DOMINANCE MODIFIERS IN *NEUROSPORA CRASSA*: PHENOCOPY SELECTION AND INFLUENCE ON CERTAIN ASCUS MUTANTS

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Manuscript received December 14, 1971
Revised copy received February 1, 1972

ABSTRACT

When homozygous in zygotes, mutant alleles at the *peak* locus in linkage group V of *Neurospora crassa* initiate aberrant asci that are nonlinear, in contrast to the linear asci characteristic of wild type. Most mutant alleles are recessive, inasmuch as crosses of the mutant strains with wild type give linear asci. However, five different mutant alleles, when heterozygous with the wild-type allele, act in varying degrees as zygote dominants, initiating both linear and nonlinear asci, the relative proportions depending on the allele. Five modifiers that act on the dominance relationships of at least one of the five possible heterozygotes of a dominant *peak* and its wild-type allele have been characterized, four of them having been obtained by selection directed against a phenocopy of these mutants induced by treatment of wild type with L-sorbose. The pattern of modifier specificity observed among the various dominant *peak* heterozygotes indicates that the phenotypic effects are produced by a complex relationship between the modifiers and the dominant *peak* alleles in relation to their wild-type allele. In all but two cases the direction of modification, where present, is towards decreasing the dominance of the mutant allele in the heterozygote, evidenced by an increase in the percentage of linear asci when compared with control data. The modifiers exert their maximum modification when they themselves are heterozygous with their wild-type alleles and when the dominant *peak* allele is heterozygous with its wild-type allele. No modification occurs when heterozygous modifiers are included in zygotes homozygous for a dominant *peak* allele, reinforcing the notion that the modifiers act on the dominance relationship existent between a dominant *peak* allele and its wild-type allele, rather than influencing some activity of the mutant allele itself. The modifiers have no detectable effect of their own on ascus morphology, since homozygous modifier zygotes initiate entirely linear asci when only wild-type alleles of *peak* are present in the zygotes. Their only detectable effect, other than dominance modification, appears to be in conferring sorbose resistance to the mycelium. The modifiers are unlinked to the *peak* locus, and, except for two of them, they are nonallelic.

THE existence of certain dominant mutants in *Neurospora crassa* provides the basis of an experimental system in which modifiers of genetic dominance may be obtained by an "indirect selection" technique. Moreover, particular properties of these mutants allow the effects of dominance modification to be described

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quantitatively. The genic alterations accounting for these dominant mutants suitable for such a system are at the *peak* locus of *Neurospora crassa* (MURRAY and SRB 1959).

A wild-type colony of *N. crassa* is a web-like, rapidly spreading mycelium with the branching only rarely being dichotomous. In haploid homokaryotic strains, the characteristic effects of both dominant and recessive mutations at the *peak* locus are extensive dichotomous branching and relatively slow increase in the vegetative colony diameter. The mutant colonies are rather button-like in appearance and in general are morphological mutants of the kind called "colonial" mutants by BARRATT and GARNJOBST (1949). The mutant phenotypic effects extend to the sexual phase of the life cycle in that abnormal, nonlinear asci are produced in contrast to the linear asci characteristic of wild type. The dominance and recessiveness of *peak* mutations are readily observed in their effects on ascus morphology. The typical recessive mutation *peak-2*, described by MURRAY and SRB (1959, 1962), produces abnormal asci when zygotes are homozygous (*pk-2/pk-2*) for the mutant gene, but asci from *pk-2*/+ zygotes, like those from +/+ zygotes, are normal. In an extensive search for abnormal ascus mutants, SRB and BASL (1969) identified three dominant *peak* alleles and in further work, as yet unpublished, two additional dominant *peak* alleles have been found. The effects of each of these five dominant *peak* alleles are to give some, but not all, nonlinear asci from zygotes that are heterozygous for the mutants and their wild-type allele (*Pk*/+). Thus both linear and nonlinear asci may be found within the same perithecium. Investigations in this laboratory have shown that at least most of such intraperithecial variability is nongenetic. That is, a cross between a dominant *peak* and a wild-type culture derived from ascospore isolations out of a linear ascus generally gives the same frequency of linear asci as a cross between a dominant *peak* and a wild-type culture derived from isolations out of a nonlinear ascus from the same perithecium. But for extremely rare exceptions, homozygous *Pk/Pk* zygotes result only in nonlinear asci. On a common genetic background, the five zygote dominant alleles may be distinguished from one another in that each shows a characteristic frequency of nonlinear asci, with a wide range of values amongst the five *Pk*/+ heterozygotes.

