4	+	+	–	–	+	–	–	–
	4	1	1	5	+	+	+	+	–	–	–	–
	4	1	2	6	+	+	–	–	–	–	–	–
	4	1	1	7	+	+	+	+	–	–	–	–
	4	1	2	8	+	+	–	–	–	–	–	–

(b)

			E Gp	A	2	2	2	2	4	4	4	4
				C	1	1	2	2	1	2	2	2
				D	1	1	1	1	1	1	1	1
N Gp	A	C	D		1	2	3	4	5	6	7	8
	2	1	2	1	–	–	–	–	+	–	–	–
	2	1	1	2	–	–	–	–	+	+	+	+
	2	1	1	3	–	–	–	–	+	+	+	+
	2	1	2	4	–	–	–	–	+	–	–	–
	4	1	1	5	+	+	+	+	–	–	–	–
	4	1	2	6	+	+	–	–	–	–	–	–
	4	1	2	7	+	+	–	–	–	–	–	–
	4	1	1	8	+	+	+	+	–	–	–	–

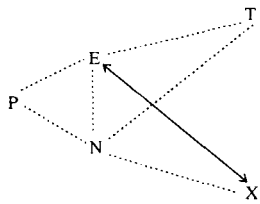
FIG. 6. *Coprinus bisporus.* (a) The pattern found when mating the progeny of E (1242) and N (1228) group isolates with one homogenic mating-type allele in common. The four monokaryons of the E group strain are those used in Fig. 5. (b) The pattern found when mating the progeny of E (1242) and N (887) group isolates with both strains having the same homogenic alleles. Four monokaryons of each homogenic mating type have been selected at random.

The E group strain is heterozygous at the C locus and the N group strain is heterozygous at the D locus. Five of the eight monokaryotic strains of the E group carried the C2 allele and four of the N group had the D2 allele. Fourteen of the 48 matings,

expected to be compatible on the basis of the homogenic alleles, were incompatible. The characteristic feature of these matings is that about half of the columns contain only compatible matings while the others have about half of the matings in each column compatible and half incompatible. When conducting these tests it may be necessary to complete every mating in the table for the pattern of incompatibility to become apparent, especially if the frequencies of the C2 and D2 alleles in the samples are low.

Figure 6b shows the result of mating monokaryotic progeny in the E and N groups when both dikaryons had the same homogenic alleles. In this case, 10 of the 32 matings expected to be compatible, because they have different homogenic alleles, were incompatible. On average the effect of heterogenic incompatibility in matings between E and N group isolates is that a quarter of the matings expected to be compatible are incompatible. With a low frequency of C2 and D2 alleles in the two samples of monokaryons from the two dikaryons the failure of a few matings to form dikaryons could be attributed to experimental error.

Figure 7a shows the pattern of compatibility found when the monokaryotic progeny of the E and X group dikaryons were mated and there is one homogenic allele in common. It is this set of matings which shows the pattern most likely to reveal



(a)

	E Gp	A	2	2	2	2	4	4	4	4
		C	1	1	2	2	1	2	2	2
		D	1	1	1	1	1	1	1	1
X Gp	A C D		1	2	3	4	5	6	7	8
	1 1 2	1	+	+	–	–	+	–	–	–
	1 1 2	2	+	+	–	–	+	–	–	–
	1 1 2	3	+	+	–	–	+	–	–	–
	1 1 2	4	+	+	–	–	+	–	–	–
	4 1 2	5	+	+	–	–	–	–	–	–
	4 1 2	6	+	+	–	–	–	–	–	–
	4 1 2	7	+	+	–	–	–	–	–	–
	4 1 2	8	+	+	–	–	–	–	–	–

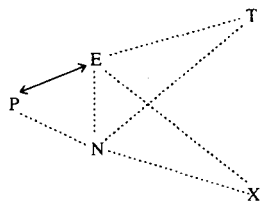
(b)

