HETEROKARYON INCOMPATIBILITY GENES IN *NEUROSPORA CRASSA* DETECTED USING DUPLICATION-PRODUCING CHROMOSOME REARRANGEMENTS[1]

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ABSTRACT

Evidence is presented for five or six previously undetected heterokaryon incompatibility (*het*) loci, bringing to about ten the number of such genes known in *Neurospora crassa*. The genes were detected using chromosome duplications (partial diploids), on the basis of properties previously known for *het* genes in duplications. Duplications *homozygous* for *het* genes are usually normal in growth and morphology, whereas those *heterozygous* are strikingly different. The heterozygotes are inhibited in their initial growth, produce brown pigment on appropriate medium, and later "escape" from their inhibition, as a result of somatic events, to produce wild-type growth.—Five normal-sequence strains were crossed to 14 duplication-producing chromosome rearrangements, and the duplication progeny were examined for properties characteristic of duplications heterozygous for known *het* genes. Each cross produced duplications for a specific region of the genome, depending on the rearrangement. Normal-sequence strains were wild types from nature, chosen from diverse geographic locations to serve as sources of genetic variation.—The duplication method was very effective. Most of the longer duplications uncovered *het* genes. The genes are: *het-5* (on linkage group IR, in the region covered by duplications produced using rearrangement *T(IR→VIR)NM103*), *het-6* (on IIL, covered by *T(IIL→VI)P2869* and *T(IIL→IIIR)AR18* duplications), *het-7* (tentatively assigned to IIIR, *T(IIIR→VIL)D305*), *het-8* (VIL, *T(VIL→IR)T39M777*), *het-9* (VIR, *T(VIR→IVR)AR209*), and *het-10* (VIIR, *T(VIIR→IL)5936*).

THREE heterokaryon incompatibility genes in *Neurospora crassa* have now been studied both in heterokaryon tests and in chromosome duplications (partial diploids). They are the genes *het-c*, *het-e*, and the mating-type locus, which acts as a *het* locus (see especially NEWMEYER and TAYLOR 1967, and PERKINS 1975). The incompatibility of the genes in heterokaryons is paralleled by their incompatibility in duplications. Duplications *homozygous* for the genes are esentially normal in growth and morphology (parallel to heterokaryon *compatibility*). Duplications *heterozygous* for these loci, however, are strikingly different. They are inhibited in their initial growth, produce brown pigment on appropriate medium, and later "escape" from their inhibition, as a result of

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somatic events, to produce wild-type growth (the inhibition parallels heterokaryon *incompatibility*).

Duplications used in studying the *het* genes were synthesized in crosses involving certain types of chromosome rearrangements, which segregate for duplications covering specific regions of the genome. Duplications facilitate the study of *het* genes because they permit the study of the *het* gene or small number of genes located in a specific duplicated region, while disregarding much of the remainder of the genome. Conventional studies, on the other hand, are complicated by the fact that a number of *het* genes have the same phenotype, and heterokaryon incompatibility of two strains does not necessarily indicate whether the strains differ at one particular locus, or another, or at many.

In the preceding paper, PERKINS (1975) documents the validity of using duplications in studying *het* genes. In this paper, a study is described in which the duplication method was applied to testing for *het* genes in regions of the *N. crassa* genome where they were not previously detected. An abstract has been published (MYLYK 1973).

MATERIALS AND METHODS

Strains: Normal sequence: wild types—74-OR23-1V *A*, ORS *a*, 3002 *a*, RL3-8 *A*, Panama *A* (FGSC 1131), Costa Rica *A* (FGSC 851), Adiopodoumé (Ivory Coast) *A* (FGSC 430), Liberia *A* (FGSC 961), Groveland (Florida, U. S. A.) *a* (FGSC 1945), conidial isolate Em v5-1 *a* from wild type Emerson *a*; mutant stocks—fl^P *A* and *a*. Rearrangements: Most rearrangement stocks are listed in Table 1. Others are: *In(IL→IR)H4250 A* (NEWMEYER stock In(506-36)) and *T(VIIL→IVR)T54M50 het-e nic-3*.

Most strains were obtained from the collection of DAVID D. PERKINS, or, in some instances, derived from his stocks. Certain strains of *In(IL→IR)NM176* and *T(IR→VIR)NM103* were provided by BARBARA C. TURNER. Crosses to illustrate *A/a* duplications (Figure 3a) and *het-E/het-e* duplications (Figure 3c) were generously provided by DOROTHY NEWMEYER and D. D. PERKINS.

The wild types 74-OR23-1V *A* (abbreviated ORV *A* and ORS *a* (MYLYK, BARRY and GALEAZZI 1974) are highly isogenic strains derived from 74-OR23-1 *A* and 74-OR8-1 *a*, and presumably have the same *het* genotype as the latter strains. Strain ORV *A* is a vegetative reisolate of 74-OR23-1 *A* obtained by eight serial single-conidial isolations of 74-OR23-1 *A*. Strain ORS *a* is an ascospore isolate obtained following seven generations of crosses of 74-OR8-1 *a* and subsequent *a* isolates to 74-OR23-1 *A*. These strains were isolated because mutations were found in heterokaryotic condition in some stocks of 74-OR23-1 *A* and 74-OR8-1 *a*, and because some phenotypic differences between these strains were also detected. Strain 3002 *a* was derived from a cross between 74-OR23-1 *A* and 74-OR8-1 *a*.

Wild types from Panama, Costa Rica, Adiopodoumé, Liberia, and Groveland are strains of the species *N. crassa* isolated from the wild. They are isosequential with laboratory stocks of normal sequence, on the basis that crosses to such stocks produce about 90–95% darkly pigmented and viable ascospores.

Media and technical methods: Media and technical methods were essentially those described by PERKINS (1975) or those found in DAVIS and DE SERRES (1970). Of particular relevance, ascospores from each cross (Tables 1-4) were isolated to 10 × 75 mm tubes of minimal medium + phenylalanine + tyrosine, which is optimal for recognizing the inhibited phenotype and dark pigment produced by duplications heterozygous for *het* loci. Additional isolates from some crosses were grown on minimal or glycerol complete medium. Barrenness was tested in crosses to fl^P strains. Most cultures were grown at 25°, with some being grown at 34°.