Our idea for a means of indirect selection of modifiers of dominant *peak* alleles is based on the capacity of the sugar L-sorbose to induce phenocopies of *peak* mutants when it is added to the culture medium of wild-type Neurospora. As shown by TATUM, BARRATT and CUTTER (1949), wild-type colonies of Neurospora grown on solidified medium containing sorbose are highly restricted in their lateral growth and have an extensive branching pattern similar to that of *peak* and other colonial mutants. Comparative chemical studies of the cell walls of wild type grown in medium with and without sorbose (DE TERRA and TATUM 1961) revealed differences in the relative amounts of particular components of the polysaccharides that make up the bulk of the cell wall. Further studies (DE TERRA and TATUM 1963; MAHADEVAN and TATUM 1965) showed that the cell wall composition of wild type grown on sorbose is similar to that of certain colonial mutants whose morphology closely resembles that of sorbose-grown wild

type. Using electrophoretic technique, BARBER, SRB and STEWARD (1969) found that sorbose-grown wild-type cultures showed a pattern of soluble proteins distinct from that of wild type grown on minimal medium but similar to that observed for minimal-grown and sorbose-grown *peak-2* examined under the same conditions. Finally, at least to some extent, the ability of sorbose to induce phenocopies of *peak* mutants extends to the sexual phase of the life cycle; i.e., homozygous wild-type crosses made on crossing medium containing sorbose often produce nonlinear asci (PINCHEIRA 1967).

Given that the effects of sorbose on wild-type Neurospora are similar to the effects of *peak* mutations, we decided that the sorbose phenocopies might provide the basis for an attempt to isolate mutations capable of modifying the dominance relationships of the dominant *peak* mutants. That is, we reasoned that mutagenesis of wild type followed by selection for resistance to the colonializing action of L-sorbose might provide mutations whose effect would be also to modify the ascus aberrancy initiated by *Pk*/+ zygotes. The anticipated direction of modification was towards decreasing dominance of the mutant allele evidenced by an increase in the percentage of linear asci among asci initiated by zygotes of the type: *Pk-mod*/+;*Pk*/+. This direction of modification will be referred to as "positive" modification. The present report deals primarily with the isolation and characterization of such modifiers, and their relevance to the evolution of dominance.

MATERIALS AND METHODS

Culture techniques: The culture techniques and the minimal and complete medium used were essentially those of BEADLE and TATUM (1945). All crosses were made on slants of DIFCO corn-meal agar incubated at 25°C.

Strains: The wild-type strains of *Neurospora crassa* used for crosses and mutagenic treatment were the St. Lawrence laboratory strains *74A* and *77a* further inbred in this laboratory. The allelic mutants *Peak-1* through *Peak-5*, isolated in *N. crassa*, are zygote dominant abnormal ascus mutants which have been shown by recombination studies to be allelic with recessive mutants at the locus called *peak* by MURRAY and SRB (1959, 1962) and *biscuit* by PERKINS (1959) in linkage group V of this species. Following the convention utilized by NOVAK and SRB (1971), dominant *peak* mutations will be symbolized with a capital letter and recessive *peak* mutations with a lower case letter.

A sorbose-resistant strain, *sor(DS)*, possibly at the same locus as *patch* in the left arm of linkage group I (PERKINS *et al.* 1969), was obtained from the Fungal Genetics Stock Center, Humboldt State College, Arcata, California, 95521. Gene *sor(DS)* when incorporated into a strain carrying the allele *Pk-1* proved to be a dominance modifier under criteria to be described.

Mutagenic technique: Mutagenesis was performed on wild-type conidia harvested from 6.5-day-old cultures grown on solid minimal medium at 25°C. The mutagens and conditions of treatment were as follows: N-methyl-N'-nitro-N-nitroso-guanidine, 15 μg/ml in H_2O, 2.5 hr exposure at 25°C; nitrous acid, 0.005M in 0.009M acetate buffer, pH 4.7, 4.0 hr exposure at 25°C. Following exposure to a mutagen, the conidia were washed three times with sterile distilled water.

Selection for modifiers: Mutagenized conidia were plated on solidified minimal medium (BEADLE and TATUM 1945) altered to contain 0.5% sucrose and supplemented with 2% sorbose (Sigma Chemical Co.) and incubated at 35°C in order to detect strains capable of "escaping" the colonializing action of sorbose. Colonies of wild type on this medium show colonial morphology for five to six days before characteristic wild-type mycelial growth is observed. Strains classified as "sorbose-resistant" were isolated from those colonies that produced rapidly spreading growth

within 48–60 hr of incubation. The sorbose-resistant strains do not immediately show wild-type-like mycelial growth when inoculated onto sorbose-containing medium. Rather, their distinction from wild type is a significant decrease in the lag time, during which they show a colonial growth habit, before they "escape." For any given sorbose-resistant strain grown on sorbose-containing medium, this lag time is longer when colonies are initiated from ascospores than when they are initiated vegetatively from conidia.

After one backcross to wild type followed by reisolation from sorbose-resistant colonies derived from ascospores, sorbose-resistant strains were crossed with a strain carrying the most dominant *peak* allele, *Pk-1*, and the relative proportions of linear and nonlinear asci were ascertained. Modifiers of the dominance of this dominant *peak* allele were identified as those mutations giving sorbose resistance and a repeatable, statistically significant increase in the percentage of linear asci over that exhibited by *Pk-1*/+ control crosses. By this technique of mutagenesis followed by "indirect selection" four modifiers of the *Pk-1*/+ heterozygote were identified. These modifiers were subsequently crossed to strains carrying the other four dominant *peak* alleles in order to test for modifier specificity.

