GENETIC CONTROL OF NUCLEAR SELECTION IN NEUROSPORA HETEROKARYONS[1]

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THE lack of stable diploids in Neurospora has made it necessary for geneticists using this organism to rely almost exclusively on heterokaryotic systems in genetic tests requiring heterozygosity. Following the pioneering work of BEADLE and COONRADT in 1944 on heterokaryosis, it has been repeatedly demonstrated that when compatible strains of the appropriate genotypes are employed, heterokaryotic systems can be exteremely useful in the study of a variety of genetic phenomena. However, if valid conclusions are to be drawn from experiments using heterokaryotic systems merely as a tool, the systems themselves must first be thoroughly understood. Although heterokaryons probably are more widely used now than ever before, many purely biological aspects of heterokaryotic growth and behavior are still incompletely understood, partially because of the scarcity of information regarding the interaction of genetically different nuclei in such systems. That the ability of different strains to form a stable heterokaryon is genetically controlled (GARNJOBST 1953, 1955; HOLLOWAY 1955) has been known for some time. Still many genetic factors effecting heterokaryotic compatibility and growth remain undescribed. More important perhaps, the modes of action of such genes, although unknown, are obviously different in some cases from the cytoplasmic incompatibility described by GARNJOBST and WILSON (1956) and WILSON, GARNJOBST and TATUM (1961). If the phenotypes of heterokaryotic cultures are to provide useful criteria for assessing nuclear interactions, more information is needed concerning the extent to which genetic factors directly control the many abnormal phenotypes regularly encountered in the growth of heterokaryotic cultures. Such incompatible phenotypes include not only complete lack of heterokaryotic growth, but also labile growth rates, cyclic patterns of growth and cessation of growth, submaximal growth rates, normal growth followed after a time by complete cessation of growth, or no growth for various lengths of time followed by growth, either maximal or submaximal.

Since some of the same growth phenotypes used to define compatibility and incompatibility are also used in assessing complementation and noncomplementation, it is most important to be able to distinguish between factors effecting

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these two phenomena. For example, in intra-allelic complementation studies it is commonly found that two mutant strains, A and B, may complement, and that A also complements strain C, but B and C do not complement. It is apparently not commonly known, however, that exactly the same situation may exist in regard to compatibility reactions, and moreover, that such reactions can be demonstrated with nonallelic nutritional mutants (HOLLOWAY 1953). Such observations suggest that homozygosity of certain genetic factors is not enough in itself to insure compatibility; interactions between nonallelic genes must also be considered as contributing to incompatibility reactions. Although it is routinely possible to avoid the more obvious complications by striving for homozygosity, it is not completely clear whether such a condition is always desirable. This may be inferred from the observations that many single gene differences arising through mutation may have a more adverse effect when they occur in homozygous strains (EMERSON 1947, 1948; EMERSON and CUSHING 1946; RYAN and LEDERBERG 1946; and DAVIS 1960b), although it remains to be shown whether such mutations would behave similarly in more heterogeneous systems in all cases. In any event it is clear that when heterokaryons are used as a tool in studying genetic phenomena, every precaution must be taken to make certain that the observed phenomena do not result from interactions of genetic factors effecting heterokaryosis itself rather than from the genetic system under investigation.

Although the many problems inherent in studying genes whose only phenotype is recognized in heterokaryons makes such an analysis a formidable one, this study was initiated with the idea of searching for genes whose interaction is manifested as nuclear competition. In studies of genetic factors effecting heterokaryon compatibility the authors have been impressed with the number of strains eventually classified as incompatible which actually grow as heterokaryons for considerable distances in growth tubes before stopping. These observations suggested that such behavior may be due to a nonadaptive increase in one of the nuclear components resulting in homozygosity. To determine more precisely if such mechanisms are commonly operative, methods especially suited for detecting such behavior were developed. This report is concerned with a description of an allelic pair of genes, designated *I* and *i*, which have just such an effect and whose phenotype at present can be detected only in heterokaryons. Nuclei carrying *I* are operationally able to inhibit the normal multiplication of *i* nuclei under specified conditions leading to a nonadaptive increase in *I* nuclei. However, *I* is classified as being weakly dominant since, if the proportion of *i* nuclei is approximately .70 or greater, *I* nuclei are then incapable of affecting the division rate of *i* nuclei and growth is normal.

Theoretical notations and descriptions of the system used to detect nuclear selection in Neurospora heterokaryons: It previously has been demonstrated that the nuclear proportions in heterokaryons among compatible strains carrying different nutritional mutants show no systematic change during prolonged growth under varying environmental conditions. The stabilizing mechanism in such balanced heterokaryons is a nonadaptive one and operates to maintain the status quo even when a change would be advantageous in terms of growth rate of

the culture. The stabilizing mechanism involves an active transport system, plus being consistent with the hypothesis that different nuclei in the same heterokaryotic culture have the same rate of nuclear division (PITTENGER and ATWOOD 1956). DAVIS (1959) confirmed that hypothesis by showing that the nuclear ratios in heterokaryons maintained on minimal agar slants through several conidial transfers stabilized around 50 percent of each nuclear type as would be expected if nuclear division rates were equal and heterokaryotic conidia were the only contributors to each new transfer generation. This does not mean, however, that labile growth rates do not occur, nor that such rates are not correlated with changes in nuclear ratios. Systematic changes in nuclear proportions may and do result from combinations of genotypes that do not fulfill the conditions of equal growth rates. The unstable type III heterokaryon described by HOLLOWAY (1955) is an excellent example, as are the non-adaptive growth reported by RYAN and LEDERBERG (1946) and a similar case reported by DAVIS (1960b).