Conventions: The female parent is listed first in designating a cross. In the text, rearrangement symbols are usually abbreviated to the isolation numbers, e.g. *T(IIL→VR)NM149* is designated

NM149. The term "duplication" is commonly used in referring to "duplication progeny". Gene symbols include changes proposed by PERKINS and BARRATT (1973) and adopted at the Seventh Neurospora Information Conference, held in 1974.

RESULTS

Experimental approach and rationale

Expected results can conveniently be illustrated with rearrangement NM149, used by PERKINS (1975) in studying *het-c* in duplications. In this paper, NM149 data will be presented along with data on other rearrangements, to provide a comparison with a system in which duplications cover a known *het* gene.

Crosses between normal sequence and translocation NM149 segregate for duplications for a *specific* portion of linkage group IIL including *het-c*, as shown in Figure 1a. The duplications result from the independent assortment of chromosomes, and constitute about one third of the viable progeny. When parental strains have identical alleles at *het-c* (e.g., *het-C*), all duplications are homozygous for the gene (e.g., *het-C/het-C*). When parental strains differ (e.g., *het-C* and *het-c*), most duplications are heterozygous (e.g., *het-C/het-c*), with some being homozygous because of crossing over during meiosis (see Figure 1a).

Crosses between normal sequence and the 14 rearrangements used in testing for new *het* genes also produce duplications covering specific regions of the genome, shown in Figure 2. Duplications from rearrangement NM176, dependent on crossing over in the inversion loop, occur in a frequency of about one fifth of the viable progeny (TURNER *et al.* 1969), whereas the duplications from other rearrangements, dependent on independent assortment of chromosomes, constitute about one third of the viable progeny, like those from NM149 (PERKINS, personal communication; for possible exception concerning D305, see later).

Heterokaryon incompatibility genes were anticipated in regions covered by duplications whenever one or more crosses between a rearrangement and the five wild types would produce inhibited duplications, resembling those heterozygous for *het-c*, *het-e*, and mating type. Wild types from diverse geographic locations were used as sources of genetic variation to maximize the possibility that at least one normal sequence strain would differ from the laboratory rearrangement stocks at any *het* locus.

Evidence for het *genes from segregation of inhibited phenotypes*

Inhibited progeny segregated in appreciable numbers in some crosses involving rearrangements NM103 (duplications covering a portion of linkage group IR), P2869 and AR18 (duplications from both covering regions of IIL, but not the locus *het-c*), D305 (IIIR), T39M777 (VIL), AR209 (VIR), and 5936 (VIIR) (as well as all five crosses to rearrangement NM149, IIL, included for comparison) (Table 1). They did not occur, or were very infrequent, in crosses involving rearrangements NM176, Y112M4, 4540, NM177, ALS159, S4342, and NM152 (Table 1). The results indicate that *het* loci occur in regions of the genome covered by duplications produced by the former group of rearrangements, which produced substantial numbers of inhibited progeny, but they provide no evidence for *het* loci in regions covered by the latter group.

TABLE 1

The incidence of inhibited progeny from crosses between duplication-producing chromosome rearrangements and wild types from different geographic locations

Rearrangements	Rearrangement stock numbers*	Wild types				
		Panama *A*	Costa Rica *A*	Adiopodoumé *A* (Ivory Coast)	Liberia *A*	Groveland *a* (Florida)
T(IIL→VR)NM149 het-C (for comparison)	755-13 *a* (M), 755-17 *A* (M)	**7/36**	**9/36**	**8/37**	**11/34**	**10/37**
T(IR→VIR)NM103	2138 *a*, 2137 *A*	**9/37**	0/39	0/32	**9/37**	0/37
T(IR→VIR)NM103 ad-9 cyh-1 trp-2	268-19 *a* (T)	**11/39**	0/38	0/37	**17/96**	
T(IIL→VI)P2869	1829 *a*, 1828 *A*	**12/35**	**17/76**	1/67	**32/75**	**17/38**
T(IIL→IIIR)AR18	1562 *a*, 1561 *A*	**22/63**	**18/58**	2/64	**24/67**	**23/66**
T(IIIR→VIL)D305	2140 *a*, 2139 *A*	1/34	0/38	2/36	**17/131**	**15/141**
T(VIL→IR)T39M777	2134 *a*, 2133 *A*	**23/63**	1/117	**30/105**	0/27	**10/34**
T(VIR→IVR)AR209	1932 *a*, 1931 *A*	**14/36**	1/67	**19/65**	1/66	0/35
T(VIR→IVR)AR209 cot-1 pan-2	64-1051 *a* (P)	**19/67**	1/69	**8/37**	2/72	
T(VIIR→IL)5936	2105 *a*, 2104 *A*	0/31	**9/38**	**38/108**	1/72	2/35
In(IL→IR)NM176† (+ + *a*, *un-5 ser-3 A*)	2374-6 *a* (P), 11-843 *A* (P)	0/111	0/106	3/92	0/35	0/35
T(IR→IIIR)Y112M4 ad-3B al-2 a, A	13-790 *a* (P), 13-789 *A* (P)	0/68	0/34	0/93	1/23	2/65
T(IR→IIIR)4540 nic-2	767 *a* (P), 766 *A* (P)	0/34	0/72	0/27	0/30	0/37

TABLE 1 (Continued)

Rearrangements	Rearrangement stock numbers*	Wild types				
		Panama *A*	Costa Rica *A*	Adiopodoumé *A* (Ivory Coast)	Liberia *A*	Groveland *a* (Florida)
T(IIR→IL)NM177	2003 *a*, 1610 *A*	0/35	0/40	0/31	0/39	0/35
T(IVR→VIR)ALS159	2101 *a*, 2100 *A*	0/67	0/73	2/73	0/37	0/37
T(IVR→IIIR)S4342	2065 *a*, 1559 *A*	0/38	1/37	0/36	1/38	0/36
T(IVR→I)NM152	1753 *a*, 1752 *A*	0/37	0/40	0/38	0/36	0/38

Results are presented as inhibited progeny/total. Bold face indicates heterozygosity for *het* loci in regions studied. For explanation of occasional inhibited progeny in other crosses, see text.