The modifier mutations isolated by the "indirect selection" technique are designated *Pk-mod-A* through *Pk-mod-D*. This symbolism was chosen in order to avoid confusion with sorbose-resistant mutants of *N. crassa* already isolated by KLINGMULLER (1967), and to emphasize that the mutations discussed in this paper have modifying effects on the dominance of particular morphological mutants.

Method of ascus classification: Perithecia were taken from crossing tubes and their contents squeezed into a drop of distilled water on a microscope slide. A cover slip was applied and the unstained asci were examined under bright field at 100 × magnification with an AO Microstar compound microscope. Asci initiated from homozygous dominant *peak* zygotes (*Pk/Pk*) were predominantly balloon-shaped with the eight spores in a biseriate arrangement, like those asci initiated from homozygous recessive *peak-2* zygotes (*pk-2/pk-2*) pictured in PINCHEIRA and SRB (1969). Asci initiated from heterozygous dominant *peak* zygotes (*Pk*/+) seldom exhibit such a high degree of aberrancy, and ascus morphologies range from those with biseriately arranged ascospores to those with all eight spores linearly arranged. Among asci initiated from zygotes heterozygous for a dominant *peak* allele, and for a modifier gene (*Pk-mod*/+;*Pk*/+) and in which positive modification is observed, the frequency of linear asci increases as compared with the control (+/+;*Pk*/+), and the relative frequency of asci which closely resemble linear also increases, for example asci with only one spore out of line. For the purposes of classification, linear asci were defined as those from which it would have been possible unambiguously to isolate ordered tetrads of eight spores. All other types of asci were classified as nonlinear. In effect, the method of classification minimizes the effects of the modifiers.

While precise data have not been collected for intra- and interperithecial variation in the frequency of linear asci, qualitative inspection does not reveal such variation to be nonrandom. In all cases, asci for classification were from perithecia taken at random to minimize possible errors from such variation. All intact asci from a given perithecium were classified and counted.

RESULTS

Effects of modifiers as heterozygotes: The procedure employed for the isolation and determination of modifier strains is shown in Figure 1. In all, 209 sorbose-resistant strains were isolated, and tetrads were examined from crosses of some of these strains to wild type. In each of these tetrads the sorbose-resistant trait segregated as a single nuclear gene. An example of a tetrad showing such a segregation is shown in Figure 2. The upper two colonies are the sorbose-sensitive wild-type segregants, and the lower two colonies are the sorbose-resistant segregants. Four different sorbose-resistant strains proved to be modifiers of the ascus phenotype initiated by a *Pk-1*/+ heterozygote when they were included in a

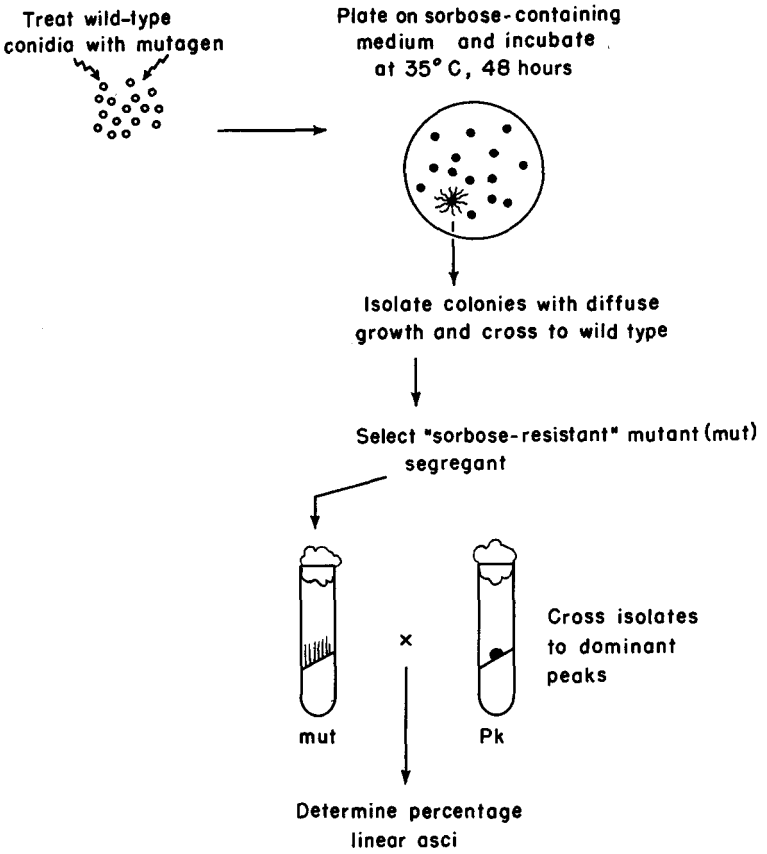

FIGURE 1.—Procedure for the isolation and determination of modifier strains.