That different nuclei in a heterokaryon normally divide at the same rate can be demonstrated by using conidial-derived heterokaryons. When conidia from a heterokaryon are plated on minimal sorbose medium, each colony is assumed to have originated from a single heterokaryotic conidium. A culture derived from such a conidium will be referred to as a conidial-derived heterokaryotic culture. It has been demonstrated that heterokaryons grown on minimal medium have an average of between two and three nuclei per conidium (PROUT, HUEBSCHMAN, LEVENE and RYAN 1953; HUEBSCHMAN 1952; ATWOOD and MUKAI 1955). If rates of nuclear division are equal, a first approximation prediction would be that cultures established from heterokaryotic conidia should have nuclear proportions similar to those found in individual heterokaryotic conidia, that is, ratios of approximately 1:1, 2:1, 1:2, and less often 3:1, 1:3, 2:3, 3:2, 4:1 and 1:4. Approximations of such ratios have been found repeatedly in our laboratory in conidial-derived heterokaryotic cultures using a variety of nutritional mutants. In certain nuclear combinations the frequency of the more disparate nuclear ratios is higher than expected. In a few cases these can be accounted for by the presence of mycelial fragments from heterokaryons with disproportionate nuclear ratios, but in most instances a lack of synchronism in the early nuclear divisions within germinating conidia is probably involved. This lack of synchronism of nuclear divisions within conidia can be demonstrated cytologically (C. SOMERS, personal communication) and undoubtedly often leads to some distortion of the expected nuclear ratio in heterokaryons derived from single conidia. For example, if a conidium has two type-A and one type-B nuclei and if both type-A nuclei divide once before the type-B nucleus divides at all, and thereafter all nuclei divide at an equal rate, the ratio in the mature heterokaryon would approximate four A to one B rather than the expected two A to one B. During this period of germination and early growth of the conidium before an equilibrium in cellular processes is reached, many possible mechanisms might distort somewhat the initial nuclear ratio present in individual conidia.

Although the nuclear ratios in conidial-derived heterokaryotic cultures can usually be accounted for by nuclear ratios known to exist in heterokaryotic

conidia, whether the exceptions are stable and have genetic bases has never been systematically determined. Under the usual experimental conditions, however, deviations from the expected ratio are detected only when special techniques involving morphological mutants are employed. Albino-1 (4637T) and albino-2 (15300) have been routinely used in our laboratory as morphological markers in conjunction with nutritional mutants used in heterokaryotic cultures. These complementary nonallelic albino mutants, when present in different nuclear components of the heterokaryon, impart a normal or near normal light orange color to the conidial mass of the cultures in a variety of nuclear ratios. However, when the heterokaryons have certain disproportionate nuclear ratios in favor of the albino-1 nuclear component, colors of the conidial mass may range from dark yellow to very light yellow or even white in the most extreme ratios. Similarly, in heterokaryons having a very high proportion of albino-2 nuclei, colors range from very light pink to white, the exact color again depending upon the nuclear ratio. The proportion of albino-2 nuclei must be quite high before a color change can be phenotypically recognized as different from the normal wild-type coloration, but a slight excess of albino-1 nuclei can usually be recognized phenotypically. This range in color of the conidial mass of heterokaryotic cultures makes it very easy to detect disproportionate nuclear ratios when albino-1 and albino-2 are present in the respective nuclear components. Although it has not been possible to correlate within narrow limits the nuclear ratios of a culture with its color, and although there is some overlap, it is nevertheless easy to detect the extreme deviate from the normal nuclear ratio in heterokaryons derived from single heterokaryotic conidia (Table 1).

In addition to albino-1 and albino-2, strains of the *nic-2* mutant (4540T) are very useful in heterokaryons because this mutant accumulates a reddish brown pigment in the media when nicotinic acid becomes limiting (BONNER and BEADLE 1946). In a heterokaryon with as much as 95 percent *nic-2* nuclei this color may not be noticeable, but if the nuclear proportion becomes more extreme in favor of the *nic-2* component and the other nucleus cannot supply a sufficient amount of the nutritional requirement, a red-brown to dark yellow pigment is found in

TABLE 1

The relationship between the color of conidial-derived heterokaryotic isolates and the proportion of al-1 *and* al-2 *nuclei in these cultures**

Color phenotype	Number of isolates tested	Proportion of albino-1 or albino-2 nuclei	
		Mean	Range
Wild type	25	.60 *al-1*	.39 *al-1*– .80 *al-1*
Dark-to-medium yellow	13	.82 *al-1*	.58 *al-1*– .91 *al-1*
Light yellow	30	.95 *al-1*	.83 *al-1*–>.99 *al-1*
White (albino-1)	9	.99 *al-1*	.98 *al-1*–>.99 *al-1*
Light pink	16	.93 *al-2*	.79 *al-2*– .98 *al-2*
White (albino-2)	34	>.99 *al-2*	.98 *al-2*–>.99 *al-2*

