6 Heterogenic Incompatibility in Fungi

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CONTENTS

I. Introduction

In biology, incompatibility is usually defined as restriction of mating competence controlled by genes other than those determining sexual differentiation. It has been long recognised that incompatibility concerns not only the sexual phase but also the vegetative phase. The latter becomes apparent especially in fungi and was first termed heterokaryon incompatibility. In both sexual and vegetative incompatibility, the action of the genetic traits involved precludes the exchange of genetic material. Thus, inhibition of recombination results by a lack of karyogamy (sexual incompatibility) as well as by an inability of the nuclei to coexist in a common cytoplasm and to undergo somatic recombination (vegetative incompatibility). Since recombination is of paramount importance in evolution, the biological significance of incompatibility as a factor controlling recombination is immediately apparent.

Nature has evolved two principal systems to control incompatibility. According to their mode of genetic determination, these have been called homogenic and heterogenic incompatibility (Esser 1962). The genetic basis of homogenic incompatibility consists in a sexual incompatibility of nuclei carrying identical incompatibility factors. **Heterogenic incompatibility consists in a genetic difference of at least one single gene which inhibits the coexistence of the nuclei concerned in a common cytoplasm.**

From these definitions, it follows that homogenic incompatibility enhances outbreeding and favours recombination and evolution of the species. Heterogenic incompatibility, however, restricts outbreeding and thereby favours the evolution of isolated groups within a single species. Both systems, despite controlling recombination in an antagonistic way, are integrated constituents of evolution.

Homogenic incompatibility has been known since Darwin's time and its various mechanisms have been analysed in great detail, in both higher plants and fungi. Its actions and its distribution are the subject of many books and reviews. This type of incompatibility is described in Freihorst et al. (2016) and Dyer et al. (2016).

The genetics of heterogenic incompatibility was first revealed 60 years ago, in studies involving the ascomycete *Podospora anserina*

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(Rizet and Esser 1953; Esser 1954). Meanwhile, this subject has attracted much attention, and many cases of heterogenic incompatibility, dealing with the vegetative and/or the sexual phases of fungi, have been described and analysed (see Table 6.1).

It is understandable that heterogenic incompatibility was less intensively studied than homogenic incompatibility, since the former rarely occurs within true breeding laboratory strains, but rather between geographical races differing in their genetic constituency. In addition, heterogenic incompatibility has often been overlooked and sometimes misinterpreted as "sterility", merely because of a failure to mate.

This review is a revised and updated version of the chapter on heterogenic incompatibility in the second edition of this volume (Esser 2006).

Other pertinent reviews on heterogenic incompatibility can be found in Esser and Kuenen (1967), Esser (1971), Lemke (1973), Carlile and Gooday (1978), Lane (1981), Esser and Meinhardt (1984), Jennings and Rayner (1984), Perkins and Turner (1988), Glass and Kuldau (1992), Leslie (1993), Bégueret et al. (1994), Leslie and Zeller (1996), Worall (1997), Glass et al. (2000), Saupe (2000) and Glass and Kaneko (2003).

II. Barrage Formation

More than 100 years ago, Reinhardt (1892) first observed that when certain fungal mycelia approached one another, sometimes an interaction phenotypically recognisable as repulsion occurred. This phenomenon was subsequently described by others (Cayley 1923, 1931; Nakata 1925), and it was probably Vandendries (1932) who introduced the term barrage to describe it. Certainly, older descriptions of barrages, based on quite different phenomena, were assigned different names. Since **barrage is a phenotypic expression of heterogenic incompatibility**, it is at first necessary to define the concept of barrage.

If fungal hyphae from different mycelia grow towards each other, in general four main types of interaction occur, and these can be easily demonstrated on agar media.

1. Mutual Intermingling = Normal Contact
   After approach, the hyphae intermingle in the zone of contact and show (with a few exceptions, as in Oomycota) numerous hyphal fusions via anastomosis. After a time, the border zone between the two mycelia becomes unrecognisable (Fig. 6.1a–d).

2. Inhibition
   When opposing hyphae approach each other, an inhibition zone free of hyphae is formed between the two mycelia. This phenomenon may be caused by unilateral or mutual interaction, due to the secretion and diffusion of inhibitory substances.

3. Mutual Intermingling and Inhibition = Barrage Formation
   When two mycelia grow into each other and intermingle, an antagonistic reaction ensues. In contrast to inhibition by diffusible substances, the barrage reaction requires cytoplasmic contact via hyphal fusions. The phenotype of the barrage varies depending on the species and mode of genetic control (Fig. 6.1). However, in all barrages known so far, nuclear exchange is not inhibited, but in most cases the two types of mycelia form abnormal and even lethal fusions. The hyphal tips may branch profusely. A clear line of contact appears with increasing age of the culture. Barrages are mainly found in intraspecific (interracial) matings. Depending on the species, the barrage may be colourless or pigmented (Fig. 6.1a, c). A recent study in *Neurospora* shows that different types of barrages may occur also within one and the same species (Micali and Smith 2003).

4. Mutual Repulsion = Border Line, Demarcation Line
   Especially noted in matings of wood-rotting basidiomycetes, a mutual repulsion and antagonistic reaction is evident which leads to the formation of a more or less strongly pigmented zone of intermingled hyphae. This demarcation line (Adams and Roth 1967) or border line (Esser and Hoffmann 1977) occurs mainly in interspecific matings. It is visible in nature on cuttings of logs (Rayner and Todd 1977; Esser and Meinhardt 1984; Fig. 6.1d) as well as in axenic cultures (Fig. 6.1c). Border lines are often used as criteria for species delineations. However, this commonly creates some problems in interpretation because, in most cases investigated to date, there are no analyses of the microscopic structure of these

Table 6.1 Examples of occurrence of heterogenic incompatibility in fungi, as interpreted from symptoms described by the investigators

Species	Symptoms	References
Oomycota		
* *Phytophthora* spp.	Restriction of mating competence	Savage et al. (1968), Boccas (1981)
P. infestans	Influence of nutrient media on incompatibility	Cherepennikova-Anikina et al. (2002)
Eumycota Glomeromycota		
Glomus mosseae	v-c groups	Giovanetti et al. (2003)
Ascomycetes, saprophytes		
**Ascobolus immersus*	Bipolar; v-c groups; barrage; sexual incompatibility	Meinhardt et al. (1984)
**Aspergillus* spp.	Self-compatible; v-c groups; up to eight *het*-genes	Grindle (1963a, b), Jinks et al. (1966), Butcher (1968, 1969), Butcher et al. (1972), Caten et al. (1971), Dales and Croft (1977, 1990), Croft and Dales (1984)
Aspergillus flavus *A. parasiticus*	i-c groups; morphological and physiological diversities	Horn et al. (1996)
A. tamarii **Aspergillus niger*	i-c groups; virus transfer inhibited Mating-type idiomorphs; up to 11 *het*-genes	Van Diepeningen et al. (1997), Pal (2005), Garnjobst and Wilson (1956), Pittenger and Brawner (1961), Wilson (1961, 1963), Wilson et al. (1961), Pittenger (1964), Wilson and Garnjobst (1966), Moreau and Moruzi (1933), Newmeyer and Taylor (1967), Newmeyer (1968, 1970), Williams and Wilson (1968), Turner et al. (1969), Mylyk (1975, 1976), Leslie (1987), Perkins and Turner (1988), Shiu and Glass (2015)
**Neurospora* spp.		
* *Podospora anserina*	Bipolar; v-c groups; barrage; up to nine *het*-genes; reciprocal and nonreciprocal sexual incompatibility	See Sect. II, this chapter
Ascomycetes, parasites		
* *Botrytis cinerea*	v-c groups; gene *Bc-hch* homolog to *het*-genes of *N. crassa* and *P. anserina*	Fournier et al. (2003)
Cochliobolus heterostrophus	Bipolar; v-c groups; *het*-genes	Leach and Yoder (1983), Nelson (1963, 1965a, b 1966, 1970)
**Cochliobolus* spp. (imperf. *Helminthosporium*)	Heterogenic incompatibility in the sexual phase	Nelson and Kline (1964), Webster and Nelson (1968)
Crumenolopsis soriora	Bipolar; restriction of mating competence between subpopulations	Ennos and Swales (1987)
Diaporthe phaseolorum **Endothia (Cryphonectria) parasitica*	Self-compatible; barrage Bipolar; barrage; v-c groups; six or seven *het*-genes identified, allelic and non-allelic mechanism	Ploetz and Shokes (1986), Anagnostakis (1977,1982a, b, 1983), Nuss and Koltin (1990), Cortesi and Milgroom (1998), Smith et al. (2006)
Erysiphe cichoracearum	Bipolar; sexual incompatibility	Morrison (1960)
Ophiostoma (Ceratocystis ulmi)	Bipolar; barrage	Brasier (1984)
Gaeumannomyces graminis	v-c groups	Jamil et al. (1984)
* *Gibberella fujikuroi* (*Fusarium moniliforme*)	Bipolar; v-c groups; *het*-genes	Kuhlmann (1982), Puhalla and Spieth (1983, 1985), Sidhu (1986), Leslie et al. (2004)

(continued)

Table 6.1 (continued)