	E Gp	A	2	2	2	2	4	4	4	4
		C	1	1	2	2	1	2	2	2
		D	1	1	1	1	1	1	1	1
X Gp	A C D		1	2	3	4	5	6	7	8
	2 1 2	1	–	–	–	–	+	–	–	–
	2 1 2	2	–	–	–	–	+	–	–	–
	2 1 2	3	–	–	–	–	+	–	–	–
	2 1 2	4	–	–	–	–	+	–	–	–
	4 1 2	5	+	+	–	–	–	–	–	–
	4 1 2	6	+	+	–	–	–	–	–	–
	4 1 2	7	+	+	–	–	–	–	–	–
	4 1 2	8	+	+	–	–	–	–	–	–

FIG. 7. *Coprinus bisporus*. (a) The pattern found when mating the progeny of E (1242) and X (353) group isolates with one homogenic allele in common. The E group strains are the same as in Figs 5 and 6. Four monokaryons of each homogenic mating type have been selected at random. (b) The pattern found when mating the progeny of E (1242) and X (892) group isolates with both homogenic mating-type alleles in common. The E group strains are the same as those used in Figs 5 and 6.

the presence of heterogenic incompatibility in a species when a small number of strains is initially isolated from the wild. Some of the strains of both homogenic mating types of the E group dikaryon mate with all strains of the X group. By contrast, other strains of the E group isolate mate with none of the X group strains. The expectation is that 50% of the strains of the E group should be compatible with those of the X group. A similar pattern would be expected in matings between the N and T group dikaryons.

Results similar to those shown in Figs 5–7 have been found when 31 wild dikaryotic isolates of *C. bisporus* were tested for homogenic and heterogenic incompatibility (Kemp, unpublished). Preliminary studies have also shown that it is present in *Coprinus trisporus* (Kemp, unpublished).



					E Gp	A	1	1	1	1	2	2	2	2	1	1	1	1	2	2	2	2
						B	1	1	1	1	2	2	2	2	2	2	2	2	1	1	1	1
						C	1	1	2	2	1	2	2	2	1	1	1	2	1	1	2	2
						D	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
P Gp	A	B	C	D		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
	1	1	1	1	1	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	
	1	1	1	1	2	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	
	1	1	1	1	3	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	
	1	1	1	1	4	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	
	2	2	1	1	5	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	
	2	2	1	1	6	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	
	2	2	1	1	7	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	
	2	2	1	1	8	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	
	1	2	1	1	9	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	
	1	2	1	1	10	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	
	1	2	1	1	11	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	
	1	2	1	1	12	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	
	2	1	1	1	13	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–	
	2	1	1	1	14	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–	
	2	1	1	1	15	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–	
	2	1	1	1	16	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–	

FIG. 8. The pattern expected in a tetrapolar species when mating the progeny of P and E group isolates with both dikaryons having the same homogenic mating-type alleles. Four monokaryons of each homogenic mating type were selected at random. When mating, in a tetrapolar species, the progeny of P and E group isolates with no homogenic mating-type alleles in common all matings will be compatible.

RESULTS PREDICTED FOR A TETRAPOLAR SPECIES

Figures 8–10 show the results expected in a similar series of tests using the monokaryotic basidiospore progeny of a species with tetrapolar homogenic incompatibility if four representatives of each homogenic mating type are used. These would involve four times as many matings as in bipolar species. The basic pattern of homogenic incompatibility would be modified in the same manner as in a bipolar species. In the test involving the progeny of E and N group dikaryons it would be possible for the heterogenic incompatibility to be overlooked or explained away as errors of inoculation or recording if every mating was not tested. The advantage of testing the monokaryotic components of the dikaryons obtained by maceration is that the minimum number of mating tests have to be made and there is no chance of missing any heterozygous alleles by sampling too small a number of monokaryotic strains of each homogenic mating type. However, species vary considerably in the ease with which the component monokaryons can be isolated by maceration. Results identical to those shown in Fig. 10a, involving E and X group dikaryons, have been found in matings between two supposedly different four-spored species of *Pleurotus* (Bresinsky et al., 1987). Starting with E and X group dikaryons it should be possible to isolate strains belonging to all five classses of dikaryons forming a 'Pentax' so that a test for the overall 'Pentax' pattern could then be made. Non-allelic heterogenic incompatibility does not seem to be confined to species with two-spored or three-spored basidia. There seems to be no reason why it should be more frequent in species which form dikaryotic spores. The formation of dikaryotic spores must reduce very considerably the amount of gene flow within a population. Non-allelic heterogenic incompatibility results in there being no direct gene flow between the T and X group strains but some gene flow is possible, at least in a four-spored species, depending on the relative frequencies of the P, E and N groups.