* The majority of these isolates were from *pan al-1 A* and *nic-2 al-2 A* heterokaryons.

the media. For example, if a heterokaryon between *pan al-1* and *nic-2 al-2* had a nuclear ratio so unequal that the culture had a white phenotype and growth was submaximal, one usually could tell whether the *pan al-1* or *nic-2 al-2* component was in excess by the presence or absence of the brown pigmentation in the media. This phenotype has proved extremely useful for detecting change in nuclear proportion during growth of the heterokaryons in growth tubes.

In the experiments described below extensive use was made of color phenotypes to detect nuclear selection that resulted in highly disproportionate nuclear ratios. More specifically, heterokaryotic conidia were isolated from a large variety of different heterokaryons between various complementary nutritional mutant strains marked with albino-1 and albino-2. When conidial-derived heterokaryotic cultures had a wild-type coloration, the nuclear ratios were considered to be within the range expected to occur in individual conidia. On the other hand, when heterokaryotic conidia produced mature cultures, the majority of which deviated significantly from wild-type coloration, such heterokaryons were investigated to determine if nuclear competition was responsible. In most cases phenotypically-white, conidial-derived heterokaryons were not stable and conidia from such white cultures produced normally colored isolates. Certain heterokaryotic cultures, however, had deviant color types that remained stable, and the *I-i* system described below was discovered in that way.

EXPERIMENTAL RESULTS

Growth behavior of I-i *heterokaryons:* In order to illustrate the interaction of an allelic pair of genes controlling nuclear selection, the growth behavior of various heterokaryotic combinations between the strains described below will be examined. The action of these allelic genes has been studied in a variety of genetic backgrounds, but the salient features of *I-i* interaction can be adequately demonstrated by using only a few strains. Although we have also used mutant strains of *lys-3* (4545), *tryp-1* (10575) and *nic-1* (3416) in combination with *al-1*, *al-2*, *I* and *i*, the behavior of *I* and *i* are described primarily with reference to the following strains: (1) *pan al-1 23A I*, (2) *nic-2 al-2 A I*, (3) *pan al-1 3A I*, (4) *nic-2 al-2 A i*, and (5) *pan al-1 24 A i*. The *pan-1* allele used in these studies was 5531, the *nic-2* allele was 4540T, and *al-1* and *al-2* were 4637T and 15300, respectively (BARRATT, NEWMEYER, PERKINS and GARNJOBST 1954). The designation *nic i*, *nic I*, *pan i*, *pan I*, or simply *I* and *i* often are used, but the reader should bear in mind that the *nic* strains always were marked with *al-2*, and the *pan* strains always carried the *al-1* marker. Strains 1 and 2 above have been used for a good many years in our laboratory, but strains 3, 4, and 5 recently have been recovered from the progeny of crosses of strains 1 and 2 to a standard wild-type strain 74-OR-8-1a. This strain is referred to as 74a and was kindly furnished by DR. F. J. DESERRES. It is also important to note that all of the strains above (except 74a) were carefully selected because they are heterokaryotically compatible with one another and with common tester strains employed in our laboratory.

In the absence of incompatibility factors it has been generally observed that heterokaryons of the same mating type grow at normal rates (BEADLE and COON-

RADT 1944) and that the proportions of the two nuclear components show no consistent change during growth (PITTENGER and ATWOOD 1956). Heterokaryons between two *I* strains, for example, *pan al-1 A I* and *nic-2 al-2 A I*, have these attributes in a wide variety of nuclear ratios (Table 2A). By using different input ratios of the two nuclear types (for methods see PITTENGER, KIMBALL and ATWOOD 1955) one can produce heterokaryons that have a high proportion of either of the two nuclear components. Nuclear ratios in such heterokaryons show no systematic change and the rate of growth is normal unless nuclear proportions are extremely disparate (Table 2A). The nuclear ratios in all such cultures were determined by the method described by ATWOOD and MUKAI (1955) and a $\bar{n}$ value of 2.5 was used in calculating all such ratios.

The growth behavior of such heterokaryons may vary widely, however, when heterozygosity of *I* and *i* is involved. Consider an experiment (results presented in Table 2B) in which *pan al-1 A I* and *nic-2 al-2 A i* were used. When a heterokaryon with a high proportion of *nic i* nuclei was prepared the growth was constant at wild-type rates and the nuclear ratios at the proximal and distal ends of the growth tubes showed no systematic change in favor of *I* or *i* nuclear types. On the other hand, when the input ratio of *I* to *i* was approximately 1:1, or when *I* nuclei were in excess, the behavior of the heterokaryons differed widely from the behavior of those with *i* in excess. The behavior of these heterokaryons (Table 2B) suggests a nonadaptive nuclear change. The heterokaryons were nearly white, indicating extreme nuclear disproportion favoring one of the albino components, and they grew only a short distance (from 55 to 170 mm) before stopping completely. The nuclear ratios, determined from the conidia produced in the proximal ends of these growth tubes, showed the cultures to be essentially homokaryotic for the *pan al-1 A I* nuclei, although a few heterokaryotic conidia were recovered. When conidia could be obtained from these cultures after growth had stopped, they were homokaryotic for *pan al-1 A I*. It is hypothesized that such cultures stopped growing because they had become homokaryotic.