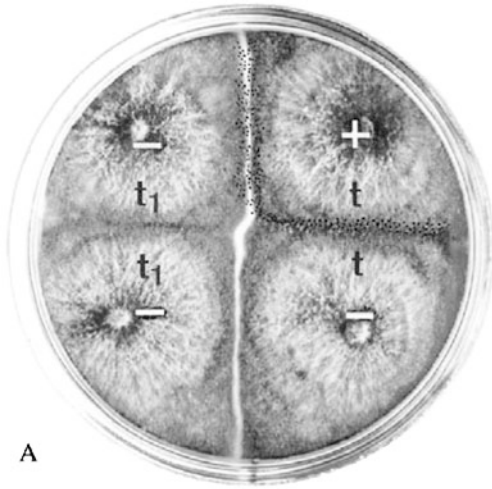
Species	Symptoms	References
Fusarium oxysporum	Imperfect; v-c groups	Correl et al. (1986), Jacobson and Gordon (1988), Katan and Katan (1988)
Fusarium spp.	Sexual and vegetative incompatibility	Hornok (2007)
Leucocytospora kunzei	v-c groups; six *het*-loci	Proffer and Hart (1988)
Monascus purpureus	Self-fertile; six v-c groups	Chaisrisook (2002)
Nannizzia spp. (imperf. *Microsporum)*	Bipolar; heterogenic incompatibility in the sexual phase?	Padhaye and Carmichel (1971)
Sclerotinia sclerotiorum	v-c groups	Kohn et al. (1991), Ford et al. (1995)
Sclerotinium rolfsii (teleomorph: *Athelia rolfsii)*	71 v-c groups	Punja and Sun (2002)
Sclerotinium delphini	Five v-c groups	
Stagonospora nodorum (teleomorph: *Leptosphaeria nodorum*)	v-c groups	Newton et al. (1998)
Venturia inaequalis	Bipolar; nonreciprocal incompatibility between different mating types of geographical races	Kiebacher and Hoffmann (1981)
Verticillium dahliae	Imperfect; v-c groups partially without barrage formation	Puhalla and Hummel (1983), Papaioannou and Typas (2015)
Basidiomycetes (in considering the wood-destroying basidiomycetes in this group, there is no distinction between saprophytes and parasites)		
Auricularia spp.	Tetrapolar; intraspecific i-s groups; barrage formation	Wong (1993)
Agaricus spp.	Border lines between species	Anderson et al. (1984)
Armillaria mellea	Bipolar; i-s groups; partially compatible	Anderson and Ullrich (1979), Anderson et al. (1980), Anderson (1986)
Athelia (*Sclerotium*) *rolfsii*	Bipolar? Barrage; i-s groups	Punja and Grogan (1983a, b)
Bjerkandera fumosa	Bipolar; i-s groups	Lombard et al. (1992)
Ceratobasidium bicorne	Self-fertile; demarcation line; i-s groups	Hietala et al. (2003)
**Collybia dryophila*	Bipolar; i-s groups; reduced sexual compatibility	Vilgalys and Miller (1987), Vilgalys and Johnson (1987)
Collybia subnuda	Bipolar; i-s groups; barrage	Murphy and Miller (1993)
Coprinus bisporus (Coprinellus bisporus)	Bipolar; heterogenic incompatibility sexual phase	Kemp (1989)
* *Coprinus cinereus* (*Coprinopsis cinerea*)	Tetrapolar; barrage, due to heterogenic mitochondrial DNA	May (1988)
Coriolus versicolor (*Trametes versicolor*)	Tetrapolar; i-s groups; barrage	Rayner and Todd (1977)
Cyathus spp.	Tetrapolar; unilateral dikaryotization within two species	Brodie (1970)
Fomes cajanderi	Barrage between geographical races	Adams and Roth (1967)
Ganoderma boninense	Tetrapolar incompatibility; i-s groups; border line	Pilotti et al. (2002), Goh et al. (2014)
Helicobasidium mompa	i-s groups; border line	Ikeda et al. (2003)
**Heterobasidion annosum*	Bipolar; i-s groups; 4–5 *het*-genes; epistatic; multiple alleles	Chase and Ullrich (1990a, b), Hansen et al. (1993a, b)
Heterobasidion insulare	Bipolar; three i-s groups	Dai et al. (2002)

(continued)

Table 6.1 (continued)

Species	Symptoms	References
Inonotus arizonicus	Self-compatible; i-s groups; barrage	Goldstein and Gilbertson (1981)
Laccaria spp.	Bipolar; i-s groups within one and between species	Fries and Mueller (1984)
Lentinula edodes	Tetrapolar; i-s groups; barrage	Yindeeyoungyeon and Triratana (1992)
Marasmiellus parasiticus	Tetrapolar; i-s groups; barrage	Murphy and Miller (1993)
Marasmius spp.	Tetrapolar; i-s groups	Gordon and Petersen (1992)
Paxillus involutus	Bipolar; i-s groups	Fries (1985)
Peniophora spp.	Bipolar; host-dependent intra- and interspecific incompatibility	McKeen (1952)
Phellinus gilvus	Tetrapolar; i-s groups; one or several *het*-genes	Rizzo et al. (1996)
Phellinus pini	Bipolar; i-s groups	Fischer (1994)
Phellinus weirii	Bipolar; barrage; i-s groups	Hansen (1979)
**Phellinus torulosus*	Bipolar; barrage; i-s groups	Fischer and Bresinsky (1992)
Phlebia spp.	Bipolar; intraspecific barrage; interspecific border line; i-s groups	Boddy and Rayner (1983)
Pisolithus arhizus (syn. *P. tinctorius)*	Tetrapolar; unilateral dikaryotization	Kope (1992)
**Pleurotus ostreatus*	Tetrapolar; barrage; i-s groups	Kay and Vilgalys (1992)
**Polyporus* spp.	Interspecific crosses sterile (border line); intraspecific crosses, tetrapolar incompatibility superimposed by heterogenic incompatibility; barrage formation caused by three genes	Macrae (1967), Barrett and Uscuplic (1971), Hoffmann and Esser (1978)
**Sistotrema brinkmannii*	Different breeding systems superimposed by heterogenic incompatibility	Lemke (1969)
**Stereum hirsutum*	Bipolar; compatibility superimposed by multiallelic *het*-genes; barrage	Coates and Rayner (1985a, b, c), Coates et al. (1981, 1985)
Stereum rugosum	Bipolar; i-s groups	Rayner and Turton (1982)
Stereum gausapatum	Bipolar; i-s groups	Boddy and Rayner (1982)
Stereum sanguinolentum	Self-compatible; i-s groups	Rayner and Turton (1982)
Stereum rameale	Self-compatible; i-s groups	Rayner and Turton (1982)
**Thanatephorus cucumeris*	Self-compatible; i-s groups; host-dependent	Stretton and Flentje (1972a, b)
Thanatephorus practicola (Rhizoctonia solani)	Self-compatible; i-s groups; barrage	Cubeta et al. (1993)
Trametes versicolor	Self-compatible i-s groups, barrage	Guler and Bicer (2014)
Typhula spp.	Tetrapolar; interspecific border line	Bruehl et al. (1975)
**Ustilago maydis*	Killer phenomenon	Puhalla (1968), Hankin and Puhalla (1971)

Because of the diversity involved in examples cited, there is risk of misinterpretations. I do not claim to present a complete list of all examples published. There are many papers, especially in basidiomycetes, which only vaguely indicate the existence of heterogenic incompatibility, and this needs to be further investigated. Abbreviations: v-c groups, fungal isolates which show vegetative compatibility, used mostly for ascomycetes; for the same concept in basidiomycetes, the terms intersterility (i-s) groups, biological races and biological species are used; *het*-genes, genes responsible for heterokaryon incompatibility; idiomorphs, mating-type genes showing slight differences of their genetic code; bipolar and tetrapolar, homogenic incompatibility controlled by a mono- and bifactorial mechanism, respectively. All these terms are also defined at appropriate places in the text. Asterisks indicate fungi treated in detail in the text



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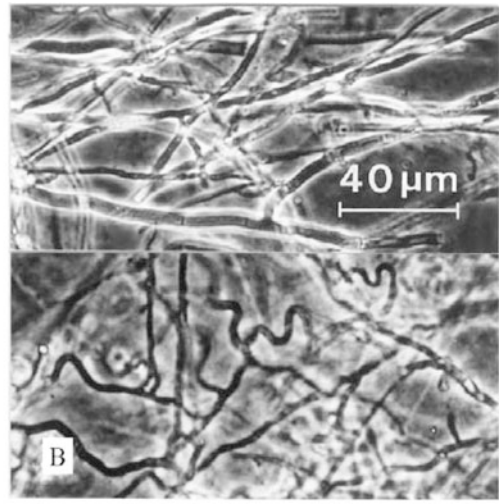

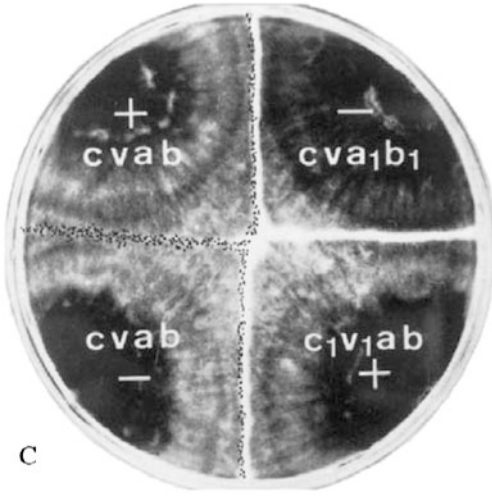

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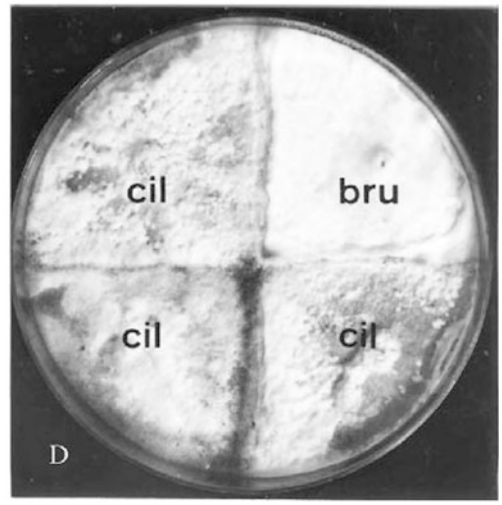