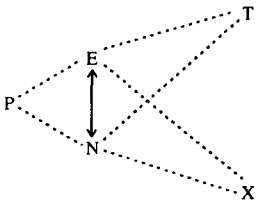
A COMPARISON WITH OTHER SYSTEMS OF INCOMPATIBILITY FOUND IN FUNGI

Now that the basic set of patterns of non-allelic heterogenic incompatibility for *Coprinus bisporus* has been described in detail it is possible to make comparisons with similar systems in ascomycetes and basidiomycetes.

The homogenic system in *C. bisporus* presents few problems, but the existence of only four mating-type alleles in a sample of 62 monokaryons is surprising. The A2 and A4 alleles were found in 21 of the 31 dikaryons isolated from the wild, so incompatible matings due to the pairing of identical homogenic alleles were frequent.

There are three other similar systems of incompatibility found in various species of fungi which may operate when matings are made between different isolates of a species rather than between sibs.

The first is usually known as heterokaryon incompatibility and has been studied in detail in the ascomycete *Neurospora crassa* by Mylyk (1976). This form of incom-



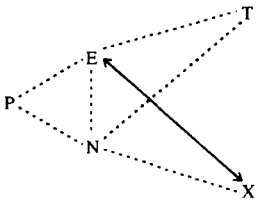
(a)

N Gp	A	B	C	D	E Gp	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	1	1	1	2		1	1	1	1	2	2	2	2	1	1	1	1	2	2	2	2
	1	1	1	2		1	1	1	1	2	2	2	2	2	2	2	2	1	1	1	1
	1	1	2	1		1	1	2	2	1	2	2	2	1	1	1	2	1	1	2	2
	1	1	2	2		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	2		–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
2	1	1	1	1		–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
3	1	1	1	1		–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
4	1	1	1	2		–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
5	2	2	1	1		+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
6	2	2	1	2		+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7	2	2	1	1		+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
8	2	2	1	2		+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
9	1	2	1	1		–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
10	1	2	1	1		–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
11	1	2	1	1		–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
12	1	2	1	2		–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–
13	2	1	1	2		–	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–
14	2	1	1	1		–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
15	2	1	1	2		–	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–
16	2	1	1	2		–	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–

(b)

N Gp	A	B	C	D	E Gp	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	3	3	1	2		1	1	1	1	2	2	2	2	1	1	1	1	2	2	2	2
	3	3	1	1		1	1	1	1	2	2	2	2	2	2	2	2	1	1	1	1
	3	3	1	1		1	1	2	2	1	2	2	2	1	1	1	2	1	1	2	2
	3	3	1	2		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	3	3	1	2		+	+	–	–	+	–	–	–	+	+	+	–	+	+	–	–
2	3	3	1	1		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	3	3	1	1		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	3	3	1	2		+	+	–	–	+	–	–	–	+	+	+	–	+	+	–	–
5	4	4	1	1		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	4	4	1	2		+	+	–	–	+	–	–	–	+	+	+	–	+	+	–	–
7	4	4	1	1		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	4	4	1	2		+	+	–	–	+	–	–	–	+	+	+	–	+	+	–	–
9	3	4	1	1		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	3	4	1	1		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	3	4	1	1		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	3	4	1	2		+	+	–	–	+	–	–	–	+	+	+	–	+	+	–	–
13	4	3	1	2		+	+	–	–	+	–	–	–	+	+	+	–	+	+	–	–
14	4	3	1	1		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	4	3	1	2		+	+	–	–	+	–	–	–	+	+	+	–	+	+	–	–
16	4	3	1	2		+	+	–	–	+	–	–	–	+	+	+	–	+	+	–	–