* Stocks are usually Fungal Genetics Stock Center (FGSC) designations, or, occasionally, MYLYK (M), TURNER (T), or PERKINS (P) designations.

† BARBARA TURNER (personal communication) now has data on crosses between rearrangement *In(IL→IR)NM176* and a number of *N. crassa* wild types collected from India. Inhibited progeny were also absent or rare in all those crosses.

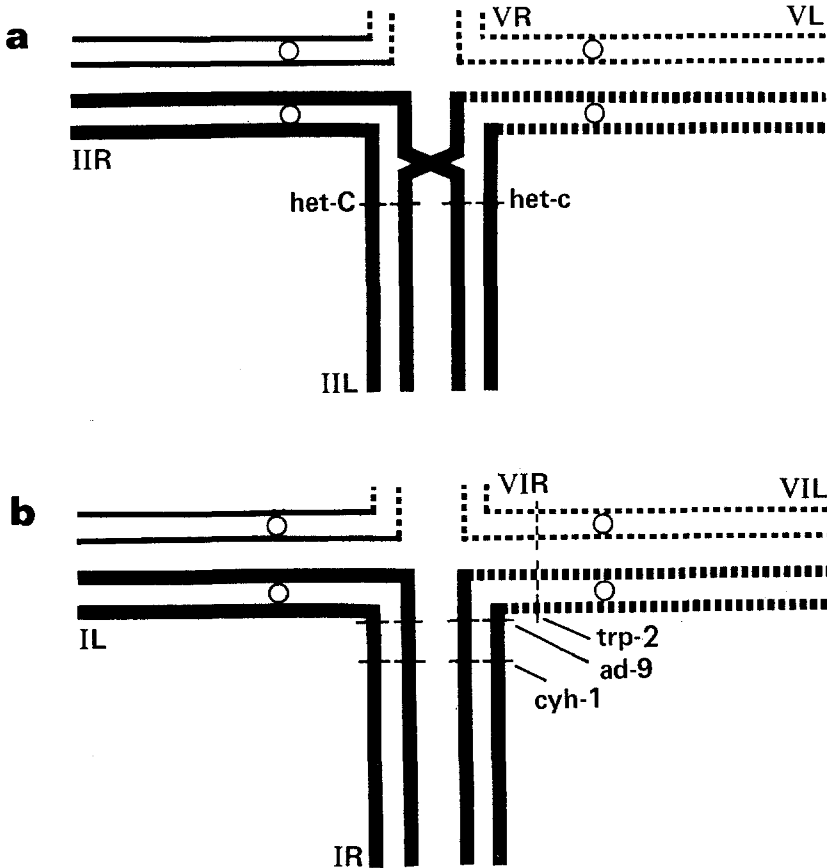

FIGURE 1.—Pairing of chromosomes and the production of duplications in crosses between normal-sequence and two chromosome rearrangements. (a) *Normal* × *T(IIL→VR)NM149*. The rearrangement has a portion of linkage group IIL translocated to or near the tip of linkage group VR. When the chromosomes designated by heavy lines (solid + broken) segregate together in meiosis I, the resulting meiotic products are duplicated for the region of linkage group IIL which includes the heterokaryon incompatibility locus *het-c*. If both parents have the same allele for *het-c*, all the duplications are homozygous for that allele. If they have different alleles, e.g. *het-C* and *het-c*, the duplications are heterozygous (*het-C/het-c*), or, if a crossover occurs as shown between this gene and the breakpoint of the arrangement, the duplications produced, following meiosis II, can be homozygous (*het-C/het-C* and *het-c/het-c*). (b) *Normal* × *T(IR→VIR)NM103*. The rearrangement has a portion of linkage group IR translocated to or near the tip of VIR. When the chromosomes designated by heavy lines (solid + broken) segregate together at meiosis I, the resulting meiotic products are duplicated for a portion of linkage group IR. Genes *ad-9*, *cyh-1*, and *het-5* (see text) are covered by the duplications; *trp-2* is not.

The frequency of *T(IIL→VR)NM149* and *T(IR→VIR)NM103* duplications, dependent on the independent assortment of chromosomes, is one quarter of all the meiotic products or one third of the viable spores (meiotic products complementary to the duplications are deficient for portions of linkage groups IIL and IR, respectively, and result in the production of hyaline and inviable spores). *Dp(IIL→VR)NM149* and *Dp(IR→VIR)NM103* progeny may be deficient for the tips of linkage groups VR and VIR, respectively, but if so, the tips are dispensable, as the duplications are viable. The drawings are not to scale.

For illustration in more detail, consider the crosses involving rearrangement T39M777. Cross Panama *A* × T39M777 produced 23 inhibited progeny out of a total of 63, Adiopodoumé *A* × T39M777 produced 30 out of 105, and Groveland *a* × T39M777 produced 10 out of 34 (Table 1). The inhibited progeny could account for most or all duplications from these crosses (approximately one third of the viable progeny). On the other hand, cross Costa Rica *A* × T39M777 produced only one inhibited progeny out of 117, and T39M777 × Liberia *A* produced none out of 27 (Table 1). Duplications from these two crosses were thus non-inhibited ("rare" inhibited progeny, such as from Costa Rica *A* × T39M777, will be considered later). The results indicate a *het* locus in the region of linkage group VIL covered by T39M777 duplications (see Figure 2). The Panama, Adiopodoumé, and Groveland wild types seem to have different alleles at this locus than the T39M777 stocks, whereas the Costa Rica and Liberia wild types seem to have the same allele.

Using this rationale, the crosses for which data are in bold print in Table 1 are interpreted to reflect allelic differences between wild types from nature and the rearrangement stocks, at *het* genes located in the respective duplicated regions.

Phenotypes of the inhibited duplications

Examples of duplications heterozygous for newly detected *het* genes, in comparison with heterozygotes for previously detected ones, are shown in Figure 3. Typically the initial growth was slow and restricted largely to the surface of the medium and below, with any aerial growth occurring as short, non-conidiating hyphae. Brown pigment was produced on medium containing phenylalanine and tyrosine. Then the cultures escaped, producing pink aerial growth, usually with conidia, and the cultures often filled the tube like a wild-type culture. As for duplications heterozygous for previously detected *het* genes, the presence or absence of phenylalanine and tyrosine in the medium had no effect on whether duplications were inhibited. Only the production of brown pigment was dependent on whether these biochemicals were present.

