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Main features of vegetative
incompatibility in *Neurospora*

Vegetative (heterokaryon) incompatibility in *Neurospora* involves adverse reactions between cells or nuclei of unlike genotype during the vegetative phase of the life cycle. The mating-type locus in *N. crassa* has both sexual and vegetative incompatibility functions. Both A and a mating type alleles have now been cloned (Vollmer and Yanofsky 1986; Glass, Metzenberg, Vollmer, Staben and Yanofsky 1988). Thus it seems useful to review what is already known of vegetative incompatibility, in anticipation of information on its molecular basis. Earlier work has been reviewed in different contexts by Davis 1966, Perkins 1975 and Hartl et al. 1975. The following statements apply to *N. crassa* unless other species are named.

1. Vegetative incompatibility may be manifested in several ways: a) Failure to form stable heterokaryons with continued growth (Garnjobst 1953; Holloway 1955). b) Protoplasmic killing following fusion, observed microscopically (Garnjobst and Wilson 1956). The hyphal segments involved are sealed off and die; incompatible nuclei do not migrate through septal pores into adjoining cells. c) Abnormal growth, morphology, and pigmentation of segmental aneuploids or disomics, when the diploid segment in heterozygous for a het gene (Newmeyer and Taylor 1967; Mylyk 1975). d) A barrage reaction following confrontation of incompatible strains inoculated separately and allowed to grow toward each other (Griffiths 1979; Griffiths and Rieck 1981; Perkins and Raju 1986). The barrage is seen as a clear zone between two rows of perithecia when the confronted strains are of opposite mating type. The barrage reaction is most clearly seen when aconidiate fluffy strains are used on crossing medium.

2. Vegetative incompatibility results when different alleles are present at one (or more than one) vegetative incompatibility locus (designated het: heterokaryon incompatibility). Identity of alleles at all het loci is required for vegetative compatibility

3. Vegetative incompatibility genes (het genes) have been identified and mapped at ten loci in five linkage groups. In addition, the mating type alleles A and a function as het genes during the vegetative phase of the life cycle. Numerous other het loci are inferred to be present (see Mylyk 1976; Perkins et al. 1982).

4. Sexual and vegetative incompatibility functions of the mating type (mt) alleles A and a have not been separated by recombination (Newmeyer, Howe and Galeazzi 1973). Both functions are usually lost together by null mutation, and both revert together. (Two exceptional mating-type mutations have been obtained, where the vegetative function has been lost while mating function is retained (Griffiths and DeLange 1978; Griffiths 1982)). The vegetative function of the mating type alleles, but not the sexual function, is suppressed by an unlinked recessive gene, tol (Newmeyer 1970). Expression of het-c and het-e is not suppressed by tol. (Suppression of genes at other het loci has not been tested.) Independent occurrences of tol have been found in wild strains and have arisen by mutation in the laboratory (see Perkins et al. 1982).

5. Vegetative incompatibility appears to be a necessary condition for a barrage reaction to occur when two strains of opposite mating type confront one another. If mating-type associated incompatibility is suppressed (by an unlinked suppressor, tol) or is absent (if the mutant a^{m1} allele is used), no barrage is seen: only a single row of perithecia if formed. But if different alleles are introduced at another vegetative incompatibility locus, the barrage occurs even though mating type alleles are not longer het-incompatible, in A x a^{m1} or A;tol x a;tol (shown for het-c x het-C by F.J. Doe, unpublished).

6. Vegetative incompatibility of mating type alleles is expressed in *N. crassa* and *N. intermedia* (Turner and Perkins 1979) but not in *N. tetrasperma*, *N. sitophila* (Whitehouse strains - Mishra 1971), or *N. discreta* (Perkins and Raju 1986). However, the mating type genes of *N. tetrasperma* and *N. sitophila* gain the ability to act as vegetative incompatibility genes when they are introgressed into *N. crassa* (Metzenberg and Ahlgren 1973; Perkins 1977). Suppressors similar to tol may be present in the strains that were tested of *N. tetrasperma*, *N. sitophila* and *N. discreta*.

7. Differences at het loci do not result in a protoplasmic incompatibility reaction during the sexual phase, from trichogyne to ascospore, and do not impair fertility. (Fertility may be enhanced.) It should be emphasized that vegetative incompatibility between partners in *Neurospora* is not a prerequisite for entering or completing the sexual cycle. Vegetative incompatibility does not in any way impede initiation of the *Neurospora* sexual phase, prevent fertilization, or preclude completion of meiosis and ascospore formation. Pairs of strains that are of opposite mating type are fully fertile when crossed, even though they are highly incompatible vegetatively, with differences at many het loci. In this respect, *Neurospora* is diametrically different from basidiomycetes, where two partners must be vegetatively as well as sexually compatible in order to form the dikaryon that is essential for fruiting and completing the sexual phase.

8. Natural populations are highly polymorphic for het genes at various loci so that heterokaryon formation is effectively excluded even between related isolates from the same collection site (Mylyk 1976, using tests for five het loci).

9. When disomic or duplication strains are heterozygous for het alleles, growth rate and morphology are abnormal. The degree and type of impairment differ, depending on what het-genes are involved (see Fig. 3 of Mylyk 1976; Newmeyer and Taylor 1967). Brown pigment is produced by such heterozygotes in the presence of tyrosine + phenylalanine (or casein hydrolysate). There is no pigment on most media without tyrosine and phenylalanine, but the abnormal morphology persists (Perkins 1972, 1975).

10. Multiple allelism at het loci has neither been proved nor excluded. Some of the results originally attributed to multiple alleles of het-c are due instead to separate, linked het loci (Perkins 1975).

11. No example has been described in *Neurospora* where major vegetative incompatibility effects require differences at two loci. The well studied cases involve alleles at single loci. However, the vegetative incompatibility function of the mating type alleles can be suppressed by the unlinked recessive gene, tol.

12. Genetic differences of mutational origin can affect the vigor and speed of formation of heterokaryons without completely preventing heterokaryosis between complementing partners. Heterozygosity at several loci can contribute, with marginal effects that are cumulative (de Serres 1962). Microscopic observations suggest the presence in different strains of inherited differences in hyphal fusion, attraction, or avoidance (Wilson and Garnjobst 1966; J. Wilson, D. Galeazzi, O. Mylyk, unpublished).

13. Differences at the i (het-i) locus do not produce protoplasmic killing but they can result in the elimination of one nuclear component from I+i heterokaryons during growth (Pittenger and Brawner 1961).

14. Injection of extract from het-incompatible but not from het-compatible strains results in protoplasmic killing resembling that from incompatible fusions (shown with het-c and (het-d). Extracts from a different species invariably kill when injected. Ability of a het-c het-d extract to kill is destroyed by proteases but not by nucleases (Wilson et al. 1961; Williams and Wilson 1966).

15. Results incidental to one study indicate that the vegetative incompatibility function of A and a is not expressed in a heterokaryon where one of the nuclear components is of the slime genotype, which is incapable of forming a cell wall (see Nelson et al. 1975). This observation should be confirmed. There is no effect on intact protoplasts of a suspension containing incompatible extract that would kill if injected (Williams and Wilson 1966).

16. Systems of vegetative (somatic) incompatibility are found also in other fungi, in protista, and in plants (reviewed by Lane 1981; Knox and Clarke 1980).