FIG. 9. (a) The pattern which might be obtained in a tetrapolar species when mating the progeny of E and N group isolates with both dikaryons having the same homogenic mating-type alleles. Four monokaryons of each homogenic mating type have been selected at random. The E group strains are the same as those in Fig. 8. (b) The pattern which might be obtained in a tetrapolar species when mating the progeny of E and N group isolates with no homogenic mating-type alleles in common. The E group strains are the same as those in Figs 8 and 9a.



(a)

X Gp	E Gp	A	B	C	D		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
		1	1	1	1		2	2	2	2	2	2	2	2	1	1	1	1	2	2	2	2
		1	1	1	1		2	2	2	2	2	2	2	2	2	2	2	2	1	1	1	1
		1	1	2	2		1	2	2	2	1	1	1	1	1	1	2	2	1	1	2	2
		1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	3	3	1	2		1	+	+	–	–	+	–	–	–	+	+	+	–	+	+	–	–
	3	3	1	2		2	+	+	–	–	+	–	–	–	+	+	+	–	+	+	–	–
	3	3	1	2		3	+	+	–	–	+	–	–	–	+	+	+	–	+	+	–	–
	3	3	1	2		4	+	+	–	–	+	–	–	–	+	+	+	–	+	+	–	–
	4	4	1	2		5	+	+	–	–	+	–	–	–	+	+	+	–	+	+	–	–
	4	4	1	2		6	+	+	–	–	+	–	–	–	+	+	+	–	+	+	–	–
	4	4	1	2		7	+	+	–	–	+	–	–	–	+	+	+	–	+	+	–	–
	4	4	1	2		8	+	+	–	–	+	–	–	–	+	+	+	–	+	+	–	–
	3	4	1	2		9	+	+	–	–	+	–	–	–	+	+	+	–	+	+	–	–
	3	4	1	2		10	+	+	–	–	+	–	–	–	+	+	+	–	+	+	–	–
	3	4	1	2		11	+	+	–	–	+	–	–	–	+	+	+	–	+	+	–	–
	3	4	1	2		12	+	+	–	–	+	–	–	–	+	+	+	–	+	+	–	–
	4	3	1	2		13	+	+	–	–	+	–	–	–	+	+	+	–	+	+	–	–
	4	3	1	2		14	+	+	–	–	+	–	–	–	+	+	+	–	+	+	–	–
	4	3	1	2		15	+	+	–	–	+	–	–	–	+	+	+	–	+	+	–	–
	4	3	1	2		16	+	+	–	–	+	–	–	–	+	+	+	–	+	+	–	–

(b)

X Gp	E Gp	A	B	C	D		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
		1	1	1	1		2	2	2	2	2	2	2	2	1	1	1	1	2	2	2	2
		1	1	1	1		2	2	2	2	2	2	2	2	2	2	2	2	1	1	1	1
		1	1	2	2		1	2	2	2	1	1	1	1	1	1	2	2	1	1	2	2
		1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1	1	1	2		1	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
	1	1	1	2		2	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
	1	1	1	2		3	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
	1	1	1	2		4	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
	2	2	1	2		5	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	2	1	2		6	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	2	1	2		7	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	2	1	2		8	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	1	2	1	2		9	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–
	1	2	1	2		10	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–
	1	2	1	2		11	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–
	1	2	1	2		12	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–
	2	1	1	2		13	–	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–
	2	1	1	2		14	–	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–
	2	1	1	2		15	–	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–
	2	1	1	2		16	–	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–

FIG. 10. (a) The pattern which might be obtained in a tetrapolar species when mating the progeny of E and X group isolates with no homogenic alleles in common. The E group strains are the same as those used in Figs 8 and 9. (b) The pattern which might be obtained in a tetrapolar species when mating the progeny of E and X group isolates with both dikaryons having the same homogenic mating-type alleles. The E group strains are the same as those used in Figs 8, 9 and 10a.