zygote of the type: *Pk-mod*/+;*Pk-1*/+. A fifth modifier was identified when the independently isolated sorbose-resistant strain, *sor(DS)*, was crossed with *Pk-1*. The results of crosses of these five modifiers, and of wild type, with the five dominant *peak* alleles available are shown in Table 1. Both the modifier gene and the dominant *peak* gene in the zygotes represented in this table are heterozygous with their respective wild-type alleles. The figures are the results of examination of asci from replicate crosses of each type. Some degree of specificity may be observed for these modifiers of *Pk-1* in that they affect the other dominant *peak* alleles in different ways. In many cases, no modification is observed, whereas in other cases there is a significant change in the percentage of linear asci when compared to the value shown by the control cross the generalized genotype of which is: +/+; *Pk*/+. *Pk-mod-A* and *Pk-mod-B*, for example, are similar in that they result in positive modification of the dominant *peak* alleles *Pk-1*, *Pk-3*, *Pk-4* and *Pk-5*, when they are included in zygotes heterozygous for these alleles. Despite this identity of modifier specificity these two modifiers have been shown to be nonallelic. The pattern of specificity shown by *Pk-mod-A* and *Pk-mod-B* differs from that of *Pk-mod-C* and from that of *Pk-mod-D*. The latter two modi-

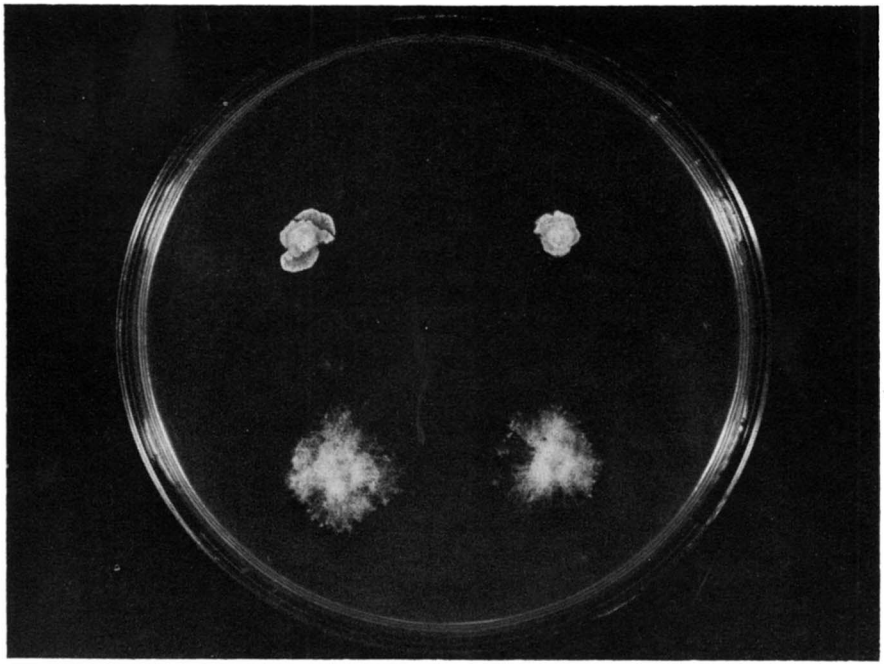

FIGURE 2.—A Petri plate of sorbose-containing medium (see MATERIALS AND METHODS) showing colonies representing the four members of a tetrad isolated from a cross of a sorbose-resistant mutant with wild type. Inocula for the colonies were vegetative transfers from the cultures derived from ascospore isolates. The upper two colonies are the sorbose-sensitive (wild-type) segregants, and the lower two colonies are the sorbose-resistant segregants. The plate was incubated for 48 hr at 35°C.

fiers also differ from one another in their specificity pattern. The independently isolated mutation for sorbose resistance, *sor(DS)*, also a modifier of *Pk-1*, does not result in positive modification of any of the other dominant *peak* alleles, although when it is included in a zygote heterozygous for *Pk-3* a very significant decrease in the percentage of linear asci is observed as compared to that shown by the control cross. Such "negative" modification may be seen to a lesser extent in zygotes heterozygous for *Pk-4* that include the modifier gene *Pk-mod-C*. Thus modification of the dominance relationships exhibited by a *Pk*/+ heterozygote may occur in either direction, towards increasing or decreasing dominance of the *peak* allele.

Presumably these five modifiers represent only part of the potential array of modifiers that exist for these dominant *peak* alleles, inasmuch as if we had initially selected for modifiers of a dominant *peak* allele other than *Pk-1*, we might well have obtained a different set of modifiers with a different pattern of modifier specificity. It is also apparent that modifiers with the effect of negative modification of these dominance relationships might be found if a suitable selection method could be devised.