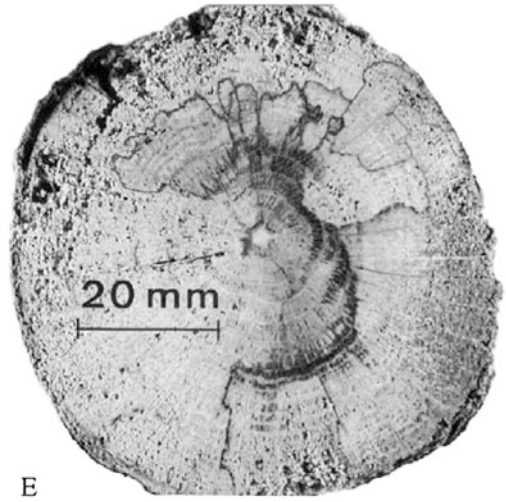

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lines, nor of whether there is hyphal fusion allowing cytoplasmic contact and nuclear exchange. Therefore, it is often rather difficult to evaluate experimental reports with respect to barrage or border line formation and, consequently, to distinguish between intraspecific and interspecific matings.

Accordingly, I shall henceforth use the term barrage only if the microscopic observations show that a zone of aversion occurring between two mycelia is associated with hyphal fusions. I am well aware that this distinction is not always possible on the basis of exact data.

III. Heterogenic Incompatibility in the Ascomycete *Podospora anserina*

A. The Phenomenon

Although barrage formation is found in mycelial interactions with various higher fungi, the best analysed case concerns the ascomycete *Podospora* anserina. As in many ascomycetes, the mating competence of *P. anserina* is controlled by the bipolar mechanism of homogenic incompatibility (heterothallism) due to an interaction of the two idiomorphs + and − of the mating-type locus (see Dyer et al. 2016). In analogy to the *Neurospora* terminology, these alleles were later renamed and termed *mat*+ and *mat*−, respectively.

In studying various races of *P. anserina* with different geographical origins, the mycelia of which showed no recognisable macroscopic differences, Rizet (1952, 1953) found that in interrace combinations a barrage was formed irrespective of mating type. As may be seen from Fig. 6.1a, this barrage is macroscopically characterised by a sharp white zone between two darkly pigmented (melanotic) paired mycelia. A microscopic examination revealed that in this zone the hyphae forming anastomoses become curled, swollen and degenerative (Fig. 6.1b). This barrage zone eventually consists of dead hyphae.

In some cases the barrage formation does not affect fruiting between different mating types, because perithecia are formed on both sides of the barrage (Fig. 6.1a).

This peculiar phenomenon is explained by the fact that the trichogynes of the + female sex organs (protoperithecia) fuse only with the − male gametes (spermatia), and vice versa. Spermatia, however, are never formed in the barrage zone. From this it follows that the trichogynes pass the barrage unimpaired, since no anastomoses involving trichogynes take place. Obviously, the fusion of the tip of the trichogyne with a spermatium does not necessarily bring about the incompatibility reaction occurring between two hyphae, although the reason for this remains obscure.

Nevertheless, as summarised in Fig. 6.2, a comprehensive study of the mating interactions between 19 geographical races revealed that in addition to the barrage formation, the fruit body formation (perithecia) also was quantitatively and/or qualitatively disturbed. In the first instance, the number of perithecia was drastically reduced on one or both sides of the barrage. In the second case, one or both of the reciprocal crosses between the two mates were incompatible. In the 13 (7.6 %) interracial combinations which showed no barrage, there was also no effect on fruiting—in other words, **sexual incompatibility in interrace crosses is always linked with barrage formation.**

In this context, it should be noted that at least one incompatibility mechanism may also

Fig. 6.1 (**a–e**) Compilation of mycelial interactions in fungi. (**a**) *Podospora anserina*. Barrage formation between different geographical races. Sexual reproduction is not affected. Perithecia are produced in any combination between different mating types. (**b**) *Podospora* anserina. Hyphal morphology. *Above hyphae* in the contact zone of an intra-race combination, *below hyphae* within the barrage zone. (**c**) *Podospora anserina*. Barrage formation linked with sexual incompatibility (for details, see Fig. 3 and text). (**d**) Intra- and interspecific interactions between monokaryons of *Polyporus ciliatus (cil)* and *Polyporus brumalis (bru)* showing normal contact *(left)*, and barrage *(bottom)* and border line *(top* and *right)*. All monokaryons are compatible in mating type. (**e**) Cross section of a log colonised by wood-destroying basidiomycetes. *Dark zones* indicate the aversion lines of mycelia. Further details are given at various sites in the text (adapted from Esser 1956, and Esser and Meinhardt 1984)

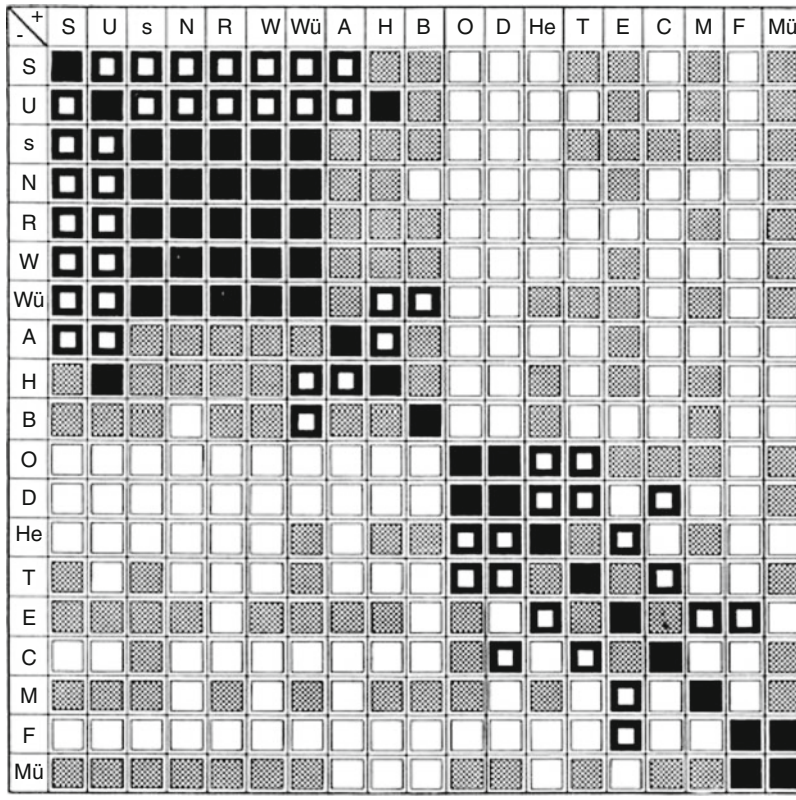

Fig. 6.2 Scheme of the mating reactions between various races of *Podospora anserina* isolated from different localities in France and Germany. +/− Mating type, *uppercase letters* designation of races, *closed squares* compatible in both vegetative and sexual phase, *closed squares with inserted open squares* sexual compatibility and vegetative incompatibility (barrage formation only), *hatched squares* vegetative incompatibility and reduced fruit body formation, *open squares* incompatibility in both vegetative and sexual phase (Adapted from Esser 1971)

lead to distortions in meiotic segregation due to spore killer effects (van der Gaag et al. 2003; Hamann and Osiewacz 2004).

B. Genetic Control

The genetic background of heterogenic incompatibility was revealed by the analysis of the two races *s* and *M* (Rizet and Esser 1953; Esser 1954, 1956). Six loci were identified as instrumental in two different mechanisms, as summarised in Fig. 6.3 and explained below:

1. The **allelic mechanism** caused by alleles of the *t* and *u* loci does not interfere with sexual compatibility. If strains differ at either one (Fig. 6.1a) or both loci, a vegetative incompatibility is provoked, showing up as barrage formation.
2. The **non-allelic mechanism** depends on the interaction of two specific alleles at two different loci. In the interracial cross of *s* and *M*, four loci *a*, *b*, *c*, *v* were identified showing an incompatibility of the alleles a_1/b and c_1/v, respectively, leading to barrage formation as well as to unilateral incompatibility (middle of Fig. 6.1c). If both mechanisms overlap in recombinants from the cross $s \times M$ in the combination a, b, c_1, $v_1 \times a_1$, b_1, c, v, then a complete sexual incompatibility is brought about (Fig. 6.1c, right side).