patibility involves the fusion of vegetative hyphae and is distinct from the incompatibility systems which control the fusion of the nuclei which will ultimately fuse during sexual reproduction. In heterokaryon incompatibility two strains are compatible only if they carry identical alleles at all *het* loci. Ten of these are now known, one of which is the homogenic mating-type locus, A/a. Homozygosity is required at each *het* locus if two strains are to form a heterokaryon. The pairing between the strains *het-c het-D* and *het-c het-D* is compatible but the strain *het-c het-D* is not compatible with *het-c het-d*. Mylyk found that no two isolates from any site were heterokaryon compatible because of the very large number of combinations of alleles of the 10 loci.

The basidiomycete *Heterobasidion annosum* (Fr.) Bref. has unifactorial homogenic incompatibility with numerous alleles. In addition it has heterogenic incompatibility involving five loci which affect the ability of non-sib homokaryotic strains to form dikaryons (Chase & Ullrich, 1990a,b). In this species two homokaryons are compatible if they have different alleles at the homogenic mating-type locus (A) and in addition are homozygous for the (+) allele at any one of the five heterogenic loci. The strain Al $V1^-$ $V2^-$ $V3^-$ S^+ P^- is compatible with the strain A2 $V1^-$ $V2^+$ $V3^-$ S^+ P^- because both strains are S^- but is not compatible with A2 $V1^-$ $V2^-$ $V3^-$ S^- P^+. The initial studies on this species in Finland (Korhonen, 1978) showed that there were two intersterile groups (ISG's) which were called P/Fin and S/Fin. In the United States, Chase & Ullrich also found isolates which could be assigned to these two ISGs. But they also found an S group isolate from Oregon which was compatible with a P group isolate from Vermont. In addition, an isolate from Montana produced monokaryons, half of which were compatible with P/Fin and half not. So starting with the two groups P and S from Finland it was later found necessary to assign some strains to a V group. As a result of further crosses this became VI and V2 and eventually the results could only be explained by having the three loci VI, V2 and V3, in addition to P and S. It was thus possible to explain the results by increasing the complexity of the system, adding one locus at a time rather than pairs of loci as would be needed for the non-allelic heterogenic system found in *C. bisporus*. A mating between the strains $V1^-$ $V2^-$ $V3^-$ S^+ P^- and $V1^-$ $V2^-$ $V3^-$ S^- P^+ was incompatible because neither the S locus nor the P locus was homozygous for the (+) allele. But if the strains were $V1^+$ $V2^-$ $V3^-$ S^+ P^- and $V1^+$ $V2^-$ $V3^-$ S^- P^- then the dikaryon could be formed despite the fact that the S and P loci were both heterozygous. The progeny from a cross of this type was found to contain the four genotypes S^+ P^-, S^- P^+, S^- P^- and S^+ P^+. In the *C. bisporus* 'Pentax' system the four equivalent genotypes cannot occur as it is not possible to form a dikaryon or monokaryon which contains the C2 and D2 alleles.

The 'Pentax' pattern of incompatibility can be found in *Heterobasidion* if a limited number of strains with particular genotypes are chosen. The progeny from the five dikaryotic strains shown in Fig. 11 will have the same mating pattern as the 'Pentax' system of *C. bisporus*.