Correlation of sorbose resistance with dominance modification: In order to test

TABLE 1

Effect of heterozygous modifier genes on the dominance relationships of Pk/+ *heterozygotes**

Genotype: Modifier locus	Genotype: *Peak* locus heterozygote (*Pk*/+)	Ascus phenotype: Percentage linear asci†
+/+ (wild type)	*Pk-1*	3.5 ± 0.9 (90)
	Pk-2	19.6 ± 7.2 (15)
	Pk-3	77.3 ± 7.1 (10)
	Pk-4	26.7 ± 5.5 (16)
	Pk-5	30.5 ± 4.4 (13)
Pk-mod-A/+	*Pk-1*	10.3 ± 4.4‡ (39)
	Pk-2	19.7 ± 6.0 (3)
	Pk-3	92.4 ± 1.7‡ (2)
	Pk-4	58.2 ± 6.1‡ (4)
	Pk-5	57.3 ± 4.4‡ (3)
Pk-mod-B/+	*Pk-1*	11.8 ± 2.5‡ (12)
	Pk-2	18.4 ± 1.4 (2)
	Pk-3	96.3 ± 2.1‡ (6)
	Pk-4	47.7 ± 3.8‡ (2)
	Pk-5	51.8 ± 8.7‡ (5)
Pk-mod-C/+	*Pk-1*	10.5 ± 3.4‡ (17)
	Pk-2	37.4 ± 2.0‡ (2)
	Pk-3	83.3 ± 2.4 (2)
	Pk-4	11.8 ± 1.6§ (2)
	Pk-5	55.0 ± 3.1‡ (2)
Pk-mod-D/+	*Pk-1*	10.3 ± 1.1‡ (13)
	Pk-2	21.5 (1)
	Pk-3	81.4 (1)
	Pk-4	41.5 ‡ (1)
	Pk-5	43.2 ‡ (1)
sor(DS)/+	*Pk-1*	10.0 ± 1.1‡ (13)
	Pk-2	15.5 (1)
	Pk-3	15.5 § (1)
	Pk-4	31.1 (1)
	Pk-5	24.4 (1)

* The percentage data were analyzed without transformation for statistically significant differences at 5% confidence limits by the use of DUNCAN's multiple range test (FEDERER 1955).

† Mean ± standard deviation. The number of replicate crosses examined in each case is indicated in parentheses. Approximately 200–300 asci were classified and counted from each cross.

‡ Significant increase in the percentage of linear asci when compared with control value (+/+ at modifier locus).

§ Significant decrease in the percentage of linear asci when compared with control value (+/+ at modifier locus).

more precisely the genetic basis of the modifier phenotype and whether or not it was directly associated with the sorbose-resistant gene mutation, tetrads were isolated from crosses of each of the five modifier strains with wild type. Cultures

TABLE 2

Tests of a tetrad segregating for a gene that determines sorbose resistance and that acts as a modifier of a Pk-1/+ *heterozygote**

Spore position in ascus	*Pk-mod-A*/+ tetrad Mating type	Phenotype on sorbose-containing medium†	Percentage linear asci‡
1	A	S	3.00
2	A	S	2.25
3	A	R	10.00
4	A	R	10.25
5	a	S	3.75
6	a	S	3.00
7	a	R	11.75
8	a	R	9.00

* The tetrad was obtained from a cross of wild type with a strain carrying a gene for sorbose resistance. Cultures derived from each ascospore of this tetrad were tested for modification of the effects of dominant *peak* and the wild-type allele of *peak* on ascus morphology by crossing them to a strain carrying the *Pk-1* allele. The same cultures were tested for sensitivity or resistance to sorbose.

† S = sensitive. R = resistant.

‡ 400 asci were classified and counted in each case.

derived from these tetrads were tested for resistance or sensitivity to sorbose, and were then crossed with *Pk-1* to test for the segregation of the modifier phenotype. For each of the five modifiers the results indicated that the modifier phenotype always segregated with resistance to sorbose, indicating that these attributes are almost certainly the effects of the same mutant gene. An example of the results obtained from such a test, that of the members of a tetrad from a cross of *Pk-mod-A* with wild type, crossed with *Pk-1* as a tester, is shown in Table 2.

Effects of modifiers as homozygotes: Table 3 shows the effects of certain homozygous modifiers on the dominance relationships of some of the different dominant *peak* alleles upon which the modifiers are active as heterozygotes. The prototype zygote represented in this table is of the genotype: *Pk-mod/Pk-mod;Pk/+*.

TABLE 3

Percentage reduction in the frequency of linear asci from Pk/+ *heterozygotes when modifiers are homozygous as compared to heterozygous*

Genotype: Modifier locus homozygote (*Pk-mod/Pk-mod*)	Genotype: *Peak* locus heterozygote (*Pk/+*)	Ascus phenotype: Percentage reduction in frequency of linear asci*	Ascus phenotype: Total number of asci counted
Pk-mod-A	*Pk-1*	37.9 (6.4)	2,731
Pk-mod-A	*Pk-4*	22.5 (45.1)	419
Pk-mod-B	*Pk-3*	53.4 (44.9)	730
Pk-mod-B	*Pk-5*	84.4 (8.1)	835
Pk-mod-C	*Pk-5*	55.3 (24.6)	122

* Control values for heterozygous modifiers used for the calculations were taken from Table 1. The figures in parentheses are the actual percentages of linear asci for the given genotypes with homozygosity at the modifier locus.