The essential neutrality of the *pan* and *nic-2* markers in the system can be shown by noting the behavior of the heterokaryon between *pan al-1 24A i* and *nic-2 al-2 A I* with the *I* and *i* reversed in regard to the nutritional and albino markers. Heterokaryons with high proportions of *pan i* nuclei had normal rates of growth with no significant systematic changes in nuclear ratios during growth (Table 2C). On the other hand, heterokaryons with initial high proportions of *nic-2 I* nuclei always stopped before reaching the end of the growth tubes; the media showed a dark brown discoloration throughout the area of growth indicating a very high proportion of *nic-2 I* nuclei. Analysis of conidia produced at the proximal ends of such growth tubes showed the cultures to be essentially homokaryotic for the *nic-2 I* component. When the initial mixing ratio was approximately 1:1, growth was normal for the first 24–36 hours. Growth then slowed and the dark brown discoloration of the media accompanied the slower rate. However, such cultures reached the ends of the 500 mm-long growth tubes. Frequencies of *nic-2 al-2 I* nuclei at the proximal ends of the growth tubes ranged from 93 to 99 percent. At the distal ends of the growth tubes only *nic-2 I* homo-

TABLE 2

The growth behavior and the nuclear proportions of various I-I, i-I, *and* i-i *heterokaryons grown in 500 mm-long growth tubes on Fries minimal medium at 30°C*

Heterokaryon and the approximate initial mixing ratio*	Proportion of specified nuclear types in opposite ends of growth tubes		Growth behavior
	Proximal	Distal	
A. *pan al-1 A I* + *nic-2 al-2 A I*			
100 *pan I*:1 *nic-2 I*	.95 *pan I*	.95 *pan I*	constant, but submaximal
1 *pan I*:1 *nic-2 I*	.64 *pan I*	.53 *pan I*	normal†
1 *pan I*:100 *nic-2 I*	.07 *pan I*	.06 *pan I*	normal
	.04 *pan I*	.04 *pan I*	normal
	.03 *pan I*	.12 *pan I*	normal
B. *nic-2 al-2 A i* + *pan al-1 23A I*			
100 *nic-2 i*:1 *pan I*	.86 *nic-2 i*	.78 *nic-2 i*	normal
	.93 *nic-2 i*	.94 *nic-2 i*	normal
	.96 *nic-2 i*	.95 *nic-2 i*	normal
1 *nic-2 i*:1 *pan I*	.01 *nic-2 i*	.00 *nic-2 i*	stops‡
1 *nic-2 i*:100 *pan I*	.01 *nic-2 i*	.00 *nic-2 i*	stops
C. *pan al-1 A i* + *nic-2 al-2 A I*			
10 *nic-2 I*:1 *pan i*	.93 *nic-2 I*	1.00 *nic-2 I*	stops
	.99 *nic-2 I*	1.00 *nic-2 I*	stops
	.98 *nic-2 I*	1.00 *nic-2 I*	stops
1 *nic-2 I*:1 *pan i*	.98 *nic-2 I*	1.00 *nic-2 I*	labile and submaximal§
	.95 *nic-2 I*	1.00 *nic-2 I*	labile and submaximal
	.93 *nic-2 I*	1.00 *nic-2 I*	labile and submaximal
1 *nic-2 I*:10 *pan i*	.90 *pan i*	.91 *pan i*	normal
	.88 *pan i*	.98 *pan i*	normal
	.71 *pan i*	.72 *pan i*	normal
D. *pan al-1 24A i* + *nic-2 al-2 A i*			
	.89 *pan i*	.90 *pan i*	normal
	.78 *pan i*	.84 *pan i*	normal
	.73 *pan i*	.67 *pan i*	normal
E. *lys-3 al-1 10a i* + *nic-1 al-2 22a i*			
	.93 *nic i*	.93 *nic i*	normal
	.83 *nic i*	.91 *nic i*	normal
	.85 *nic i*	.95 *nic i*	normal
F. *lys-3 al-1 10a i* + *nic-1 al-2 10a i*			
	.83 *nic i*	.84 *nic i*	normal
	.86 *nic i*	.85 *nic i*	normal
	.83 *nic i*	.85 *nic i*	normal

* When heterokaryons were prepared by superimposing conidia of the two components, no data were available for initial mixing ratios.
† Normal indicates growth was constant and ca. 4 mm/hr.
‡ Stops is interpreted to mean heterokaryon has become homokaryotic.
§ Culture probably would have stopped if growth tubes had been longer.

karyotic conidia could be recovered, though *pan i* nuclei should have been recovered even if only one in 10^6 was present. It seems clear that although these mixtures were initially heterokaryotic, they became homokaryotic as growth

proceeded and would have stopped had growth not been limited by the length of the growth tube.