In *C. bisporus* all strains in the T group are incompatible with all strains in the X group. The reaction between the C2 and D2 alleles is dominant. But in *Heterobasidion*

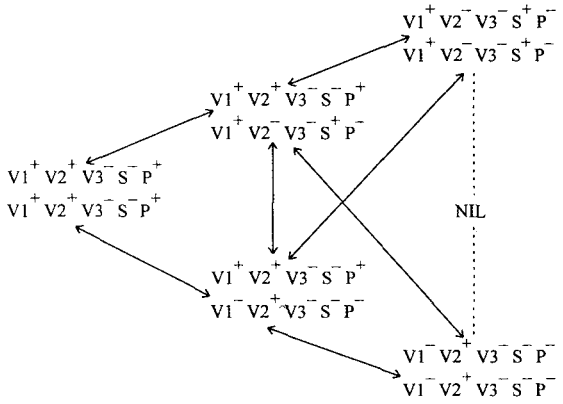


FIG. 11. The 'Pentax' pattern of matings in *Heterobasidion* shown by choosing particular dikaryons.

two strains may be compatible by being homozygous (+/+) at only one of the five loci. The incompatible reaction due to the other four loci is thus marked. Using strains selected at random it is very unlikely that the 'Pentax' pattern would be found in *Heterobasidion*.

In *C. bisporus* the presence of additional pairs of heterogenic incompatibility loci, or loci for other systems, would have to be looked for by mating together all homogenically compatible monokaryotic strains in the P, E and T groups, or similarly by mating the monokaryotic strains in the P, N and X groups. The appearance of distinct patterns of incompatibility not accounted for by an overlap of homogenic alleles would suggest that a second pair of heterogenic loci might be present or some other form of incompatibility could be in operation.

The non-allelic heterogenic incompatibility in *C. bisporus* has similarities with the heterogenic system found in the ascomycete *Podospora anserina* (Esser & Blaich, 1973). In this species sexually mature perithecia can be formed along one side of a mating between homogenically compatible strains if one strain carries the a1 allele at the a/a1 locus and the other has the b allele of the b1/b locus. Fertilization can occur only in the direction a1 to b. There is a similar reaction between c1 and v alleles at a second pair of loci. The mating between the two genotypes a1 b1 and a b is not fully incompatible, as a heterokaryon with the genotype a1 b1/a b can occur and the nuclei fuse and go through meiosis but perithecia are formed on only one side of the mating. This type of unilateral mating is said to be hemi-compatible.

By contrast, the mating between the two homokaryotic strains C1 D2 and C2 D1 in *C. bisporus* is completely incompatible and a dikaryon cannot be formed in either direction. Unilateral nuclear migration is common in many species of *Coprinus* but in no case has it been found to be under heterogenic control. It is always due to the presence in one strain of a Mendelian allele which blocks nuclear migration whatever the genotype of the opposing strain. There are no detailed studies which show that

unilateral nuclear migration in basidiomycetes is heterogenically controlled. Unilateral nuclear migration or hemi-compatible matings are not a specific feature of non-allelic heterogenic incompatibility.

Originally the 'Pentax' pattern of incompatibility in *C. bisporus* was thought to be a form of heterokaryon incompatibility, with a het-0 allele being compatible with both the het-1 and het-2 alleles which were incompatible with each other (Kemp, 1980). A system of this type has not been suggested for any other species, and as non-allelic heterogenic incompatibility gives the pattern found in the 'Pentax' system in *C. bisporus* this latter interpretation is now favoured.

CONCLUSIONS

The interpretation presented suggests that non-allelic heterogenic incompatibility, which overlies homogenic incompatibility, best explains the results found in *C. bisporus*. It also exists in four-spored species of *Pleurotus*, as the initial results of Bresinsky et al. (1987) can be interpreted on this basis. It could indeed exist undetected in other species of basidiomycetes and ascomycetes, as detection of the system requires rigorous sampling and mating methods which are not always practised.

In a species having the 'Pentax' system of heterogenic incompatibility there can be no direct gene flow between the T and X groups. Depending on the relative frequencies of the 'Pentax' genotypes the system could lead to isolation in both sympatric and allopatric situations. Future studies of both the taxonomy and the incompatibility systems of closely related groups of species are needed to see to what extent this system might be associated with speciation in basidiomycetes.

ACKNOWLEDGEMENTS

I thank Dr M. K. Chamberlain (M. K. Jurand) for help in the preparation of the manuscript and Sir John Burnett for his interest and encouragement. I thank Dr T. E. Chase for kindly sending me a copy of a large part of his thesis and the President and officers of the British Mycological Society for a Centenary Award which has enabled this research to be continued.