Since the observed frequency of linear asci when the modifiers were homozygous was always lower than when they were heterozygous, the data are presented in this table as the percentage reduction in the frequency of linear asci. When homozygous, different modifiers showed different degrees in the reduction in the frequency of linear asci. Nevertheless, the results as a whole, at least for the particular genotypes tested, indicate that the optimal positive modification of the *Pk*/+ dominance relationship occurs when the modifier is heterozygous with its wild-type allele. Since not all possible genotypes were tested, we cannot as yet give a firmer generalization.

Absence of effect of modifiers on homozygous dominant or recessive peak: For five zygote types in which the modifier was heterozygous and the dominant *peak* allele was homozygous, *Pk-mod*/+; *Pk/Pk*, no effect of the modifier was observed; that is, the number of linear asci initiated by these zygotes was not larger than that from control zygotes of the type: +/+; *Pk/Pk*. Similarly, in comparable representative zygotes, but where the modifier was also homozygous (*Pk-mod/Pk-mod*; *Pk/Pk*), no effect of the modifier was observed. From these results we may conclude that the modifiers change the dominance relationship between the dominant *peak* allele and its wild-type allele, but do not affect the morphogenetic system when only the dominant *peak* allele is present.

Although none of the modifiers had any effect on dominant *peak* alleles *per se*, we thought it worthwhile to test the modifiers for possible effect in the presence of a recessive *peak* allele, *pk-2*. Asci initiated by a homozygous *pk-2/pk-2* zygote are predominantly nonlinear, with only a rare linear ascus being produced. Thus, any positive modification resulting from a modifier being included in a homozygous *pk-2* zygote would be readily detectable. The results of counting asci derived from three such zygotes are presented in Table 4. These results show no significant increase in the percentage of linear asci over that shown by the control zygotes, indicating that the modifiers had no detectable effect on the activity of the recessive *peak* allele, *pk-2*. No other recessive *peak* alleles were similarly tested and we do not yet know whether or not this absence of modifier effect is general for all recessive alleles at the *peak* locus. All present evidence, however, indicates that a wild-type allele at the *peak* locus must be present if the modifier effects on ascus morphology are to be manifested.

TABLE 4

Effect of modifier genes on the recessive peak *allele* pk-2

Genotype		Ascus phenotype
Modifier locus	*Peak* locus	Percentage linear asci*
+/+	*pk-2/pk-2*	0.46 (3)
Pk-mod-A/+	*pk-2/pk-2*	0.50 (8)
Pk-mod-B/+	*pk-2/pk-2*	0.25 (20)
Pk-mod-C/+	*pk-2/pk-2*	0.35 (3)

* The number of replicate crosses examined in each case is indicated in parentheses. Approximately 100–200 asci were classified and counted from each cross.

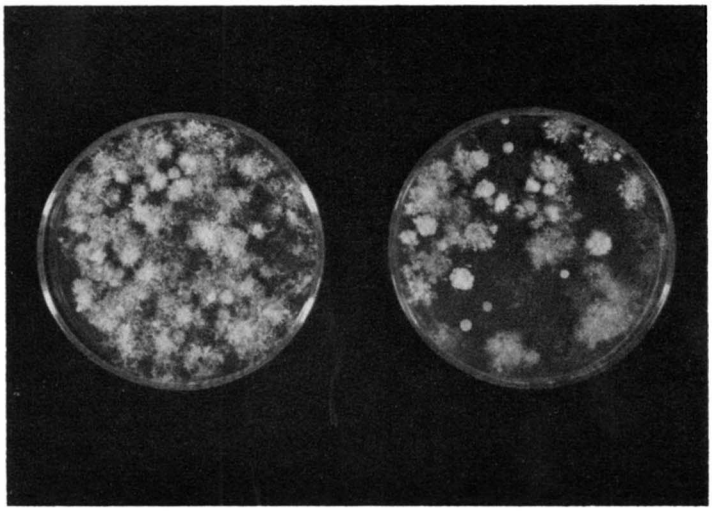

FIGURE 3.—The basis for determining allelism and nonallelism of modifier (sorbose-resistant) mutations. The sorbose-containing plates (see MATERIALS AND METHODS) were incubated for 60 hr at 35°C. Sorbose-sensitive (wild-type) and sorbose-resistant colonies may be identified by referring to Figure 2. Left plate: Colonies derived from ascospores from a cross of two sorbose-resistant mutants. All colonies are sorbose-resistant. The mutations are probably allelic. Right plate: Colonies derived from ascospores from a different cross of two sorbose-resistant mutants. Both sorbose-sensitive (wild-type) and sorbose-resistant colonies are seen. The mutations are nonallelic.

In all cases in which both a modifier and a *peak* allele, either recessive or dominant, were present in the same strain, the phenotype of the mycelium on minimal medium was always that of the *peak* mutant. This suggests that any pronounced effect of the modifiers is limited to the sexual phase of the life cycle. None of the modifier genes was found to be linked to the *peak* locus.