The data presented indicate that normal growth and constancy of nuclear ratios in *I-i* heterokaryons depend on the initial ratio of *I* to *i* nuclei. Furthermore, Table 2 data suggest that when *i* nuclei are present in proportions greater than 70 percent, the nuclear ratios and growth rates are constant, or at least show no systematic changes. On the other hand when the proportion of *i* nuclei is less than 70 percent, the nuclear ratios change rapidly in favor of *I* nuclei, the nuclear mixture rapidly becomes homokaryotic in favor of *I* nuclei, and growth stops for lack of the growth factor needed by the now homokaryotic *I* nuclear component.

Will heterokaryons initially with high enough proportions of *i* nuclei to insure normal growth eventually show an increase in *I* nuclei? Such behavior has not yet been observed. For example, in one experiment a *pan al-1 24A i* strain was combined with a *nic-2 al-2 A I* strain with an initial frequency of 92 percent *pan i* nuclei. The heterokaryon was grown for one month in a continuous growth tube without being transferred and the nuclear proportions were regularly determined 12 different times. Although fluctuations in the nuclear ratio occurred during growth, the proportion of *pan i* nuclei at the end of one month's growth was 96 percent. The significance of this increase in *i* nuclei was reduced by fluctuations almost twice as great in the opposite direction during growth.

Many heterokaryons between *i* strains have been examined. The behavior of three are presented in Table 2 to show the variation encountered. Some nonsignificant (t test) changes in nuclear ratios were found. Thus, observations of *i-i* and *I-I* combinations have shown no systematic change in nuclear proportions like those found in *I-i* combinations.

The behavior of conidial-derived heterokaryotic cultures from I-i *combinations:* The demonstrated nonadaptive increase of *I* nuclei in heterokaryotic combination with *i* nuclei, should make possible the prediction of the growth behavior of cultures derived from heterokaryotic conidia. From the preliminary results presented, one would predict that heterokaryons with 70 percent or more *i* nuclei should be capable of normal growth. Expressing this same relationship in whole numbers, any conidium with a ratio of 3 *i*:1 *I* or greater should give rise to a culture with normal growth and most should have normal or near normal coloration. On the other hand, all heterokaryotic conidia with a greater proportion of *I* to *i* nuclei should show a nonadaptive increase in the *I* component and be nearly white from nuclear disproportion.

Analyses of unpublished data by PITTENGER and ATWOOD on the number of nuclei per conidium from a variety of different heterokaryons grown on minimal medium, including nuclear determinations of over 41,000 conidia, showed only 13.7 percent of the conidia with four or more nuclei. In an *I-i* heterokaryon, the percentage of such multinucleate conidia that are heterokaryotic is unknown, as is the percentage with ratios of *i* to *I* favorable to normal growth. Conceivably both percentages could be very small. Conversely one would expect a large percentage of heterokaryotic conidia to have ratios of *I* to *i* within the range where

I nuclei could inhibit *i* nuclei. Such cultures should have high proportions of *I* nuclei and abnormal color phenotypes.

In the course of this investigation thousands of heterokaryotic conidia were isolated from a variety of *I-I*, *I-i*, and *i-i* combinations which were heterozygous for *al-1* and *al-2* as well as for two different nutritional markers. The number of such isolates with wild-type coloration were compared with the number with colors indicating extreme nuclear disproportion. Classifying conidial-derived cultures according to color phenotypes has been the most satisfactory way to distinguish *I-i* heterokaryons from either of the homozygous combinations. A tabulation of some typical results is given in Table 3 and summarized as follows: When a heterokaryon is homozygous for either *I* or *i*, nearly all combinations analyzed showed that a majority of the cultures derived from single conidia had normal wild-type coloration, or the color was within the range expected from ratios known to occur regularly in conidia. Some exceptions, discussed later, were found; however, they most certainly resulted from factors other than *i*. If a heterokaryotic culture is isolated from an *I-I* or *i-i* combination and found to have a high proportion of one of the nuclear components, conidial-derived heterokaryotic isolates recovered from this culture usually will exhibit the normal color phenotype. In other words, these deviant types are not stable.

TABLE 3

The percentage of conidial-derived heterokaryotic isolates with normal color from various al-1 *and* al-2 *heterokaryons*

Heterokaryon	Number of isolates tested	Percent normal*	Percent abnormal
	I-I combinations		
pan al-1 23A I + nic-2 al-2 A I	232	76.0	24.0
nic-1 al-2 88a I + lys-3 al-1 a I	100	100.0	0.0
pan al-1 3A I + nic-1 al-2 55A I	145	100.0	0.0
	I-i combinations		
tryp-1 al-1 a i + nic-1 al-2 88a I	10	10.0	90.0
nic-2 al-2 A i + pan al-1 3A I	194	3.6	76.4
lys-3 al-1 10a i + nic-1 al-2 88a I	15	0.0	100.0
nic-2 al-2 A i + pan al-1 23A I	55	16.0	84.0
nic-2 al-2 A i + lys-3 A I	20	15.0	85.0
pan al-1 24A i + nic-2 al-2 A I	90	26.0	74.0
nic-1 al-2 22a i + lys-3 al-1 a I	64	3.1	96.9
	i-i combinations		
pan al-1 24A i + nic-2 al-2 A i	20	85.0	15.0
lys-3 al-1 10a i + nic-1 al-2 22a i	15	100.0	0.0
lys-3 al-1 10a i + nic-1 al-2 10a i	15	100.0	0.0
lys-3 al-1 10a i + nic-2 al-2 a i	20	95.0	5.0
lys-3 al-1 22a i + nic-2 al-2 a i	15	100.0	0.0
lys-3 al-1 18a i + nic-2 al-2 a i	14	100.0	0.0
lys-3 al-1 27a i + nic-2 al-2 a i	15	93.3	6.7
lys-3 al-1 29a i + nic-2 al-2 a i	15	100.0	0.0