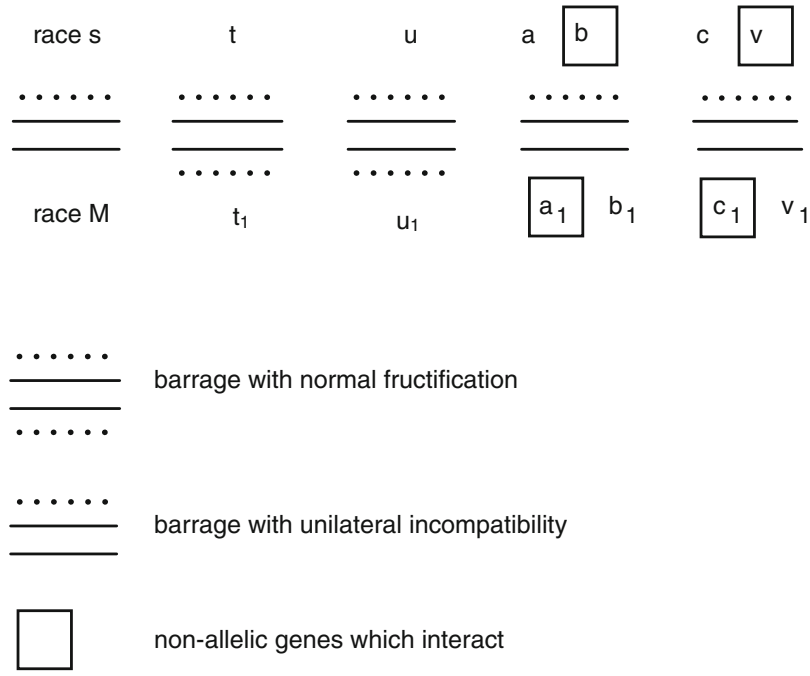

Fig. 6.3 *Podospora anserina*. Scheme of the action of the two mechanisms of heterogenic incompatibility operating in a cross of the races *s* and *M*. The different alleles are symbolised by *lowercase letters*. For further information, see text (Adapted from Blaich and Esser 1970)

C. Physiological Expression

As mentioned above, a prerequisite for the expression of heterogenic incompatibility is that two nuclei showing a specific allelic and/or non-allelic difference are brought together in a common cytoplasm. This occurs very frequently because in *Podospora* as in many other ascomycetes, hyphae fuse by anastomosis when they come into contact. This is followed by a mutual nuclear migration leading to heterokaryosis.

Incompatibility of heterogenic nuclei can be brought about by a unilateral or by a bilateral action. It was found that in those heterokaryons in which the allelic mechanism was effective, a destabilisation took place, leading to a formation of homokaryotic sectors of either nuclear type, separated by barrage formation. In heterokaryons in which the non-allelic mechanism was instrumental, however, no sectoring occurred, because one nuclear species was eliminated. For example, in the combination $ab+a_1b_1$, only the *ab* nuclei survived. Thus, it follows that the allelic mechanism brings about a mutual interaction, whereas the non-allelic mechanism is realised through unilateral gene action (Esser 1956, 1959a, b).

This explains as well the unilateral sexual incompatibility in the non-allelic mechanism present, for instance, in the crossing of strains $ab \times a_1b_1$ (Fig. 6.3). It may be deduced from these heterokaryon experiments that *b* is the "aggressive" allele and that a_1 is its target. Since during the mating procedure in *Podospora* in the differentiated trichogynes the nuclei degenerate in the combination $ab \times a_1b_1$, the active *b*-nuclei are no longer present and there is no inhibition for the sensitive a_1-nucleus, which may thus migrate into the ascogonial cell of the protoperithecium. In the alternate case, the active *b*-nucleus of the spermatium is able to initiate destruction when entering the trichogyne. As in the case of the allelic mechanism, there is also no explanation why the non-allelic mechanism does not become effective during the steps of sexual differentiation and is expressed only when the ascospores germinate, as demonstrated by the assay of heterokaryons.

If as a result of sexual propagation, two *het*-genes come together in one nucleus and in comparable heterokaryons, respectively, they are not viable (a phenomenon found also in other fungi). This has resulted to use in recent publications programmed cell death or apoptosis for heterokaryon incompatibility (Glass and Demnthon 2006; Goncalves and Videira 2014).

These findings were later confirmed by Bernet (1965), who studied the same races *s* and *M*. By analysis of other races, six more incompatibility loci were identified (Bernet 1967; Bourges et al. 1998), one being involved in the allelic and five in the non-allelic mechanism. Unfortunately, Bernet did not use our gene designations. All these genes were later summarised under the heading *het*-genes, e.g. *het-c*. In the following, I shall give the original names of these genes in parentheses.

D. Function of the *het*-Genes

During the last years, many efforts were undertaken to understand the function of the *het*-genes on the molecular and biochemical level.

1. The Non-allelic Mechanism

a) Application of Biochemical Techniques
In heterokaryons, specific proteins (Esser 1959b; Blaich and Esser 1970), catabolic enzymes (Blaich and Esser 1971) and new enzyme activities, such as for several proteases, phenoloxidases, malate and NADH dehydrogenase and amino acid oxidase (Boucherie and Bernet 1978; Boucherie et al. 1981; Paoletti et al. 1998), were found, but their involvement with the function of the *het*-genes was not evident.

b) Mutations Which Interfere with het-Genes
A series of modifier mutants (mod-genes) were found, most of which suppress in specific combinations the barrage formation caused by the allelic or by the non-allelic mechanism. In active combinations, these mutants also suppress the formation of female sex organs (protoperithecia). One of these mod-genes, *mod-E* (*a*), codes for a member of the Hsp90 family of heat-shock proteins acting in various types of stress responses (Loubradou et al. 1997). A second cloned gene, the *mod-D* gene *(v)*, encodes a Gα protein. The gene displays functional interactions with *mod-E* and *Pa AC*, a gene for an adenylate cyclase. Although addition of cyclic AMP can partially suppress growth defects caused by *mod-D* mutations, a molecular connection of cAMP with the effect of *mod-D* mutations on the *het*-genes was not found (Loubradou et al. 1999).

c) Analyses of mRNA
During the incompatibility reaction, a strong decrease of mRNA synthesis and the appearance of a new set of proteins occur. Therefore, the vegetative incompatibility is regulated, at least in part, by variation of the mRNA content of specific genes. Genes induced during the incompatibility reactions have been termed *idi*.

- idi-1 is a cell wall protein and resides in the septum during normal growth (Dementhon et al. 2003).
- idi-4 is abZIP transcription factor regulating autophagy and cell fate and also expression of other *idi*-genes (Dementhon et al. 2004).
- *idi-6/pspA* encodes a vacuolar protease involved in autophagy (Pinan-Lucarré et al. 2003).
- *idi-7* acts in the formation of autophagosomes, vesicles which target cytoplasmic material to the vacuole (Pinan-Lucarré et al. 2003).

Rapamycin treatment of *Podospora anserina* causes *idi*-gene expression and cellular effects typical for heterogenic incompatibility (Dementhon et al. 2003).

d) Molecular Analysis of the *het-c*, *het-d* and *het-e* Genes
Alleles of *het-c* and *het-e* genes correspond to the genes b/b_1 and a/a_1 in Esser's studies. Alleles of the *het-c* locus have similar ORFs but lead to protein products with some amino acid differences (Saupe et al. 1995). The *het-c* gene products are members of a family of ancestral sphingolipid transfer proteins (Mattjus et al. 2003). By inactivation of the *het-c* gene, abnormal ascospores are formed. *het-c* alleles

interact in different ways with the *het-e* and *het-d* alleles (Saupe et al. 1995). Bastiaans et al. (2014) identified 11 *het-c* alleles, which define 7 distinct incompatibility specifities.

Both the *het-d* and the *het-e* genes code for proteins which display a GTP-binding site and a WD40 repeat domain, typical for a β-subunit of a G-protein. Sequence comparison of different *het-e* alleles showed that *het-e* specificity is determined by the sequence of the WD40 domain, which may confer the incompatibility interactions (for references, see Espagne et al. 2002). Physical interactions with the *het-c* proteins need to be demonstrated to clarify this.

2. The Allelic Mechanism

The first *het*-genes of all described are the alleles *het-s* and *het-S* (Rizet 1952). They cause barrage formation by the allelic mechanism but do not interfere with fruit body production. Their co-expression in a common cytoplasm causes cell death. Both alleles encode 30-kd proteins consisting of 289 amino acids. The alleles differ in 43 amino acid positions (Turcq et al. 1990, 1991).

A disruption of either gene resulted in a lack of the 30-kd protein. When mated, these strains no longer formed a barrage. Sexual compatibility was not affected. It was further shown by detailed analyses of the *het-s/S* locus of 13 wild strains that the specificity of the *s* and *S* proteins to provoke heterogenic incompatibility depends on a single amino acid difference only (Deleu et al. 1993).

Strains with the genotype *het-s* exist in two phenotypic states: the neutral phenotype *het-s** and the active phenotype *het-s*. The neutral phenotype is characterised by the fact that it does not show the barrage reaction, when crossed with *het-S* strains. The *het-s* phenotype is infective and is able to transform, via hyphal fusions, a neutral *het-s** strain.

The *het-s* protein which provokes the transformation is a prion which adopts an amyloid structure and propagates in vivo as a self-perpetuating amyloid aggregate (Nazabal et al. 2003; Balguerie et al. 2004). Amyloid structures formed in vitro were shown to be infectious, in contrast to soluble *het-s* protein and amorphous aggregates, supporting the prion nature of the amyloid fibres (Maddelein et al. 2002).