Within this general description, there was a considerable amount of variation in specific morphology, degree of pigmentation, and time of escape, but the variation was largely between crosses, with most inhibited progeny from a particular cross resembling each other. In some crosses, the progeny were so strongly inhibited that pigmentation could not be observed before escape (Panama *A* × NM103—both crosses), or the cultures had to be maintained in plastic bags to prevent drying (crosses Panama *A* × P2869, Groveland *a* × P2869, Groveland *a* × D305, Panama *A* × NM149, and Groveland *a* × NM-149) in order to observe pigmentation and escape (the majority of inhibited progeny from the latter crosses, except Panama *A* × NM149, produced pigment, and most of the pigmented cultures escaped, when grown under such conditions). (Also see later for special circumstances concerning AR18 duplications.)

At least one cross involving each of rearrangements NM103, P2869, D305, T39M777, AR209, and 5936, providing evidence for all *het* loci detected, segre-

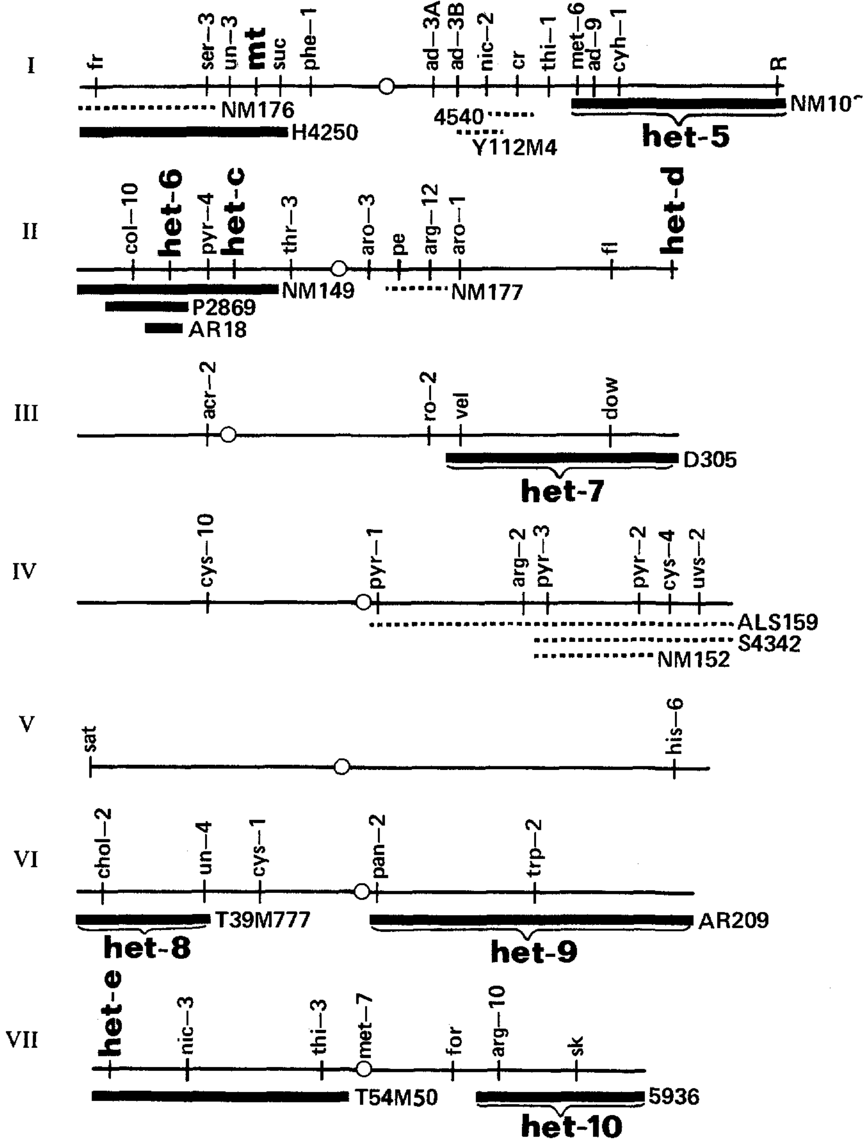

FIGURE 2.—*Neurospora crassa* genome, showing regions included in duplications produced using various chromosome rearrangements, and showing location of heterokaryon incompatibility (*het*) genes. Lines below linkage groups show portions of chromosomes occurring in non-tandem duplications produced from crosses between normal sequence and the respective chromosome rearrangements: Normal sequence × Rearrangement *In(IL→IR)NM176* (abbreviated NM176) segregates for duplications covering a portion of linkage group IL distal to *un-3*; Normal sequence × *T(IR→VIR)NM103* (abbreviated NM103) segregates for duplications covering a portion of linkage group IR distal to *thi-1*, etc. Duplications produced by pericentric inversions NM176 and H4250, dependent on crossing over in the inversion loop, segregate in a frequency of approximately 20–25% of the viable progeny; those produced using all other rearrangements, which are insertional or effectively terminal translocations, are dependent on independent as-