* Normal means any phenotype expected on the basis of the nuclear ratios known to be normally present in conidia; dark to medium-yellow phenotypes were classified as normal.

The situation is quite different when heterokaryotic colonies are isolated from *I-i* combinations (Table 3). In such heterokaryons, regardless of the nuclear ratios present in the "parent heterokaryon," the majority of the conidia from such combinations give rise to new cultures having proportions of *I* nuclei far in excess of the nuclear proportions present in individual conidia. When *I-i* conidial-derived cultures were not white, they usually had a high proportion of *i* nuclei. The degree of nuclear disproportion attained in small test tube slants depended somewhat upon the nutritional mutants involved. For example, heterokaryotic colonies between *nic-2* and *pan* usually had a greater proportion of *I* nuclei when the *nic-2*, rather than the *pan* component, was *I*. The behavior of conidial-derived *I-i* cultures can also be determined further by observing the growth of heterokaryotic colonies transferred to growth tubes rather than to slants. Since the results are according to expectations, a detailed presentation of the data is not warranted. It will suffice to say that some single heterokaryotic colonies, when transferred to 500 mm-long growth tubes, were normal in color and growth rate, and the frequency of *i* nuclei in such cultures was always over 70 percent. The majority of the isolates, however, grew for varying distances but growth in the tubes always ceased as a result of homozygosity of the *I* component. Thus, the expected nonadaptive increase in the *I* nuclei was observed except in cases where the initial frequency of *i* nuclei was high enough to insure normal growth.

Descriptions of other I-i *and* i-i *interactions in various heterokaryotic combinations:* The *pan al-1 24A i* and *nic-2 al-2 A i* strains used extensively in the previous sections of this report were selected because the presence of *i* in them has been verified by its segregation in the progeny of crosses to *I* strains. Also a number of *i* strains have been isolated by crossing *I* strains to both ST 74A and 74a and also by crossing *nic-2 al-2 A i* with *lys-3 al-1 a I*. The progeny of these crosses have provided a large number of additional isolates that have been tested in many heterokaryotic combinations. Some are shown in Table 3. For example, *nic-1 al-2 88a I* and *nic-1 al-2 55A I* have been used extensively as *I* tester strains with many *i* isolates of various types. By crossing these two strains with 74A and 74a, it has been possible to recover *nic-1 al-2* strains (see *nic-1 al-2 22a i* and *nic-1 al-2 10a i*, Table 3) among random isolates which were *i*, demonstrating that both of these wild-type strains carry the *i* gene. That many mutants in common usage have been induced in 74A backgrounds and still form normal heterokaryons is ample evidence that *i-i* heterokaryotic combinations can show normal heterokaryotic behavior. That *i-i* combinations not in the more uniform 74A background also may behave normally is shown by data in Tables 2 and 3. Nevertheless, other genetic factors clearly affect *i-i* and *I-i* interactions because some such combinations fail to show the expected phenotypes. Nearly all of the exceptional phenotypes encountered involve one strain, *lys-3 al-1 a I*, or strains which were recovered from progeny of crosses in which this strain was one of the parents. For example, in a heterokaryon between *lys-3 al-1 a I* and *nic-1 al-2 22a i*, the *I* component undergoes the expected increase in proportion during growth until approximately 70 percent of the nuclei are *I* and then

no further increase in the *I* component takes place. Such heterokaryons, however, were white rather than the expected wild-type color when this equilibirium in the nuclear ratio was reached and the growth rate was constant but approximately half maximal. As an illustration, the behavior of one such heterokaryon follows. Conidia were initially mixed in a ratio of nine *nic-1 al-2 22a i* to one *lys-3 al-1 a I* and the conidial mixtures placed directly into a 1000 mm-long growth tube with nine sampling ports along its top at 100-mm intervals to facilitate removal of conidia to be used in the determination of nuclear ratios. The percentages of *lys al-1 a I* nuclei from these nine sampling ports, starting from the proximal end, were 30, 57, 64, 72, 73, 73, 69, 69, and 71. The conidia formed below the first three sampling ports had approximately normal wild-type color, below the fourth they were light yellow, and thereafter they were white. Initially the growth rate was 4 mm/hr, but approximately a third of the way down the tube growth slowed to ca. 2.0 mm/hr and remained constant thereafter. Similar results have been found using other *i* strains in combination with this one *lys* strain. In a cross of this same *lys*-requiring strain with *nic-2 al-2 A i*, approximately half of the *lys-3 al-1 a i* isolates behaved as one would predict in combination with several *i* and *I* strains but the remainder showed a variety of unexpected results in combination with both *i* and *I* testers. The genetic analysis of these deviant cases has not yet been completed, but it seems clear that the expected interaction betwen *i* and *i*, or *I* and *i* genotype may not be manifested in all cases probably because of the residual genotypes of the nuclei involved. Initially, attempts have been made to describe the phenotypic interactions which seem paramount without analyzing each deviant case, however, attention should be called to the deviant behavior if only to emphasize that other genetic factors often may mask the *i-I* interaction or perhaps be epistatic to these alleles.