The analysis of deletion constructs and site-directed mutants showed that a short C-terminal peptide (112 amino acids) allows the propagation of the prion analogue (Cousteau et al. 1997). This part of the protein contains the amyloid core regions of the *het-s* prion protein (Balguerie et al. 2003, 2004).

In conclusion: The many studies of heterogenic incompatibility performed with *Podospora anserina* have given deep insights into the genetic mechanism of the *het*-genes and shown many aspects of their action. However, a complete understanding of their function in causing their mutual antagonism still needs further research.

IV. Further Examples of Heterogenic Incompatibility

As one may suppose, the discovery of heterogenic incompatibility was not through focussed research. By contrast, in the ascomycetes it was observed as a "by-product" of genetic research on breeding competence. In the basidiomycetes, as discussed below, data on heterogenic incompatibility are very often a result of studies in population genetics dealing with intergeneric and interspecific delineation and evolution. In the literature, there is a diversity of names, definitions and gene symbols for effects which can be interpreted as manifestations of heterogenic incompatibility. Thus, it is understandable that in describing the antagonistic mycelial interactions and the various groups of natural isolates showing compatibility or incompatibility, different terms and expressions are used (as stated in the legend in Table 6.1), which I consider also as a source of information for a reader who is not familiar with this area of research and who wishes to gain more detailed information. Therefore, I shall discuss only some other cases of heterogenic incompatibility which have been studied in more detail.

Although the **myxozoa** are no longer grouped with the fungi, they have at least to be briefly mentioned, because these organisms have been the subject of intensive studies of heterogenic incompatibility. Vegetative incompatibility is widespread between geographical races of the genera and species analysed to date. In analogy with *Podospora*, plasmodial matings may lead to a visible zone of aversion, which does not allow nuclear migration, because there is either unilateral or mutual disintegration of the nuclei. Collectively from these studies, various genes acting according to the allelic mechanism have been identified. There are no data concerning the physiological actions of these genes.

For pertinent information, the reader is referred to literature on *Didymium iridis* and related species (Betterley and Collins 1984; Clark 1984, 2003), *Physarum polycephalum* (Knowles and Carlile 1978a,b; Lane and Carlile 1979; Schrauwen 1979; Lane 1981), and *Dictyostelium discoideum* (Robson and Williams 1979, 1980).

A. Oomycota

Oomycota have scarcely been used for genetic studies. This may be partially due to the fact that in contrast to ascomycetes and basidiomycetes, they are vegetative diploids, thus complicating genetic analysis of progeny. It is not surprising that our knowledge of genetic control of their breeding systems, and especially evidence for heterogenic incompatibility, is very limited. Furthermore, anastomoses between the coenocytic hyphae having cellulose walls do not occur. The only indication for the existence of heterogenic incompatibility in Oomycota concerns the genus *Phytophthora* (for details, this is referenced in Table 6.1).

B. Glomeromycota

In the recently defined Glomeromycota, heterogenic incompatibility has been reported between isolates of the arbuscular mycorrhizal fungus *Glomus mosseae* (Giovanetti et al. 2003).

C. Dikaryomycota

1. Ascomycotina

This class includes some of the most thoroughly studied saprophytic genera, for example, *Neurospora*, *Aspergillus* and *Podospora*. There are also numerous data proving the existence of heterogenic incompatibility in a great number of parasitic ascomycetes, but detailed genetic data are lacking in many cases.

a) Saprophytic Ascomycetes

Neurospora: Apart from *Podospora*, the genus *Neurospora* is the best analysed taxonomic entity for heterogenic incompatibility in fungi (Table 6.1).

Over 3900 isolates have been collected from nature from over 500 sampling sites. Perkins and his collaborators have classified and assessed this impressive dataset in terms of inter- and intraspecies mating relations (Perkins et al. 1976; Perkins and Turner 1988). The species most studied is *Neurospora crassa*, but the closely related species *Neurospora sitophila* is also used for investigating heterogenic incompatibility.

Heterogenic incompatibility in *Neurospora* has, according to my knowledge, been reported only as **heterokaryon incompatibility** concerning the vegetative phase. It is under polygenic control and involves **several allelic mechanisms**. According to Debets et al. (1994), mitochondrial plasmids might be involved in heterogenic incompatibility.

1. The Mating-Type Locus

   As early as 1933, Moreau and Moruzi reported "cross sterility" between opposite mating types in interracial crosses of *N. sitophila*. A comparable phenomenon of "cross sterility" was also described by Lindegren (1934) for *N. crassa*. In both cases, no genetic analyses were performed. A more substantial indication for the occurrence of heterogenic incompatibility originates from the classical "heterokaryon paper" of Beadle and Coonradt (1944), showing that strains of *N. crassa* must be of the same mating type in order to form a vigorous and stable heterokaryon.

Newmeyer and her collaborators (cf. Table 6.1) proved that the two mating types MAT A and MAT ***a*** are not able to coexist in a common cytoplasm. They found that the gene ***tol*** (linkage group IV), which is not linked with the mating-type locus, suppresses this vegetative incompatibility. ***tol*** does not interfere with the sexual compatibility initiated in an ***A/a*** cross. These authors proposed that the mating-type locus is a complex genetic trait

controlling both heterokaryon formation and sexual compatibility.

The *tol*-gene encodes a putative reading frame for a 1011-amino-acid polypeptide with a coiled-coil domain and a leucine-rich repeat. It is suggested that the TOL-locus may interact with the mating-type proteins MAT *A*-1 and/or MAT *a*-1 to form a death-triggering complex (Shiu and Glass 1999).

The concept of complex mating-type loci was verified by molecular analyses. The MAT *A* gene consists of 5301 bp (Glass et al. 1990), whereas the MAT *a* gene is much smaller and comprises 3225 bp only (Staben and Yanofsky 1990). However, sexual compatibility and heterokaryon incompatibility were found to be inseparable. Thus, a single gene product in both mating types MAT *A* and MAT *a* is responsible for the completion of the sexual cycle and for heterogenic incompatibility in the vegetative phase (Shiu and GlassFdeets 2015). In order to emphasise the rather strong structural dissimilarity of the mating-type alleles, Metzenberg (1990) has proposed to use the term **idiomorphs**, rather than alleles.

The structure of the mating-type alleles in *Podospora anserina* is very similar to those of *Neurospora crassa* (Debuchy and Coppin 1992, Dyer et al. 2016). However, as mentioned above, the mating idiomorphs in *P. anserina* control only homogenic incompatibility.

The involvement of mating loci in heterogenic incompatibility is rather unusual in fungi. It seems to be restricted to some of the self-incompatible *Neurospora* species. Here again, the same question as in *Podospora* is raised: why do the genes responsible for heterokaryon incompatibility not interfere with the overall sexual process? It is not possible at present to answer this question. Maybe there are additional genes which, like the above-mentioned *tol*-gene, are able to act as switches to stop the interaction as soon the nuclei enter the sexual phase.

In this context, the spore killer genes of *Neurospora* should be mentioned (Raju 1979, 2002; Turner and Perkins 1979). These *sk*-genes are widely distributed in wild-type collections. They cause (albeit only in crosses with sensitive strains), after meiotic segregation, lethality of the four ascospores carrying the genes. This phenomenon does not occur in *sk/sk* matings. This observation also strengthens the idea that there are two different, genetically controlled phases in the sexual cycle of fungi: the bringing together of genetic material via cytoplasmic contact and the true sexual cycle leading eventually to recombination.

2. The *het*-Genes
   There are some other genes, apart from the mating idiomorphs, which control heterokaryon formation in *Neurospora*. This was earlier postulated by Gross (1952) and Holloway (1955). A detailed analysis of these so-called *het*-genes was performed by Garnjobst, Wilson and collaborators (cf. Table 6.1). At present, 11 *het*-genes are known.

Two unlinked allelic pairs *(C/c* and *D/d)* were identified which control heterokaryon formation according to the allelic mechanism. Heterokaryons are formed only if the two partners have identical alleles at both loci. If one or both factors are heterogenic, then there will be an incompatibility reaction, as in the barrage zone of *Podospora*, leading to a destruction of the hyphae which have anastomosed. The *C* and *D* genes are neither linked with the mating-type locus nor suppressed by the tol-gene, nor do they interfere with sexual compatibility of different mating types. A third locus *(E/e)* was identified which resembles in its effect the *C* and *D* genes. Subsequently, these genes were given the prefix *het.*

In analysing different geographical races, Perkins (1968) has detected multiple alleles at the *het-C* locus. The similarity of the action of the *het*-genes to that of the *Podospora* barrage genes is also supported by the observation that a clear barrage zone between the two lines of perithecia may be seen when strains of opposite mating types, but heteroallelic for the *het*-genes, meet (Griffith and Rieck 1981; Perkins 1988). Degradation of nuclear DNA indicating a form of programmed cell death has also been visualised (Marek et al. 2003).

Another *het*-gene, but not leading to cell death, was found by Pittenger (cf. Table 1). The allelic pair *I/i* controls the capacity of nuclei to divide. Allele *I* is weakly dominant over allele *i*. When the proportion of *I* nuclei in an (*I*+*i*) heterokaryon is more than 30 %, the *i* nuclei are lost and the heterokaryon becomes an *I* homokaryon. By use of different marker genes, it became evident that this incompatibility is independent of the genetic background and, hence, from the action of the *C*, *D*, *E het*-genes mentioned above.

In recent years, more detailed knowledge about the structure and function of the *het*-genes has accumulated. By deletions within *het*-genes, their action is suppressed and compatibility achieved (Smith et al. 1996). Two *het*-loci were studied in more detail.