Allelism and nonallelism of modifiers: In order to test whether or not the modifier genes were allelic, all possible pairwise crosses of the five modifiers were made. Ascospores resulting from these crosses were plated on sorbose-containing minimal medium and incubated at 35°C. After 60 hr the plates were examined for sorbose-sensitive colonies. Since the modifier and sorbose-resistant phenotypes have been shown probably to be the result of the same gene mutation, for practical purposes two modifiers would be considered to be alleles if most, if not all, the colonies were sorbose-resistant. Nonallelic modifiers would be identified by the presence of many sorbose-sensitive (wild-type) colonies on the sorbose-containing minimal medium. This criterion for the distinction of allelic and nonallelic modifiers is illustrated in Figure 3. One probable case of allelism was discerned, that between *Pk-mod-D* and *sor(DS)*, on the basis that no sorbose-sensitive colonies were seen among 3,420 colonies derived from ascospores obtained after crossing these two sorbose-resistant strains. Crosses involving other pairs of modifier mutations gave evidence for substantial recombination.

DISCUSSION

Modifier genes may be defined as those that affect the phenotypic expression of genes at other loci. Since the definition is loose and phenotypic interactions are likely, one might expect modifiers to turn up frequently and in all sorts of organisms, as is indeed the case. Classical examples are the dominance modifiers described in mice by MATHER and NORTH (1940) and FISHER and HOLT (1944), and in fowl by DUNN and LANDAUER (1934, 1936) and FISHER (1935). Many modifiers are detected only by their effects on the expression of other, nonallelic genes, but certain modifiers, such as those described in this report of work with Neurospora, have phenotypic effects of their own.

In earlier work with Neurospora, GOOD, HEILBRONNER and MITCHELL (1950) reported that quantitative differences in growth substance utilization among different reisolates of a mutant strain were due to the influence of modifier genes. HASKINS and MITCHELL (1962) found a number of modifier genes that altered the ability of appropriate auxotrophs to grow on various compounds related to the tryptophan-nicotinic acid pathway. SRB (1953), from selection experiments, obtained modifier genes that caused changes in heterokaryotic dominance relations between two biochemical mutants of *Neurospora crassa*. DAVIS (1960) found an unlinked modifier of mutant alleles at the *pan-1* and *pan-2* loci of Neurospora that altered the uptake, but not the synthesis, of pantothenate.

Our experiments have been directed toward the modification of the effects of dominant mutant genes. In particular, we have asked whether, in a suitable situation, selection against the effects of an agent that induces phenocopies might not provide modifiers of phenotypically corresponding dominant mutants. The ideological background of these studies lies in FISHER's (1958) hypothesis to explain the usual dominance of wild-type over mutant alleles. He supposed that, at least in part, dominance has evolved from heterozygote intermediacy by means of selection of appropriate modifier genes. Since FISHER's time, increased understanding of the molecular attributes of gene action has gone far in removing the dilemma that led to his hypothesis (KACSER 1963; SRB 1966). Nevertheless, the reality of modifier effects on dominance continues to present implications for evolution in relation to dominance. And our results perhaps have special relevance to MULLER's (1932) concept of the selection of modifier genes to reduce the variability of characters influenced either by environmental or genic perturbations. The experiments reported here do show that selecting for modification of phenocopies may have the effect of providing modifiers for appropriate dominant mutations that are not involved in the selection process *per se* but are active in the same physiological and morphological system affected by the phenocopy-inducing agent. The possibility exists, therefore, that in nature the genetic adjustment of a population to particular environmental influences might provide and maintain an indirectly selected set of modifiers for certain potentially dominant mutations. In a sense, such modifiers might be preselected in advance of the occurrence of the mutation. The existence of dominance modifiers in natural populations has been shown by, among others, HELFER (1939).

The results reported here reveal the possibilities for extreme complexity in dominance relationships. These possibilities emerge most obviously from the varying patterns of specificity of particular modifiers for particular dominant mutant alleles. In most instances where modification occurs it is "positive," in that the effects are an increase in the dominance of the wild-type allele and therefore a decrease in the dominance of the mutant allele when heterozygotes are examined. However, in two instances "negative" modification could be observed. Thus the presence of the same modifier gene in a population might have the effect either of increasing or decreasing dominance, depending upon the presence of a particular mutant allele. Moreover, different modifiers show different effects depending upon whether they are present as heterozygotes or homozygotes. Inasmuch as the results strongly indicate that the modifiers are acting against the *Pk*/+ heterozygote rather than influencing the action of the mutant allele *per se*, one can readily imagine further complexity of dominance relationships depending upon modifier specificities in relation to different wild-type isoalleles in heterozygotes with mutants. We emphasize also that the modifiers described here represent only part of the potential array of modifiers for the five dominant mutations that have been studied, inasmuch as our selection methods almost surely have provided a restricted sample.

The lack of visible effect of the modifiers as heterozygotes when included in zygotes homozygous for a dominant *peak* allele does not preclude modification at a level different than that at which the observations were made. If the primary biochemical lesions were known, modification of homozygotes might be detectable at the biochemical level. In any case, knowledge of the mechanism of action of the modifiers must await elucidation of the biochemistry of the *peak* mutants.

The research was supported by Grant GM 12953 from the National Institute of General Medical Sciences, USPHS.