Inheritance studies: Because inheritance studies of a gene whose phenotype can only be detected in heterokaryons is extremely laborious and complicated by the large number of tester strains needed, our data regarding the inheritance of *I* and *i* are not extensive. The results, however, appear to be straightforward and indicate that *i* and *I* are determined by a single factor which is located either on linkage group I or II .

The *I* or *i* genotype of a particular strain was determined by making a heterokaryon between the unknown strain and the appropriate *I* and *i* tester strains. Most of these heterokaryons were then placed in growth tubes and their behavior observed. Heterokaryotic conidia from each tested combination were isolated as colonies from minimal plates and placed on minimal agar slants. Color phenotypes were then used in conjunction with their growth behavior in growth tubes to determine the genotypes of the strains.

Since both *al-1* and *al-2* are needed in tests to simplify the identification of *i* and *I*, the segregation of these mutants was analyzed in a cross of *pan al-1 A I* and *nic-1 al-2 -10a i*. Although use of *al-1* complicates the analyses somewhat since it is inseparable from a reciprocal translocation involving linkage groups I and II (BARRATT *et al.* 1954), the use of this marker turned out to be quite fortunate.

Asci with eight normal spores were collected as unordered tetrads by the method suggested by STRICKLAND (1960). Such asci have no single crossovers in either interstitial segment of the translocation heterozygote and result from alternate segregation of the ring-of-four chromosomes at metaphase I. Analysis of 13 asci showed a 1:1 segregation of *I* to *i* in each ascus. Since the analysis of unordered tetrads gives no evidence of centromere distance and since the data is not extensive enough to definitely prove the location of *i*, the genotypes of each spore pair will not be enumerated here. It will suffice to mention that 16 *pan* isolates were *I* and 10 were *i* suggesting independent segregation of *pan* and *I*. However 18 of the *nic* isolates were *i* and 8 were *I*. Likewise 20 *a* isolates and 20 *al-2* isolates were *i*, and only six *A* and six *al-2* isolates were *I*. In a similar cross (*nic-1 al-2 a I* × *pan al-1 A i*) in which the *nic* marker came into the cross with *I*, 20 of the 24 random *nic*-isolates were *I* and only four were *i*. Although these results clearly show an association of the markers on linkage group I and the *i* locus, they do not prove that the locus is on linkage group I. The apparent ambiguous results are due to the pseudolinkage brought about by the association of *al-1* with linkage groups I and II as a result of the reciprocal translocation. On the basis of the limited tetrad data available, DR. DAVID PERKINS, who was good enough to examine the data, has suggested that linkage group II is an even more likely site of *i* than is linkage group I. However, the data are insufficient to warrant any definite conclusion and a detailed presentation of the data will be deferred until a more exact location of *i* is determined.

DISCUSSION

The experimental results clearly show that when *I* and *i* nuclei are present in a heterokaryon the proportion of the *I* nuclear component may undergo a nonadaptive increase which eventually leads to homozygosity of the *I* nuclei. Although it is possible to demonstrate under what conditions the increase in *I* nuclei takes place, it is not clear what mechanism is responsible for the increase. Several obvious possibilities were examined. An explanation based on heterokaryotic incompatibility of the type described by GARNJOBST and WILSON, (1956) and WILSON *et al.* (1961) was dismissed when it was found that *I-i* combinations could grow normally in certain ratios. If there is a direct relationship in heterokaryons between nuclear and cytoplasmic proportions, as suggested by certain experiments involving a mixture of a normal cytoplasm with a cytoplasmic mutant (PITTENGER, unpublished), then it is quite probable that the cytoplasm can be ruled out as having a controlling function in the division of *i* nuclei. It is known that conidial-derived cultures show a nonadaptive increase of *I* nuclei regardless of whether the conidia were formed by a culture with a high proportion of *i* nuclei and *i* cytoplasm or by a culture with a high proportion of *I* nuclei and *I* cytoplasm. Consequently, it appears that the cytoplasm of the parent culture, which is present in the conidia formed by such a culture, does not have an inherent role in regulating division rates of the two nuclear components.