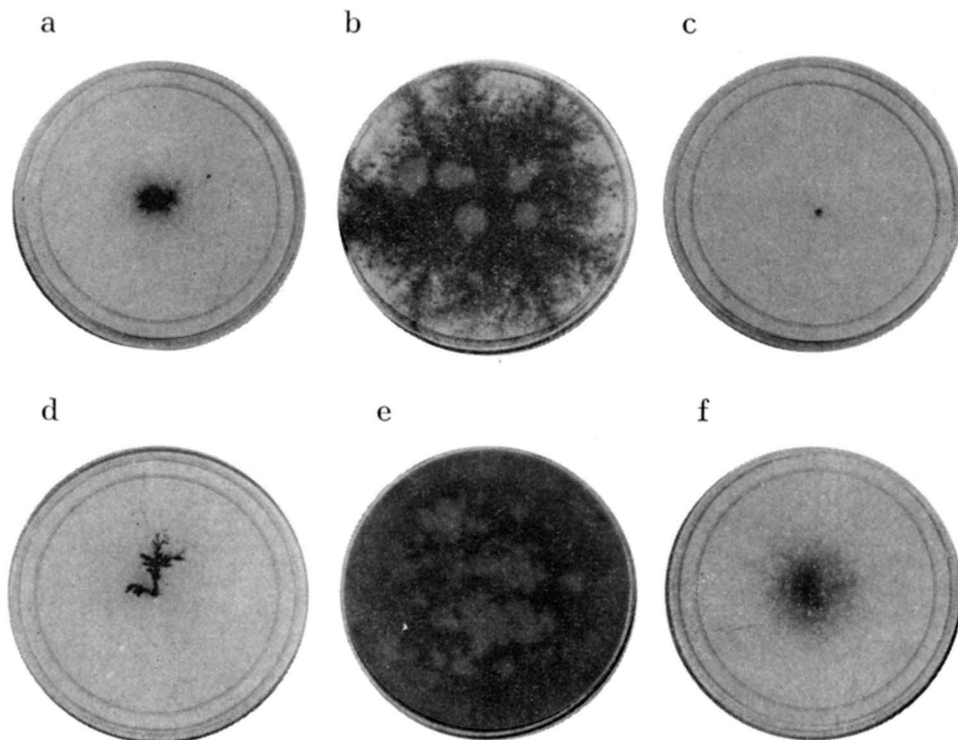

FIGURE 3.—Duplications heterozygous for different *het* loci, showing a variety of morphologies. (a) *A/a* duplication, from cross 3002 *a* × *In(IL→IR)H4250 A*. (b) *het-C/het-c*, from *T(IIL→VR)NM149 het-C* × Em v5-1 *het-c a*. (c) *het-E/het-e*, from RL3-8 *het-E A* × *T(VIIL→IVR)T54M50 het-e nic-3*. (d) *het-8* heterozygote, from Adiopodoumé *A* × *T-(VIL→IR)T39M777 het-8*^{OR}. (e) *het-9*^{OR}/*het-9*^{PA}, from Panama *het-9*^{PA} *A* × *T(VIR→IVR)-AR209 het-9*^{OR}. (f) *het-10* heterozygote, from Adiopodoumé *A* ×*T(VIIR→IL)5936 het-10*^{OR}. Pale-colored patchiness in (b) and (e) is escaped growth on the surface of brown-pigmented inhibited growth.

Cultures are from single ascospores germinated in the 55-mm diameter petri dishes containing minimal medium + phenylalanine + tyrosine. Cultural conditions: (a), (d), (e), and (f) were grown 5 days at 25°, (b) was grown 5 days at 34° (c) was grown 6 days at 34°; the more strongly inhibited duplications, in (c) and (d) were maintained in plastic bags to reduce drying of the medium, and thus enhancing growth. High contrast photographic paper was used. (Petri dishes were used only for growing cultures to be photographed. All other isolates, in the text, were to 10 × 75 mm tubes of medium.)

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sortment of chromosomes, and constitute about one third of the viable progeny (see Figure 1 for NM149 and NM103; for possible exception concerning D305, see text). Heavy, solid lines indicate regions covering *het* loci; lighter, broken lines show regions in which such genes have not been detected. Rearrangement P2869 was previously designated Y16329i (PERKINS 1972). AR18 and P2869 duplications have not been precisely mapped, but data in the text indicate that they at least overlap, both covering *het-6*. Linkage groups are not to scale. The figure has been adapted from PERKINS (1972).

gated for duplications having two growth phases that could clearly be observed: an inhibited, brown-pigmented phase prior to escape, and essentially a wild-type phase following escape.

Confirmation that inhibited classes represent duplications

A sample of crosses were tested to see if the inhibited progeny considered heterozygous for *het* loci were in fact duplications, and, as a control, to see if duplications were segregating among crosses where inhibited types did not occur or were infrequent. Usually this was accomplished by testing for barrenness, as described below. For two rearrangements, confirmation of duplications was accomplished using appropriate genetic markers.

Crosses involving Neurospora duplications are commonly "barren"; i.e., they may produce many perithecia but only few if any spores (NEWMEYER and TAYLOR 1967; TURNER *et al.* 1969; PERKINS 1972, 1974). The ability to detect duplications on this basis, however, depends on their stability. In general, there is a tendency for the extra genetic material to be eliminated from Neurospora duplications and for haploidy to be restored (PERKINS, NEWMEYER and TURNER 1972, personal communication). Once haploid nuclei accumulate in a "duplication" culture, the culture may test as non-barren when crossed to a haploid because the haploid × haploid component of the cross produces both perithecia and spores. The duplications produced from some rearrangements are relatively stable and can usually be detected on the basis of barrenness, whereas those from other rearrangements cannot.

In crosses between normal sequence and rearrangements P2869, AR18, T39-M777, and 5936 (as well as NM149), a majority or all inhibited progeny were barren (Table 2), confirming that they were duplications. (They were tested following escape. *A/a* and *het-C/het-c* duplications are commonly barren following escape.) In crosses involving these rearrangements, but where inhibited progeny did not occur or were very infrequent, substantial numbers of non-inhibited progeny were barren, confirming that duplications were segregating. This was also true for crosses involving the seven rearrangements which did not yield inhibited progeny in crosses to any of the five wild types, or produced them only rarely (rearrangements NM176, Y112M4, etc.; Tables 1 and 2).

In the crosses which produced substantial numbers of inhibited progeny, a few non-inhibited progeny were occasionally barren, and some inhibited progeny were non-barren. The non-inhibited progeny from crosses P2869 × Liberia *A* and Costa Rica *A* × 5936 (also Adiopodoumé *A* × NM149) (Table 2) were probably duplications homozygous for *het* loci in the respective regions, resulting from appropriate crossovers during meiosis, as illustrated for NM149 duplications in Figure 1a. On the other hand, the inhibited progeny from cross P2869 × Liberia *A* (also Adiopodoumé *A* × NM149) (Table 2) were probably duplications heterozygous for *het* loci in which the extra genetic material was eliminated, restoring haploidy, so that the cultures tested as non-barren or yielded ambiguous tests. Elimination of extra genetic material could have resulted in the escape of the inhibited cultures.