The ***het-c* gene** encodes a 966-amino-acid polypeptide with a putative signal peptide, a coiled-coil motif and a C-terminal glycine-rich domain, found also in cell wall proteins. Deletions showed that this region is responsible for the activity of the *het-c* gene (Saupe et al. 1996). *het-c* specificities reside in a 38–48 aa domain at the N-terminal end (Saupe and Glass 1997; Wu and Glass 2001). *Het-c* alleles of *N. crassa* function also in *Podospora anserina* in cell death reaction related to heterokaryon incompatibility, whilst the *P. abserina* homolog *Pahch* causes no heterokaryon incompatibility in its host (Saupe 2000).

Three deletion mutants were identified within an open reading frame (named vib = vegetative incompatibility blocked). These mutants relieved growth inhibition and repression of conidiation caused by the *het-c* gene. Thus, it was suggested that the vib region is a regulator for conidiation (Xiang and Glass 2002). Rather often, these suppressor mutants exhibited chromosome rearrangements (Xiang and Glass 2004).

The ***het-6* gene** maps to a region of 250 kbp. Within this region, two genes were identified which show incompatibility activity. One of these shows sequence similarity to the *het-e* product of *Podospora anserina* and the *tol*-gene product. The other encodes the large subunit of the ribonucleotide reductase. Both genes are inherited as a block. Thus, it was suggested that these genes act through a non-allelic mechanism to cause heterogenic incompatibility (Smith et al. 2000; Mir-Rashed et al. 2000).

Regarding the comprehensive new data obtained for *Neurospora*, like in *Podospora*, a breakthrough in understanding the molecular mechanism of the mutual interaction of the *het*-genes requires still further research.

Aspergillus: Some *Aspergillus* species were also studied for heterogenic incompatibility. The failure of heterokaryon formation between various natural isolates was already described by Gossop et al. (1940) for *Aspergillus niger* and by Raper and Fennell (1953) for *Aspergillus fonsecaeus* (both imperfect). Comprehensive studies with the perfect (teleomorphic) species *Aspergillus nidulans* were performed by Jinks and his coworkers (cf. Table 6.1). A synopsis of these observations and experiments, including numerous related and unrelated isolates from all over the world, allows the following conclusions:

1. Heterokaryon incompatibility is not due to geographical isolation, since the various vegetative compatibility (v-c) groups comprise isolates from adjacent as well as from distant areas. Nor is it linked with minor morphological differences of the isolates.
2. Vegetative incompatibility does not prevent heterokaryon formation. Heterokaryons seem to have selective disadvantage and are supposed to be overgrown by the homokaryons.
3. Fruit body formation for teleomorphic species is not inhibited. However, the number of fruit bodies is reduced, as observed also in *Podospora* (Fig. 6.2).
4. Eight *het*-loci were identified, two of which are multiallelic; *het-B* has four and *het-C* has three alleles. The interaction of the *het*-genes follows the allelic mechanism, as described above for *Podospora* and *Neurospora.*
5. The physiological action of these genes is not yet understood. A "killing reaction" like the one in *Podospora* and *Neurospora* seems not to take place.

Comparable results were obtained with two other teleomorphic species, *A. glaucus* (Jones 1965) and *A. heterothallicus* (Kwon and Raper 1967).

Studies with some anamorphic species (*A. versicolor*, *A. terreus*, *A. amstelodami)* performed by Caten (cf. Table 6.1) in general confirmed the observations made on the teleomorphic species, although without having the opportunity to identify *het*-genes.

A comprehensive study of the species *Aspergillus flavus*, *A. parasiticus* and *A. tamarii* revealed an association of morphology and mycotoxin production with the various i-c groups within each species (Horn et al. 1996).

Ascobolus: In *Ascobolus immersus* (cf. Table 6.1) the mating pattern of 38 strains collected at various places in Europe and southern India has been determined. There were at least three compatibility groups: A (23 strains) and B

(nine strains) comprise the European isolates, and C, the Indian isolates. Within each group sexual reproduction is, as expected, controlled by a bipolar mechanism of homogenic incompatibility. No fertile offspring are obtained in any intergroup crossing, showing that there is genetic separation by heterogenic incompatibility. However, the European group B seems to be more closely related to the Indian group (C) in that sterile fruit bodies are produced between + and − mating types. An indication for further subdivision is the occurrence of barrages between representatives of all three groups. These data thus indicate how speciation may be initiated in *Ascobolus immersus* by means of both spatial and genetic isolation, the latter mediated by heterogenic incompatibility.

b) Parasitic Ascomycetes

For a number of plant pathogenic fungi, heterokaryon tests between different isolates led to barrage or to border line formation and to the classification of the so-called vegetative compatibility groups (**v-c**). Partially due to the difficulty of breeding pathogens under laboratory conditions, genetic data are often not available (see Table 6.1). However, in some of the recently published papers, biochemical techniques such as RAPD analysis were used to characterise the v-c groups, e.g. Punja and Sun (2002).

More comprehensive data are available for *Botrytis cinerea*. It was found that heterokaryon incompatibility in this fungus is caused by the gene Bc-*hch* which is homolog to Nc-*het-c* and the Pa-*hch* loci of *Neurospora crassa* and *Podospora anserina*, respectively. A PCR-RFLP analysis on a 1171-bp section was used to screen for polymorphism for this locus among 117 wild isolates and revealed two allelic types, thus allowing scientists to structure the natural populations into two groups.

For some other parasites, more detailed studies are available. In the case of the chestnut blight, *Cryphonectria (Endothia) parasitica*, different v-c groups characterised by barrage formation of varied intensity were detected. Weak barrages did not inhibit heterokaryon formation. Over 75 v-c groups were identified, controlled by at least seven incompatibility loci, some with multiple alleles. The heterogenic incompatibility followed mostly an allelic mechanism, but a non-allelic mechanism was also observed. Sexual compatibility was not affected by these genes (Choi et al. 2012).

According to Nuss and Koltin (1990), hypovirulence is related to the presence of a virus-like double-stranded RNA which can be transmitted via heterokaryosis. The efficiency of the transfer is highly reduced between incompatible strains and leads therefore to a lack of horizontal transfer of this parasite and contributes to its biocontrol (Milgroom and Cortesi 2004; Smith et al. 2006).

In the *Gibberella fujikuroi* species complex, in addition to the mating-type genes (+/−), mating groups termed A, B, C and D are recognised. They are considered varieties according to their host specificity. Within each group, heterogenic incompatibility was found. This allelic mechanism is controlled by at least 10 loci in group A, five loci in group B and three loci each in both groups C and D.

Within parasitic ascomycetes, there are only two indications for **heterogenic incompatibility which affect the sexual phase**. Both are not linked with heterokaryon incompatibility. The genus *Cochliobolus* includes plant parasites causing leaf and inflorescence diseases in Gramineae (anamorphic: *Helminthosporium)*. Nelson and collaborators have studied extensively the mating system within this genus. A detailed analysis of the mating reactions of nearly 10,000 isolates from North and South America, comprising more than 40,000 matings, has led to the following results:

1. The bipolar mechanism of homogenic incompatibility is responsible for the basic control of mating, insofar as only the combination of the alleles *A* and *a* leads to fructification.
2. Fertility in crosses between opposite mating types originating from different hosts or origin is about 29 %. Most of the infertile crosses produce perithecia with immature or sterile ascospores. The others show no fruit body formation.
3. Several sterility genes blocking the normal ontogenesis at different stages were identified and were predominantly responsible for the formation of sterile perithecia.

4. The fact that some strains exhibiting incompatibility in certain combinations were compatible in all others can be explained only by the action of heterogenic incompatibility, despite the fact that the appropriate genes have not yet been identified.
5. The objection that the incompatibility might be provoked by gross genetic diversities or species differences could be excluded. Furthermore, Nelson was able to assign 92.2 % of his strains to five distinct morphological types which might correspond to a single species.

2. Basidiomycotina

The first phenomena which may be attributed to heterogenic incompatibility came from this group of fungi.

Apart from the description of barrage phenomena between geographical races of *Fomes* species (Mounce 1929; Mounce and Macrae 1938), Bauch (1927) found in the smut fungus, *Microbotryum violaceum (Ustilago violacea)*, that in interracial crosses additional genes interfere with the bipolar mating system and cause unilateral or reciprocal incompatibility. Similar findings were later reported by Grasso (1955) who studied interracial crosses in two other species, *U. avenae* and *U. levis*, originating from Italy and the United States, respectively.

Many phenomena resulting in heterokaryon incompatibility or inhibition of fruit body formation were poorly understood in older publications. They were mostly referred to as demarcation lines, barrages and/or crossing barriers. Thus, it is understandable that in a very comprehensive review (Burnett 1965), the mating restrictions in 17 species of basidiomycetes were treated only under the general heading "restrictions of outbreeding".

In this review, I distinguish between those cases in which the existence of heterogenic incompatibility is proved and supported by genetic data and cases in which antagonistic mycelial interactions may only be interpreted as the expression of heterogenic incompatibility. Only the better-analysed cases will be presented in detail here (for others, see Table 6.1).

In this context, it needs to be stressed that in contrast to ascomycetes where heterogenic incompatibility may concern either the vegetative and/or the sexual phase, in basidiomycetes it is instrumental only in the vegetative phase, because basidiomycetes do not form sex organs. From this it follows that if a heterokaryon incompatibility occurs, then the sexual propagation is automatically inhibited.