LITERATURE CITED

BARBER, J. T., A. M. SRB and F. C. STEWARD, 1969 Proteins, morphology and genetics in Neurospora. Develop. Biol. **20**: 105–124.

BARRATT, R. W. and L. GARNJOBST, 1949 Genetics of a colonial microconidiating mutant strain of *Neurospora crassa*. Genetics **34**: 351–369.

BEADLE, G. W. and E. L. TATUM, 1945 Neurospora. II: Methods of producing and detecting mutations concerned with nutritional requirements. Am. J. Botany **32**: 678–686.

DAVIS, R. H., 1960 Adaptation in pantothenate-requiring Neurospora. I. A gene modifying pantothenate mutants. Am. J. Botany **47**: 351–357.

DE TERRA, N. and E. L. TATUM, 1961 Colonial growth of Neurospora. Science **134**: 1066–1068. ——, 1963 A relationship between cell wall structure and colonial growth in *Neurospora crassa*. Am. J. Botany **50**: 669–677.

DUNN, L. C. and W. LANDAUER, 1934 The genetics of the rumpless fowl with evidence of a case of changing dominance. J. Genet. **29**: 217–243. ——, 1936 Further data on genetic modification of rumplessness in the fowl. J. Genet. **33**: 401–405.

FEDERER, W. T., 1955 *Experimental Design*. Macmillan, New York.

FISHER, R. A., 1945 Dominance in poultry. Trans. Roy. Soc. London B **225**: 195–226. ——, 1958 *The Genetical Theory of Natural Selection.* 2nd Edition. Dover, New York.

FISHER, R. A. and S. B. HOLT, 1944 The experimental modification of dominance in Danforth's short-tailed mutant mice. Ann. Eugenics **12**: 102–120.

GOOD, N., R. HEILBRONNER and H. K. MITCHELL, 1950 ε-hydroxynorleucine as a substitute for lysine in Neurospora. Arch. Biochem. **28**: 464–465.

HASKINS, F. A. and H. K. MITCHELL, 1952 An example of the influence of modifying genes in Neurospora. Am. Naturalist **86**: 231–238.

HELFER, R. G., 1939 Dominance modifiers of *Scute* in *Drosophila pseudoobscura.* Genetics **24**: 278–301.

KACSER, H., 1963 The kinetic structure of organisms. pp. 25–41. In: *Biological Organization at the Cellular and Supercellular Level.* Edited by R. J. C. HARRIS. Academic Press, New York.

KLINGMULLER, W., 1967 Kreuzungs-Analyse Sorbose-resistenter Mutanten von *Neurospora crassa.* Mol. Gen. Genet. **100**: 109–116.

MAHADEVAN, P. R. and E. L. TATUM, 1965 Relationship of the major constituents of the *Neurospora crassa* cell wall to wild-type and colonial morphology. J. Bacteriol. **90**: 1073–1081.

MATHER, K. and S. B. NORTH, 1940 Umbrous: a case of dominance modification in mice. J. Genet. **40**: 229–241.

MULLER, H. J., 1932 Further studies on the nature and causes of gene mutations. Proc. 6th Intern. Congr. Genet. **1**: 213–255.

MURRAY, J. C. and A. M. SRB, 1959 A recessive gene determining colonial growth of the mycelium and aberrant asci in *Neurospora crassa.* Proc. 9th Intern. Botany Congr. **2**: 276.

——, 1962 The morphology and genetics of wild-type and seven morphological mutant strains of *Neurospora crassa.* Can. J. Botany **40**: 337–349.

NOVAK, D. R. and A. M. SRB, 1971 Genetic alterations of ascus development in *Neurospora tetrasperma.* Genetics **67**: 189–199.

PERKINS, D. D., 1959 New markers and multiple point linkage data in Neurospora. Genetics **44**: 1185–1208.

PERKINS, D .D., D. NEWMEYER, C. W. TAYLOR and D. C. BENNETT, 1969 New markers and map sequences in *Neurospora crassa,* with a description of mapping by duplication coverage, and of multiple translocation stocks for testing linkage. Genetica **40**: 247–278.

PINCHEIRA, G., 1967 Studies of ascus differentiation in wild types and mutants of Neurospora. Ph.D. thesis, Cornell University, Ithaca, New York.

PINCHEIRA, G., and A. M. SRB, 1969 Genetic variation in the orientation of nuclear spindles during the development of asci in Neurospora. Am. J. Botany **56**: 846–852.

SRB, A. M., 1953 Shifts in heterocaryotic dominance relations effected by modifier genes in Neurospora. Genetics (Abstr.) **38**: 694. ——, 1966 Genetic control of physiologic processes. pp. 355–389. In: *Plant Breeding.* Edited by K. J. FREY. Iowa State University Press.

SRB, A. M. and M. BASL, 1969 The isolation of mutants affecting ascus development in *Neurospora crassa* and their analysis by a zygote complementation test. Genet. Res. **13**: 303–311.

TATUM, E. L., R. W. BARRATT and V. M. CUTTER, JR. 1949 Chemical induction of colonial paramorphs in Neurospora and Syncephalastrum. Science **109**: 509–511.