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Abstract

It has never been determined in Neurospora whether multiple alleles exist at individual het loci such that interaction between any two unlike alleles will result in an incompatibility reaction. The evidence summarized here from recombination genetics and from sampling natural populations suggests multiple allelism at two of the best studied het loci. However, an alternate explanation is not excluded that invokes linked multiple loci rather than multiple alleles.

Putative multiple alleles at the vegetative (heterokaryon) incompatibility loci het-c and het-8 in Neurospora crassa.

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It has never been determined in Neurospora whether multiple alleles exist at individual het loci such that interaction between any two unlike alleles will result in an incompatibility reaction. The evidence summarized here from recombination genetics and from sampling natural populations suggests multiple allelism at two of the best studied het loci. However, an alternate explanation is not excluded that invokes linked multiple loci rather than multiple alleles.

A genotypic het difference may be manifested phenotypically not only by failure to form stable heterokaryons between haploid strains with unlike alleles but also by typical abnormal growth and morphology of partial diploid strains containing duplications that are heterozygous for het alleles (reviewed by Perkins 1988 Fungal Genet. Newsl. 35:44- 46). The rationale and methodology for using partial diploids to study het genes are summarized by Perkins, Leslie, and Jacobson (1993 Fungal Genet. Newsl. 40). Partial diploids of defined extent and gene content can be obtained as recombinant progeny from crosses heterozygous for insertional or terminal translocations (see Perkins and Barry 1977 Adv. Genet. 19:133-285). The partial-diploid technique enables putative multiple alleles of het genes to be identified readily because a visible incompatibility phenotype signals that unlike alleles are heterozygous.

The het-c locus is included in a segment of linkage group IIL that is diploid in duplication progeny from crosses of the terminal translocation T(IIL->VR)NM149 Normal sequence. (The translocation symbol will be abbreviated as T(NM149).) When numerous wild strains were tested by crossing them with both T(NM149) het-C and T(NM149) het-c testers, certain strains were anomalous in their behavior, producing a typical incompatibility phenotype in progeny of both test crosses (Perkins 1972 Neurospora Newsl. 19:27-28). The results could be explained either by multiple het-c alleles or by the presence of two different alleles at another het locus within the T(NM149) duplication. New translocations were subsequently discovered which generated IIL duplications that were shorter than those from T(NM149) and that did not contain the het-c locus. These translocations, P2869 and AR18, revealed the presence in IIL of a second locus designated het-6 (Mylyk 1975 Genetics 80:107-124), located left of het-c (Figure 1). The anomalous incompatibility behavior of several strains could be attributed to heterozygosity at het-6, making it unnecessary to invoke multiple het-c alleles. For example, Groveland-1c a (FGSC 1945), Panama A (FGSC 1731), Costa Rica A (FGSC 851), Marrero-1d a (FGSC 2224), and Mauriceville-1c A (FGSC 2225) (Table 2 of Perkins 1975 Genetics 80:87-105) all differed from the OR wild type at het-6. However, this explanation was not adequate for all strains with anomalous incompatibility behavior. Adiopodoume A (FGSC 430) provides the best analyzed example. This strain was shown to be het-6OR by crosses with a T(AR18) tester. When

Adiopodoume was crossed to testers het-6OR het-C T(NM149) and het-6OR het-c T(NM149), the resulting duplication progeny displayed an incompatible phenotype regardless of whether the T(NM149) laboratory tester was het-C or het-c. It appeared that Adiopodoume either carried a third het-c allele ("het-cAD"), or differed from the laboratory testers at yet a third het locus in IIL ("het-x").

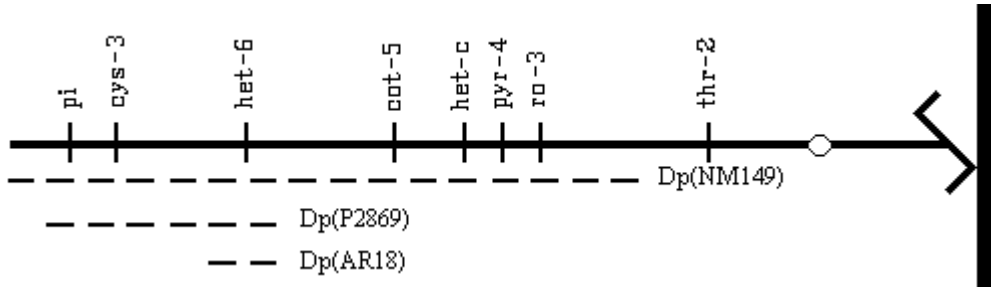

Figure 1. Map of linkage group IIL showing the most likely order based on previous data. Only those gene loci and translocation breakpoints are shown that are relevant to analysis of het-c and het-6. Interval lengths are not necessarily to scale. The dashed lines show the extent of segments duplicated in partial diploid progeny of crosses heterozygous for each of the translocations.

Conceivably, the anomalous result might be explained by frequent nondisjunction of a het-gene located outside the T(NM149) duplication. For example, the het-d locus is in the opposite arm at the far right end of linkage group II. If the het-d allele in the Adiopodoume strain differed from that in both the het-C and het-c T(NM149) testers, and if 3:1 segregation occurred frequently from the translocation quadrivalent in crosses heterozygous for T(NM149), then progeny might be obtained that were inhibited because they were D/d heterozygotes or (D+d) heterokaryons.