An impetus to study heterogenic incompatibility stems from the interest in the population structure of saprophytic basidiomycetes, involving matings between natural isolates in order to obtain information for classification of genera and species (cf. Boidin 1986). These studies have not only revealed basic mating systems but also indicate a variety of antagonistic mycelial interactions correlated with heterokaryon and/or sexual incompatibility.

However, during the last years the interest in heterogenic incompatibility of basidiomycetes seems to have decreased, because there have been fewer publications describing this phenomenon. Instead, many comprehensive studies were published in establishing intra- or interspecific relationships by using molecular techniques. Thus, there is not much progress in understanding the genetic and physiological control of heterogenic incompatibility within this group of fungi.

In **wood-rotting fungi** the antagonistic interaction is easily recognised in cross sections from logs, as a narrow zone of interwoven hyphae in a region of relatively undecayed wood. These **border lines**, also called interaction zones or demarcation lines, are usually darkly pigmented, in contrast to the adjacent decay zones (Fig. 6.1e). They also show up on agar-grown cultures, depending on the composition of the medium. Without microscopic examination, it is not possible to say whether hyphal interactions inhibiting heterokaryon formation take place, as is evident in the barrage zone of *Podospora*, or simply antagonistic repulsions occur. Thus, a distinction between delimitation of species or races is a priori not possible. In the literature, the terms **biological**

races and **intersterility groups** (**i-c**) are often used to characterise the interacting mycelia.

In analogy to the evaluation of data concerning the ascomycetes, I shall start the discussion of the basidiomycetes with a subject for which information on both heterogenic incompatibility and genetic control of speciation has been obtained. This is the wood-rotting fungus *Polyporus*.

Macrae (1967) studied the mating reactions of 31 single spore isolates of the tetrapolar *Polyporus abietinus* (syn. *Hirschioporus abietinus)* collected in different places in North America and Europe. According to differences in the morphology of their hymenial surfaces, the isolates were assigned to three morphological groups. The North American strains could be subdivided into the two classes A and B which are incompatible with each other, but which are both compatible with a third class C comprising the European strains. Since geographical isolation could be excluded, Macrae concluded that genes additional to the mating-type factors were involved. Similar data were also reported for *P. schweinitzii* (Barrett and Uscuplic 1971). Unfortunately, in both cases no genetic data are available.

Comparable phenomena were found and could be interpreted after comprehensive studies of other species of the genus (Hoffmann and Esser 1978). We had chosen the wood-rotting genus *Polyporus* in order to investigate, by genetic parameters, the validity of the classical species concept based on typological characters. In performing these studies, we "accidentally" detected evidence for heterogenic incompatibility.

As a result of matings of single spore-derived mycelia from 26 races of different origin, all races could unequivocally be grouped into three separate entities corresponding with the typological species *P. arcularius*, *P. brumalis* and *P. ciliatus*, on the basis of the following results (Fig. 6.4):

1. As expected, the basic breeding system in *Polyporus* is the tetrapolar mechanism of homogenic incompatibility controlled by multiple alleles of the mating-type factors *A* and *B*.

2. All intraspecific combinations were fertile. A conspicuous barrage formed in those crosses where dikaryotisation and fruiting were impaired. This barrage is characterised by a clear zone, about 1–2 mm wide, free of aerial hyphae, and of reduced hyphal density in the medium (Fig. 6.1d).

3. Using two races of *P. ciliatus* as an example, it was revealed that barrage formation is induced by the specific interaction of three unlinked genes (b^+/b^- = barrage initiation, bfI_1/bfI_2 and $bfII_1/bfII_2$ = barrage formation) in a way characteristic for systems of heterogenic incompatibility. Barrage formation requires the presence of the allele bi^+ in at least one mating partner, in addition to heterogeneity of both *bf*-genes.

4. Interspecific combinations were sterile. There is no hyphal fusion between mating partners, and because of the mutual repulsion, a sharp border line is formed in the area of contact. Its formation is independent of both mating type and the nuclear status (monokaryons or dikaryons) of the confronted mycelia (see also Silveira et al. 2002).

From the experimental data, the following conclusions may be drawn:

1. The analysis of intraspecific matings has shown that within each species, the so-called biological races (intersterility groups, i-s) exist. They are delineated by barrage formation, which, as deduced from the genetic data, is an unequivocal example of heterogenic incompatibility. The unilateral inhibition of fruiting, not caused by the mating-type factors, can also be considered as an expression of heterogenic incompatibility, although genetic data for this are not yet available.
2. The analysis of interspecific matings, all characterised by a strong macroscopic border line (Fig. 6.1d), is in good agreement with the species limits derived from morphological data. This indicates the validity of both the typological and the biological species concept. The latter, however, proved superior in compensating the variability of morphological characters, at least in higher fungi.

The **biological species concept** can thus be modified as follows: **populations (races) belong to different species if the failure to interbreed and to produce viable offspring is caused by**

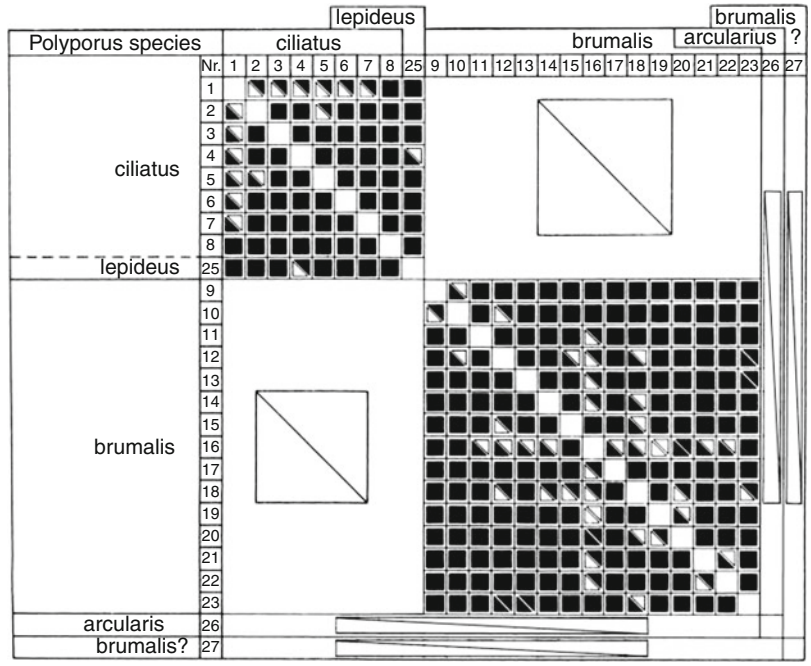

Fig. 6.4 Mating relations in intra- and interspecies combinations of monokaryons from 26 races of different species of *Polyporus*. ■ Normal contact, clamp connections and fruit bodies formed when $A \neq B \neq$; ◥ barrage formation, fruit body production delayed; ◥ barrage formation with unilateral nuclear migration (only in *dark part)*; ◹ *border line*, neither clamp connections nor dikaryotic fruit bodies formed in any combination (Adapted from Esser and Hoffmann 1977)

genetic mechanisms other than those operating upon completion of the sexual cycle.

There are some more examples where genetic control of heterogenic incompatibility is available.

In *Sistotrema brinkmannii* (syn. *Corticium coronilla)*, strains obtained from different geographical locations exhibit three types of basic sexual control:

1. Homokaryotic fruiting, i.e. homokaryons produce dikaryons and fruit bodies with viable spores.
2. Homogenic incompatibility determined by the bipolar mechanism, i.e. one mating-type locus with multiple alleles.
3. Homogenic incompatibility determined by the tetrapolar mechanism, i.e. two incompatibility factors, each with multiple alleles (Biggs 1937).

Lemke (1969) has confirmed and extended the work of Biggs by analysing the interstrain relations of 11 isolates from different parts of the world. In using the technique of forced heterokaryons between auxotrophs, he found that there are fertility barriers within each of the three above-mentioned fruiting classes as well as in interclass crosses. This phenomenon was interpreted by Lemke as heterogenic incompatibility for two reasons:

1. In incompatible interracial matings, the two auxotrophic partners form unbalanced mycelia with poor vegetative vigour and no clamp connections. This points to an antagonistic reaction similar to that in *Podospora* heterokaryons.
2. In one compatible interracial mating, recombinant types were obtained with an altered incompatibility pattern. This excluded the presence of sterility genes, which were sometimes found in other combinations.

In the oyster mushroom, *Pleurotus ostreatus*, 60 heterokaryons of a natural population were examined by pair-

wise matings for mycelial antagonisms (Kay and Vilgalys 1992). Most pairings (93–100 %) between sib-composed heterokaryons gave somatic incompatibility responses, showing that most isolates represent discrete individuals, with as many as 15 individuals occupying a single log. A total of 53 somatically distinct individuals were identified from the population, distributed among 21 logs. Test with homokaryons showed that genetic elements not identical with the incompatibility factors of the tetrapolar system are responsible for the formation of intersterility groups.

In *Stereum hirsutum*, mating is controlled by a bipolar mechanism of homogenic incompatibility (C-factor with multiple alleles). In analysing mating homokaryons from different geographical isolates, a mycelial aversion (bow-tie reaction) was observed. This involved the formation of a migrating or stationary band of suppressed mycelium. This was followed by partial or complete replacement of one homokaryon by another. This reaction is brought about by a heterozygosity at a single locus (B-factor), which is not linked with the mating-type locus. The fact that the heterogenic nuclei reject each other, reminiscent of the barrage formation of *Podospora*, is a further example for heterogenic incompatibility as an isolation mechanism within a single species. In four other species of *Stereum*, as in *Polyporus*, all interspecific matings were sterile and delineated by strong border lines.