REFERENCES

BRESINSKY, A., FISCHER, M., MEIXNER, B. & PAULUS, W. (1987). Speciation in *Pleurotus*. *Mycologia* 79: 234–245.

BURNETT, J. H. (1975). *Mycogenetics*. Wiley, London.

CHASE, T. E. & ULLRICH, R. C. (1990a). Genetic basis of biological species in *Heterobasidion annosum*: Mendelian determinants. *Mycologia* 82: 67–72.

CHASE, T. E. & ULLRICH, R. C. (1990b). Five genes determining intersterility in *Heterobasidion annosum*. *Mycologia* 82: 73–81.

ESSER, K. & BLAICH, R. (1973). Heterogenic incompatibility in plants and animals. *Adv. Genetics* 17: 107–152.

FULTON, I. W. (1950). Unilateral nuclear migration and the interactions of haploid mycelia in the fungus *Cyathus stercoreus*. *Proc. Natl Acad. Sci. USA* 36: 306–312.

JURAND, M. K. (1975). Breeding biology of the genus *Psathyrella*. PhD thesis, University of Edinburgh.

KEMP, R. F. O. (1974). Bifactorial incompatibility in the two-spored basidiomycetes *Coprinus sassii* and *C. bilanatus*. *Trans. Brit. Mycol. Soc.* 62: 547–555.

KEMP, R. F. O. (1980). Genetics of A-B-C type heterokaryon incompatibility in *Coprinus bisporus*. *Trans. Brit. Mycol. Soc.* 75: 29–35.

KORHONEN, K. (1978). Intersterility groups of *Heterobasidion annosum*. *Comm. Inst. Forest. Fenn.* 94: 1–25.

MYLYK, O. M. (1976). Heteromorphism for heterokaryon incompatibility genes in natural populations of *Neurospora crassa*. *Genetics* 83: 275–284.

RAPER, J. R. (1966). *Genetics of sexuality in higher fungi*. The Ronald Press Company, New York.

ROYAL BOTANIC GARDEN EDINBURGH

and

UNIVERSITY OF EDINBURGH
Division of Biological Sciences

MSc/Diploma

The Biodiversity and Taxonomy of Plants

Principles, Practice and Management

This one-year course, bridging traditional and modern approaches, equips biologists, conservationists and ecologists with a high level of skill in pure and applied plant taxonomy, a wide knowledge of plant biodiversity and its investigation, combined with instruction in the function, management and funding of taxonomic collections and institutes.

The following major areas are covered:

- Functions and philosophy of taxonomy
- Evolution and biodiversity of plant groups
- Adaptive radiation in angiosperms
- Elements and origins of biodiversity
- Plant geography and biodiversity
- Introduction to selected environments
- Surveys and monitoring
- Expeditions and plant collecting
- Production and use of taxonomic tools
- Handling and processing data by computer
- Taximetrics and cladistics
- Nomenclature of wild and cultivated plants
- Artificial selection, breeding strategies and genetic manipulation
- Tissue culture and micropropagation
- Chemical estimation of variation
- Managing and funding taxonomic institutes
- Herbarium curation
- Taxonomic libraries
- Curation of living collections
- Public services and public relations

There are also workshops or training sessions on cladistics, identification, cytology, computing, numerical taxonomy, molecular methods, herbarium and horticultural techniques, and bibliographic methodology.

A programme of visits to other institutes and fieldwork includes a 10-day field course in Portugal.

MSc students proceed to a 5-month research project in April, leading to submission of a written dissertation in September; Diploma students finish in June following a 2-month project.

Entry requirements: university degree or equivalent in a biological or environmental science; evidence of proficiency in English.

Further details and application forms from: Fiona Curle, Institute of Cell and Molecular Biology, University of Edinburgh, Daniel Rutherford Building, Mayfield Road, Edinburgh EH9 3JH, UK. Tel: 0131 650 5329. Fax: 0131 650 5392.