TABLE 2

Occurrence of barren progeny in crosses between duplication-producing rearrangements and wild types

het loci *not* heterozygous in zygote		*het* locus heterozygous in zygote		
			Frequency of barrens among	
Crosses	Frequency of barrens among non-inhibited progeny	Crosses	Non-inhibited progeny	Inhibited progeny
ORV *A* × *T(IIL→VR)NM149 het-C* (for comparison)	9/36	Adiopodoumé *A* × *T(IIL→VR)NM149 het-C*	3/29	7/8
Adiopodoumé *A* × *T(IIL→VI)P2869*	28/67	*T(IIL→VI)P2869* × Liberia *A*	1/43	≥22/32*
Adiopodoumé *A* × *T(IIL→IIIR)AR18*	7/28	Panama *A* × *T(IIL→IIIR)AR18*	0/27	12/12
Costa Rica *A* × *T(VIL→IR)T39M777*	15/39	Panama *A* × *T (VIL→IR)T39M777*	0/21	17/17
Panama *A* × *T(VIIR→IL)5936*	14/31	Costa Rica *A* × *T(VIIR→IL)5936*	2/29	9/9
Panama *A* × *In(IL→IR)NM176*	29/76			
Adiopodoumé *A* × *In(IL→IR)NM176*	12/38			
Panama *A* × *T(IR→IIIR)Y112M4 ad-3B al-2*	8/31			
Adiopodoumé *A* × *T(IR→IIIR)Y112M4 ad-3B al-2*	8/30			
Panama *A* × *T(IR→IIIR)4540 nic-2*	6/34			
Adiopodoumé *A* × *T(IR→IIIR)4540 nic-2*	13/34			
Panama *A* × *T(IIR→IL)NM177*	15/35			
Adiopodoumé *A* × *T(IIR→IL)NM177*	9/31			
Panama *A* × *T(IVR→VIR)ALS159*	7/36			
Adiopodoumé *A* × *T(IVR→VIR)ALS159*	9/40			
Panama *A* × *T(IVR→IIIR)S4342*	7/38			
Adiopodoumé *A* × *T(IVR→IIIR)S4342*	14/36			
Panama *A* × *T(IVR→I)NM152*	7/26			
Adiopodoumé *A* × *T(IVR→I)NM152*	10/38			

Results are presented as barren progeny/number tested.

* The tests indicate more than 22 duplications. The other ten inhibited progeny included borderline cases as well as some discretely non-barren tests. Non-duplication cultures should not have resulted in ambiguous tests.

Confirmation that inhibited progeny from NM103 and AR209 crosses were duplications was either not possible or not directly possible on the basis of barrenness, because of the instability of duplications or special properties concerned with the elimination of extra genetic material. Duplications from these rearrangements were identified using genetic markers, in the crosses (Table 1) segregating for such markers. Consider first the case for NM103. The production of duplications using this rearrangement, and location of markers, is shown in Figure 1b. The mutant *ad-9* is very close to the breakpoint of the translocation and occurs inside the region covered by NM103 duplications, whereas *trp-2* is a bit further from the breakpoint, but outside the duplicated region (BARBARA TURNER, personal communication; see Figure 1b). In crosses between wild type and NM103 *ad-9 cyh-1 trp-2*, most duplication cultures were expected to be heterozygous for *ad-9* and hemizygous for *trp-2*, hence having the phenotype ad^+ trp^- (*cyh* can be ignored), with occasional ones from appropriate crossovers being ad^+ trp^+, or, very rarely, ad^- trp^-. From the cross NM103 *ad-9 cyh-1 trp-2* × Liberia *A*, all 12 inhibited, pigmented progeny were ad^+ trp^- (Table 3), providing evidence that they were duplications (ad^+ trp^- non-duplications were expected to be infrequent). (The inhibited duplications were tested for markers before escape.) Six non-inhibited progeny were also duplications (ad^+ trp^-, Table 3), presumably homozygous for the *het* locus as a result of meiotic crossing over (they were recognized as duplications on the basis of producing large colonies on plates of sorbose medium, as is characteristic of NM103 duplications; TURNER, personal communication). The control cross Adiopodoumé × NM103 *ad-9 cyh-1 trp-2* produced 17 ad^+ trp^- progeny (Table 3), most or all of which must have been duplications.

The crosses involving strain AR209 *cot-1 pan-2* (Table 3) similarly provide evidence that most, or probably all, inhibited and pigmented progeny from AR209 crosses represent duplications. The genes *cot-1* and *pan-2* both map close to the breakpoint of the translocation; *pan-2* is covered by the duplications and *cot-1* is not. Most duplications from wild type × AR209 *cot-1 pan-2* were expected to have the phenotype cot^- pan^+, with a small proportion resulting from meiotic crossovers being cot^+ pan^+ and cot^- pan^-. The inhibited progeny from cross Panama *A* × AR209 *cot-1 pan-2* were mostly cot^- pan^+, with a few being cot^+ pan^+ (Table 3), as expected of duplications. The control cross AR209 *cot-1 pan-2* × Liberia *A* segregated for a substantial number of non-inhibited cot^- pan^+ progeny (Table 3), most of which were necessarily duplications.

Duplications from D305 crosses could not easily be identified by barrenness because of their instability, and were not tested using genetic markers. Although more work is needed, there is some evidence that the inhibited cultures were duplications. In tests for barrenness, the cultures were often borderline cases rather than yielding discrete results, whereas non-duplications should have been discretely non-barren.