In the litter-decomposing bipolar *Collybia dryophila*, collected from different continents, several intersterility groups (i-s) were identified, three of which are distributed over two or more continents. In some matings within one i-c, reduced sexual compatibility was found. Genetic diversity of some strains was proved by DNA-DNA hybridisation.

In the bipolar *Heterobasidion annosum* are at least three intersterility groups, P and S (from pine and spruce) and F (from firs), the representatives of which in general show no compatibility of different mating types. However, there are exceptions, because there is a significant degree of fertility in i-s matings. Five loci were identified controlling this system, superimposed upon the mating-type alleles. In contrast to the results obtained with *Podospora*, for instance, the heterogenic incompatibility between the i-s strains requires a heterogeneity of all five loci. A homogeneity at only one locus acts epistatically and suppresses the mating barrier. This does not exclude that under "fully" heterogenic conditions, interracial incompatibility is present. Comparable data for the *Heterobasidion insulare* complex were reported by Dai et al. (2002).

Hansen et al. (1993a, b) published data which lead to a contradictory interpretation. They suggested that mating between the incompatibility groups is controlled at 3–4 multiallelic loci. Each genotype acts independently in causing vegetative incompatibility. Thus, in accordance with the *Podospora* system, a single genetic difference would be sufficient to cause heterogenic incompatibility.

Perhaps the **control of heterogenic incompatibility is not restricted to nuclear genes.** In the tetrapolar *Coprinus cinereus (Coprinopsis cinerea)*, barrage formation was observed in matings between heterokaryons from different geographical locations having different mitochondrial genomes but common nuclear genomes (May 1988). Unfortunately, no further details of this novel interaction were given.

Yet another example should be mentioned, which is caused by an interaction of nuclear genes and cytoplasmic genetic elements. In the tetrapolar *Ustilago maydis*, an antagonism between genetically different strains, which does not depend on the mating-type genes, was observed which is similar to the killer phenomenon of yeast (cf. Stark et al. 1990). There are three genotypes:

1. Antagonistic strains, producing a heat-labile protein which inhibits the growth of sensitive strains but does not interfere with growth of the producing strain. Genetic configurations: a nuclear gene with the alleles *s* or s^+, and cytoplasmic elements *I* and *S*. The s^+ allele confers insensitivity, the *s* allele sensitivity which is suppressed by the cytoplasmic element *S*; the element *I* is responsible for the production of the killer substance.
2. Sensitive strains, which do not produce inhibitor protein but are sensitive to it. Genetic configuration: gene *s*, but no cytoplasmic element *O*.

3. Neutral strains, which do not produce inhibitor protein and are insensitive to it. Genetic configuration: s^+ *O*, *s S* or s^+ *S*.

There are phenotypic differences to the killer system in yeasts, since only growth inhibition of the sensitive cells occurs with no cell death, and there is no interference with the fusion of different mating types; hence, sexual propagation is not prevented. Thus, this phenomenon reveals similarity to heterogenic vegetative incompatibility in *Neurospora* and *Podospora*. Moreover, in the yeast killer system the genetic determinate for killer protein is a viral-related double-stranded RNA or DNA (cf. Tipper and Bostian 1984 and Stark et al. 1990, respectively).

Conclusion: The evaluation of the experimental data obtained with fungi with respect to the occurrence, distribution and mechanisms of heterogenic incompatibility allows one to make the following statements:

1. The existence of heterogenic incompatibility is unequivocally proved among Dikaryomycota. Although *het*-genes were identified for a number of species, its genetic control certainly needs more experimental investigation.
2. This holds even more true for an understanding of the expression and functions of the *het*-genes.
3. The existence of many v-c and i-s groups in ascomycetes and basidiomycetes, respectively, shows the necessity to support taxonomic classification for speciation by means of comprehensive genetic data, and not just morphological criteria.

V. Correlations with Heterogenic Incompatibility in Plants and Animals, with DNA Restriction in Bacteria and with Histoincompatibility

As reviewed earlier (Esser and Blaich 1973), **in plants** there are also many examples for the existence of heterogenic incompatibility, manifesting as either unilateral or bilateral failures of matings between individuals of different isolates or races. Sometimes the genes responsible for homogenic incompatibility are involved, but mostly the action of other genes is superimposed. In addition, extrachromosomal genetic elements such as plastid-derived DNA have been found as determinative agents (de Nettancourt 1977; Barrett 1992). In plants, according to my knowledge, vegetative incompatibility has not been described.

In comparison to the predominantly hermaphroditic plants, sexual incompatibility of the homogenic type does not play a role in **animal breeding systems**. Increasing in outbreeding is in general achieved in animals by dioecism. There are some examples known in which karyogamy between female and male nuclei is prevented by genetic differences not identical with sex factors (cf. Esser and Blaich 1973).

The spectrum of heterogenic incompatibility comprises not only eukaryotes but also **prokaryotes**. The destruction of bacterial DNA by endonucleases, when brought into a genetically different host and as a defence mechanism to escape phage infection, is also a manifestation of this phenomenon.

Heterogenic incompatibility is not restricted to cell fusion and subsequent nuclear migration, because there is also a close correlation between heterogenic incompatibility and **histoincompatibility**, occurring after tissue transplantation. In the latter case, however, a complicated immune-response mechanism is involved. It seems justifiable to conclude that both heterogenic incompatibility and histoincompatibility, which seem to have convergently developed during evolution, exhibit one and the same effect, i.e. inability of genetically different material to coexist or tolerate a common physiological machinery, and simply represent different mechanisms of a fundamental biological process.

VI. Conclusions

As shown by this survey of the literature, most cases of heterogenic incompatibility can be

supported by genetic data. A number of important special cases are known under different names. In other cases, however, effects indicative of heterogenic incompatibility have been attributed to other causes or relegated by investigators as inexplicable secondary effects. In any case, **heterogenic incompatibility** must be regarded as a **basic biological phenomenon** controlling the coexistence of different genetic determinants, whose impact may be summarised as follows.

1. Occurrence
   Heterogenic incompatibility is widespread in both prokaryotes and eukaryotes. Special cases such as DNA restriction and histoincompatibility may be considered different expressions of one basic biological phenomenon.
2. Nature of Genetic Determinants
   The widespread occurrence is also indicative of the general importance of heterogenic incompatibility, with a varied genetic basis ranging from single to multiple nuclear genes and even to extranuclear genetic elements.
3. Biochemical Basis
   There are not yet sufficient biochemical data regarding the action of the nuclear genes which bring about heterogenic incompatibility in fungi. By contrast, the molecular mechanisms of heterogenic incompatibility provoked by extranuclear genetic traits, such as bacterial DNA restriction, are well known. Evidently, many of the genetic mechanisms leading to heterogenic incompatibility have developed independently, and this may be reflected by a variety of mechanisms at the molecular level.
4. Biological Impact
   The effect of heterogenic incompatibility is threefold:

   (a) As stated in the Introduction, heterogenic incompatibility has to be considered a **breeding system** which, in contrast to homogenic incompatibility, **favours inbreeding** by restricting the exchange of genetic material. Since there is no fundamental difference between recombinational events in the sexual and the parasexual cycle, it is not surprising that heterogenic incompatibility influences both. This also applies to the initiation of plasmogamy, which may occur either by sexual processes or simply through heterokaryosis. This mode of isolating strains or races leads to further speciation. Thus, heterogenic incompatibility must have been and still is one of the **basic genetic events acting in evolution**.

   (b) Genetic isolation has a second effect which should not be overlooked. The suppression of cell fusion **stops the transfer of harmful cytoplasmic components**, such as mutated mitochondria, viruses or plasmids, between individuals and thereby inhibits the spread of cell diseases and favours the survival of uninfected cells or tissues. This is particularly important for organisms without strict cellular compartmentation, such as the majority of fungi.

   (c) **Consequences for taxonomy**: In many studies of natural isolates of fungi, the formation of antagonistic zones of mycelial aversion, such as barrages and/or border lines, is used by taxonomists as criteria for speciation. Although being a valuable tool, this parameter as a taxonomic criterion should be judged with great care to avoid creating taxonomic distinctions which are not valid and which could depend on only a single gene difference. This especially concerns the term "biological species", often used without any genetic basis. It is better to use the terms "race" or "geographical isolate", without a detailed evaluation of breeding patterns.

5. Practical Implications
   During the last decades, concerted breeding for biotechnologically relevant fungi has gained more and more importance (Esser 1985; Esser and Mohr 1990). Breed-

ing techniques employing new isolates from nature in order to exploit varied genetic backgrounds require a profound knowledge of the breeding systems involved. The existence of heterogenic incompatibility could be a serious handicap for any genetic exchange via either sexual or parasexual matings. Detailed experimental work would allow one in most cases to reach the desired goal, if based on alternative genetic manipulations such as DNA-mediated transformation.

6. Relation with Histoincompatibility and DNA Restriction

   Both of these phenomena have the same effect: hostile interaction of different genetic material originating from closely related organisms. This brings up the question: will it be possible in the future, based on further experimental work, to interrelate these events and the many manifestations of heterogenic incompatibility in considering the diversity of the genetic mechanisms promulgating the **failure of coexistence of genetically different material?**

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