Evidence from recombination

In the present study, markers bracketing het-c were employed to test the hypothesis which states that the Adiopodoume strain differs from the tester strains (and from labora-tory wild types) at het-x, a separate locus linked to het-c. Progeny recombinant for the flanking markers were scored for het-incompatibility by progeny- tests, using three T(NM149) translocation testers containing either het-C or het-c or their Adiopodoume counterpart "het-cAD". Multiple allelism would be disproved if a fourth apparent "allele" was produced when crossing over occurred to the left or right of het-c. If the apparent fourth allele proved to be incompatible with all three previous "alleles", the simplest explanation would be existence of another locus, het-x. If the Adiopodoume genotype were het-xAD het-c, the four underlying genotypes would be het-xAD het-C, het- xAD het-c, het-xOR het-C, and het-xOR het-c.

In testing the two-locus null hypothesis, it must be recognized that we do not know whether the hypothetical het-xAD is het-C or het-c. A critical test would therefore require that the Adiopodoume strain be crossed both with het-C and with het-c in the presence of flanking markers. From both of these crosses, it would be necessary to determine the het-genotype of progeny that had undergone crossing over of flankers, using T(NM149) testers of all three genotypes het-C, het-c, and "het-cAD". This exhaustive series has not been completed, but crosses that have been made are nevertheless informative.

Crossovers in the region between flankers (cot-5 and pyr-4) failed to reveal any nonparental het genotype in crosses of het-C Adiopodoume (carried out by B.J.H.). All progeny were compatible with one or the other parent. The data are as follows. In a cross of het-C pyr-4 thr-2 Adiopodoume, 14 pyr-4 thr-2+ recombinants were progeny tested by crossing to both T(NM149) het-C and T(NM149) het-c. All were het-C, showing that the Adiopodoume factor is either left of pyr-4 or close to it. This result seems to eliminate the right-arm locus het-d from consideration.

In a normal-sequence cross of cot-5 het-C pyr-4 thr-2 Adiopodoume, seven cot- 5+ pyr-4 thr-2 recombinants (in a total of 150 cot+ progeny) were scored for het- compatibility by progeny testing. Six were het-C, giving inhibited duplication progeny when crossed with the T(NM149) het-c tester but not when crossed with T(NM149) het- C; one was like the Adiopodoume parent, giving inhibited duplication progeny with both het-c and het-C T(NM149) testers but giving no inhibited duplications among 141 progeny of a test cross with a T(NM149) tester of Adiopodoume het genotype. On the multiple allele hypothesis, the recombinant genotype was cot+ het-cAD pyr-4 thr-2 and it resulted from a single crossover in the region between het-c and pyr-4. On the two-locus hypothesis, however, the interpretation is uncertain because the hypothetical genotype of the Adiopodoume parent could be either het-xAD het-C or het-xAD het-c. The marked parent of the original cross was het-C. If the Adiopodoume parent was also het-C (i.e., of genotype het-xAD het-C rather than het-xAD het-c), the cross could provide no information about het-x - het-c recombination. A complementary cross, cot-5 het-c pyr-4 thr-2 Adiopodoume, would be required before firm conclusions could be drawn.

While the present data cannot disprove the two-locus model, close linkage of both het-c and het-cAD to pyr-4 seems to favor the multiple-allele hypothesis. A rough estimate of marker distances is pi-4 (4) cys-3 (15) cot-5 (3) het-c (2) pyr-4 (1) ro-3 (12) thr-2. Evidence for location of het-c just left of pyr-4 was provided by a cross of cot-5 het-C pyr-4 thr-2 het-C. Seven progeny in 149 were crossovers between cot-5 and pyr- 4. These consisted of 1 cot-5+ het-c pyr-4 thr-2, 1 cot-5 het-C pyr-4+ thr-2+, and 5 cot-5 het-c pyr+ thr-2+. One crossover progeny from another cross, pi het-C pyr-4 thr-2 T(NM149), proved to be pi+ het-c pyr-4 thr-2 normal sequence, again consistent with location of het-c left of pyr-4. Two complementary crossovers between pyr-4 and ro-3 were obtained among 236 progeny from het-C pyr-4 ro-3 het-c. The pyr-4+ ro-3 crossover was het-c, and the pyr-4 ro-3+ was het-C, showing that the het-c locus is either very close to pyr-4 or left of it.

For the Adiopodoume factor, the one het-cAD - pyr-4 recombinant among seven crossovers in the short cot-5 pyr-4 interval indicated that it too is located left of pyr-4. Close linkage of the Adiopodoume factor to pyr-4 is also indicated by the absence of recombina-tion among 14 progeny that had undergone crossing over in the pyr-4 - thr-2 interval right of pyr-4. Thus, the Adiopodoume factor cannot be far from het-c, regardless of which hypo-thesis is correct, and multiple allelism remains a likely explan- ation. The alternative would be another het locus tightly linked to het-c. This in itself would be of significant interest.

Genetic analysis has now defined the problem, focused attention on het-c and its adjoining regions, and provided strains that are suitably marked for further analysis. Genetic resolution of the two alternatives by fine-structure recombination analysis is feasible, but it would be

laborious. Further genetic analysis might well be deferred until it is seen whether molecular data from the cloned het-c region provide a basis for deciding between the two hypotheses.

Evidence from natural populations

Two alleles at each of two loci would be expected to generate four genotypes. When a large sample of natural isolates is tested using the partial diploid technique, finding three but not more than three incompatibility types may therefore be taken as tentative evidence favoring multiple allelism rather than dual het loci. For het-c, few natural isolates have been tested. For het-8, however, data are much more extensive. Forty wild strains originating from many localities have been tested (by J.F.L.) using the OR-derived translocation T(VIL->IR)T39M777 to generate partial diploid progeny that would be heterozygous for polymorphic het genes in the VIL region that contains het-8. Only three genotypes have been found, designated het-8PA (22 isolates), het-8HO (7 isolates), and het-8OR (11 isolates). The results are consistent with a single-locus model, though once again the two-locus alternative is not excluded.

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