The distinction between duplications heterozygous for het *loci and otherwise slowly growing cultures*

Some difficulties were encountered in distinguishing cultures believed to represent heterozygosity for *het* loci from those which either grew slowly or

TABLE 3

Segregation of progeny in crosses involving rearrangements T(IR→VIR)NM103 *and* T(VIR→IVR)AR209

Crosses	Phenotypes of progeny	Numbers	Expected constitution of *most* progeny having the phenotype
*T(IR→VIR)NM103 ad-9 trp-2**	Non-inhibited + +	22	Parental, Normal sequence
×	Non-inhibited *ad trp*	17	Parental, *T(IR→VIR)NM103*
Liberia *A*	Non-inhibited + *trp*	6	Duplications homozygous for *het-5*, and/or crossovers between *trp-2* and translocation breakpoint
	Inhibited + *trp*	12	Duplications heterozygous for *het-5*
Adiopodoumé *A*	Non-inhibited + +	7	Parental, Normal sequence
×	Non-inhibited *ad trp*	12	Parental, *T(IR→VIR)NM103*
*T(IR→VIR)NM103 ad-9 trp-2**	Non-inhibited *ad* +	1	Crossover between *trp-2* and translocation breakpoint
	Non-inhibited + *trp*	17	Duplications homozygous for *het-5*
Panama *A*	Non-inhibited + +	23	Parental, Normal sequence
×	Non-inhibited *pan cot*	15	Parental, *T(VIR→IVR)AR209*
T(VIR→IVR)AR209 cot-1 pan-2	Non-inhibited *pan* +	6	Crossovers between *cot-1* and *pan-2*
	Non-inhibited + *cot*	4	Duplications homozygous for *het-9*, and/or crossovers between *cot-1* and *pan-2*
	Inhibited + *cot*	16	Duplications heterozygous for *het-9*
	Inhibited + +	3	Duplications heterozygous for *het-9*, with crossovers between *cot-1* and translocation breakpoint
T(VIR→IVR)AR209 cot-1 pan-2	Non-inhibited + +	16	Parental, Normal sequence
×	Non-inhibited *pan cot*	8	Parental, *T(VIR→IVR)AR209*
Liberia *A*	Non-inhibited *pan*	1	Crossover between *cot-1* and *pan-2*
	Non-inhibited + *cot*	11	Duplications homozygous for *het-9*

* The *T(IR→VIR)NM103* strain also has mutant *cyh-1*, which can be ignored.

ceased to grow soon after ascospore germination for other reasons. The difficulties involved progeny which did not have distinct pre- and post-escape phases, or in which these phases were difficult to observe.

Originally all five crosses involving rearrangement AR18 (Table 1) were believed to represent parental differences for a *het* locus in the duplicated region. However, a property of AR18 duplications is that they are ordinarily slow-growing (first observed by ANNA KRUSZEWSKA; personal communication to D. D. PERKINS). The duplications from Adiopodoumé *A* × AR18 were merely a slow-growing class, requiring 9–10 days' growth at 25° under conditions to prevent drying to produce substantial aerial growth. Duplications from crosses of AR18 to the other four wild strains produced tiny buttons of growth that eventually became darkly pigmented (in 2–3 weeks). Those from cross Panama *A* × AR18 *a* were maintained still longer, and were found to escape, to the phenotype ordinarily characteristic of AR18 duplications. Only the latter four crosses were finally interpreted to represent parental differences for a *het* locus in the region studied.

Original indications that 4540 and S4342 duplications may cover *het* loci (MYLYK 1973) were in error. Classes of progeny from 4540 crosses failed to grow appreciably. However, they were not duplications, but were *nic-2* 4540 cultures improperly or inadequately supplemented for *nic-2*. Tests carried out properly yielded the results in Table 1. Some classes of progeny from S4342 crosses also failed to grow appreciably. However, they were found not to be duplications, but were attributable to a temperature-sensitive mutant that went undetected in the S4342 parent. A replacement stock yielded the data in Table 1.

Occasionally, for unknown reasons, crosses between strains differing in genetic background produce a few progeny which stop growing soon after ascospore germination, before a macroscopically visible culture is produced. These inviable progeny segregate both from isosequential crosses and from those heterozygous for chromosome rearrangements. Such phenotypes were scored as inhibited in Table 1 crosses, so that some individual progeny may incorrectly have been classified as heterozygous for *het* loci. However these mysterious inviable progeny are too infrequent to affect the interpretation of whether particular crosses represented parental differences for *het* loci.

The occurrence of "rare" inhibited progeny

Some inhibited progeny occurring in very small numbers (Table 1) were of the type that stopped growing soon after ascospore germination, and may have been of the mysterious type just described. However, other rare inhibited progeny had properties characteristic of *het-C/het-c*, *het-E/het-e*, and *A/a* duplications. They produced restricted, pigmented cultures which escaped. These latter types have an attractive possible explanation.

In various organisms, including the ascomycete Aspergillus (POLLARD, KÄFER and JOHNSTON 1968; UPSHALL and KÄFER 1974), it has been established that crosses heterozygous for a chromosome rearrangement, in which chromosomes involved in the rearrangement pair as quadrivalents, the incidence of nondis-

junction (3:1 segregation) involving chromosomes so paired is relatively high in comparison with that for chromosomes from bivalents. The occasional pigmented cultures, and perhaps some of the more strongly inhibited ones also, may be the products of such 3:1 segregation. Those having an extra chromosome would be effectively duplications for regions of the genome in addition to or different from the specific region being studied. Heterozygosity for a *het* locus occurring in such a region could result in an inhibited phenotype. PERKINS (1974) found phenotypes characteristic of *A/a* and *het-C/het-c* duplications segregating from a cross heterozygous for the reciprocal translocation *T(I;II) NM129*, in which the parents had different alleles for *het-c* as well as for mating type. The rearrangement does not produce duplications as a result of a regular meiosis. The inhibited progeny were interpreted to be tertiary disomics heterozygous *A/a*, or *het-C/het-c*, or both, as a result of 3:1 segregation.

Evidence for two het *genes in the region of linkage group IIL covered by* T(IIL→VR)NM149 *duplications*

Although NM149 duplications cover *het-c*, duplications from P2869 (PERKINS, personal communication) and AR18 do not, although they cover portions of the same chromosome region present in NM149 duplications (Figure 2). Rearrangements P2869 and AR18 have failed to produce inhibited progeny when crossed to both *het-C* and *het-c* testers. These rearrangements did, however, produce inhibited progeny when crossed to certain of the wild types from nature (Table 1). Hence at least one *het* locus, in addition to *het-c*, must be present in the region covered by NM149 duplications.

Rearrangements P2869 and AR18 yielded the same pattern of results in crosses to the wild types from nature (Table 1). Furthermore, as will be shown later, the P2869, AR18, and NM149 stocks have the same *het* genotype in regions common to the three duplications. The data can most easily be explained by suggesting that the region common to all three duplications, between *col-10* and *pyr-4*, contains one previously undetected *het* locus.

Alternative explanations in D305 data

Translocation D305 was originally considered to have one breakpoint effectively terminal (like NM149, Figure 1a), so that crosses to normal sequence would theoretically produce IIIR duplications constituting one third of the viable progeny (PERKINS 1972, APPENDIX). In the present study, only 13% of the progeny from cross D305 × Liberia *A*, and 11% from Groveland *a* × D305, were inhibited. Assuming these to represent a substantial proportion of the duplications, D305 duplications may not occur in a frequency of one third. This could reflect reduced viability. Alternatively, PERKINS (personal communication) has more recently considered the possibility that D305 duplications may not be a product of a regular meiosis, but that they may reflect a high incidence of 3:1 segregation. If so, then a region different from IIIR may also become duplicated, and the *het* gene detected using D305 may be located in that other region.

Nomenclature of the newly detected genes

Although the genes detected in duplications so far remain untested in heterokaryons, it is expected that different alleles for any one will prevent stable heterokaryon formation. On this basis, they are tentatively being designated *het*, like the genes first detected in heterokaryon tests.

In the light of present knowledge, the designation "heterokaryon incompatibility" or "*het*" is not fully accurate. The incompatibility of the *het* genes so far studied both in heterokaryons and duplications is not restricted to heterokaryons, but is just as much a property of *homokaryons* heterozygous for the genes in duplications. A more accurate and operational term would be "vegetative incompatibility" or "*veg*", which encompasses the incompatibility in both situations. The designation *het* is being applied to the newly detected genes because it is preferred by other workers, to avoid multiple designations for a common class of genes.

It is simplest to assume one *het* locus in any specific duplicated region unless shown otherwise. It is also simplest to number the newly detected genes, beginning with *het-5* (for the fifth such gene, other than mating type, to be described) and to use superscripts to designate alleles, allowing for easy future designation of multiple alleles. The *het* locus on linkage group IR (in the region covered by NM103 duplications; see Figure 2) is being designated *het-5*; that on IIL between *col-10* and *pyr-4* (P2869 and AR18 duplications) is being designated *het-6*; that tentatively assigned to linkage group IIIR (D305 duplications) is being designated *het-7*; that on VIL (T39M777 duplications) *het-8*; that on VIR (AR209 duplications) *het-9*; and that on VIIR (5936 duplications) *het-10*.

Allele designations are being made using Oak Ridge wild types as references, since the wild types and strains derived from them are commonly used in Neurospora laboratories. Stocks of rearrangements with which *het* genes were detected were crossed to the Oak Ridge derivatives ORV *A* and ORS *a*. Inhibited progeny were absent or rare (Table 4). Hence the Oak Ridge wild types and the

TABLE 4

Tests for inhibited progeny from crosses between duplication-producing rearrangements and the wild types ORV A *and ORS* a

Rearrangements	Wild types	
	ORV *A*	ORS *a*
T(IIL→VR)NM149 het-C (for comparison)	0/36	0/36
T(IR→VIR)NM103	0/35	0/35
T(IR→VIR)NM103 ad-9 cyh-1 trp-2	0/34	
T(IIL→VI)P2869	0/35	0/39
T(IIL→IIIR)AR18	0/37	0/38
T(IIIR→VIL)D305	1/34	1/36
T(VIL→IR)T39M777	0/35	0/29
T(VIR→IVR)AR209	0/70	0/72
T(VIR→IVR)AR209 cot-1 pan-2	0/70	
T(VIIR→IL)5936	1/29	0/29

Results are presented as number of inhibited progeny/total. Rearrangement stocks are those listed in Table 1.

rearrangement stocks have identical *het* alleles *in the specific regions of the genome occurring in the duplications* (they could differ at *het* loci elsewhere). Wild types from nature differ both from rearrangement stocks and Oak Ridge wild types wherever inhibited duplications were produced (Table 1). The alleles occurring in Oak Ridge wild types are being designated by superscript OR ($het\text{-}5^{OR}$, $het\text{-}6^{OR}$, . . ., $het\text{-}10^{OR}$). The alleles for *het-5*, *het-6*, *het-8*, and *het-9* occurring in wild type Panama *A* are being designated by superscript PA ($het\text{-}5^{PA}$, etc.), the allele for *het-7* in Liberia *A* is being designated $het\text{-}7^{LI}$, and the allele for *het-10* in Costa Rica *A* is being designated $het\text{-}10^{CR}$.

DISCUSSION

Heterokaryon incompatibility loci seem more numerous in the *N. crassa* genome than could earlier be anticipated. The genes detected using duplications, and those previously detected in heterokaryon tests, total about ten. (The exact number is not known, since *I/i* described by PITTENGER and BRAWNER (1961) could be the same as one of the newly detected genes, and since more information on *het-7*, covered by D305 duplications, is required. It must also be kept in mind that some genes detected in duplications may not prove incompatible in heterokaryons, even though each of the three genes first found incompatible in heterokaryons also proved incompatible in duplications.) As more duplication-producing rearrangements become available, and are used in the testing for *het* genes, more such genes can be expected. So far most of the longer duplications have uncovered *het* loci (see Figure 2).

The biological role of *het* genes may depend, in part, on the special properties of an organism. However, *het* or similar genes are present in fungal species having a variety of characteristics. This suggests a primary role of an encompassing nature. Particularly attractive is the possibility that such genes act in the defense of the organism (see, for instance, CATEN 1971; CLARK and COLLINS 1973).

Heterokaryon incompatibility genes are potentially useful for a number of experimental purposes, other than in understanding their nature: (1) They may be especially useful in studying the elimination of extra genetic material from Neurospora nuclei, since "escape" from inhibition of duplications indicates, in part, the elimination of such material (NEWMEYER and TAYLOR 1967), and since *het* genes occur in duplications having several different properties concerning the restoration of haploidy. (2) Study of mechanisms of escape may be informative in understanding mitotic recombination. (3) *het* genes may facilitate the detection of nondisjunction events. (4) Study of *het* genes in nature should provide useful information on genetic variation in populations, complementing information from the use of electrophoretic variants.

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Note added in proof: Evidence has been found in microscopic heterokaryon tests, to be described later, that different alleles for *het-5* are incompatible in heterokaryons.

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