Certain observations could be interpreted to inidicate that *I* and *i* nuclei have inherently different rates of nuclear division. If such an explanation were true,

however, one would expect always to find a systematic increase in the proportion of *I* nuclei during growth of heterokaryotic cultures. Such an increase in *I* nuclei was not found when the proportion of *i* nuclei was greater than .70. Heterokaryons with over .70 *i* nuclei, observed for over a month in continuous growth-tube cultures, showed no systematic increase in the *I* component. Thus, an interpretation based solely on an inherent difference in division rates of the nuclear components does not explain the data. On the other hand, a possible explanation for such behavior, suggested by PONTECORVO (1946) on theoretical grounds, is that nuclei of different kinds within a heterokaryon multiply at different rates, the rates depending on the proportion of the two nuclear types. Although such a postulate may explain the interaction of *I* and *i* in heterokaryons and may account for other instances of labile growth rates that have been observed, it is difficult to prove because of an unequivocal way of measuring nuclear division rates.

These observations are somewhat similar to another case of nonadaptive growth described by RYAN and LEDERBERG (1946), and as RYAN (1946) has pointed out, it is not known whether the minority nuclear component is actually destroyed, merely inhibited, or simply left behind as growth proceeds. In the present case, it is clear that *i* nuclei are neither destroyed nor left behind completely, although frequently the change from a heterokaryon to a homokaryon appears to be unusually rapid. Inhibition, at least in an operational sense, seems to be the most likely of the three alternatives. For example, in a *nic-2 al-2 A i* + *pan al-1 A I* heterokaryon grown in a growth tube with sampling ports, it was found that 25 mm from the onset of growth there were 47 percent *i* nuclei; at 100 mm only 11 percent *i* nuclei were found and at 250 mm no *i* or heterokaryotic *i-I* conidia were found among the 3690 viable conidia plated. Since the number of *i* nuclei estimated to be in the conidia alone at the two sampling ports is far in excess of the number added at the beginning of the growth tube, it is clear that they divided in the presence of *I* nuclei, but apparently at a rate different from *I* nuclei. Likewise cultures derived from heterokaryotic conidia may have very disproportionate numbers of *I* nuclei, and still have far in excess of the actual number of *i* nuclei present in the original conidium. Consequently, *i* nuclei can divide in the presence of *I* nuclei undergoing a nonadaptive increase in number. Growth rates and purely practical considerations, however, lead one to believe that it is the division rate of *i*, rather than *I*, nuclei that is altered, at least until the growth of *I* nuclei in the balanced heterokaryon is limited by the availability of the growth factor supplied by *i* nuclei. At the same time it appears reasonable to assume that different proportions of *I* nuclei might have different effects on the rate of multiplication of *i* nuclei.

Although it appears that the multiplication of *i* nuclei is inhibited in the presence of excess *I* nuclei, that *I* nuclei produce any substance capable of inhibiting or altering the division rate of *i* nuclei as measured by growth rates of *i* cultures has not been demonstrated in this laboratory. For example, homokaryons of *nic-2 al-2 A i* and *nic-2 al-2 A I* strains have equal growth rates as measured in growth tubes and as determined by dry weight of mycelia from

liquid shake cultures. *i* strains grow at the same rate in growth tubes supplemented with autolysates of either *I* or *i* strains. No effect on growth was noted when growing *i* cultures in sterile filtrates of liquid medium taken from shake cultures or *I* and *i* cultures. If the mycelia of *I* isolates are homogenized and added to wells in agar plates, the homogenates fail to inhibit the growth of *i* conidia seeded on the plates. Although every attempt to show that *I* strains inhibit the growth of *i* strains has failed, such an inhibitory substance may exist and be difficult to demonstrate because of the weakly dominant condition of *I* over *i*. Likewise growth rate in Neurospora need not show a good correlation with rates of nuclear division.

Though the possibility of an inhibitor's being involved in the interaction of *I* and *i* nuclei has not been completely ruled out, there is no compelling reason to prefer this explanation over others. For example, the data can be interpreted to indicate that the interaction of *I* and *i* involves competition for a substrate, conceivably one necessary for nuclear replication. Under this hypothesis *i* nuclei could successfully compete for such a substrate only when the proportion of *i* nuclei was greater than approximately .70. If an excess of such a substrate could be added to the growth media, normal growth of both nuclear types might be expected over a wide range of nuclear ratios. At present, however, such a hypothesis is unsupported because no supplement has been found that will prevent the nonadaptive increase of *I* nuclei. 1:1 mixtures of *I* and *i* conidia in two different heterokaryotic mixtures showed the usual increase of *I* nuclei on several different concentrations of yeast extract, vitamin mixtures, and amino acid mixtures as well as on single supplements of the sulfur-containing amino acids. Supplementing the medium with the growth factor of the *i* nuclear component had no stabilizing effect on the nuclear ratios. Likewise, supplementation with various DNA precursors has given only negative results. A search is continuing for some clue to the actual mechanism involved, whether it be inhibition, substrate competition or some other metabolic control system. But the operational behavior of these alleles in heterokaryotic combination is our only clue so far.

Heterokaryotic systems in Neurospora provide an excellent tool to determine dominance of the wild-type alleles of nutritional mutants, but very little work has been done on this problem. BEADLE and COONRADT (1944) were able to show that the normal allele of *pan-1* (5531) was strongly dominant. PITTENGER and ATWOOD (1956) refined that discovery by showing that only 3–4 percent of the *pan*$^+$ allele was needed to give normal growth. In comparison to the above case it would appear that *I* is only weakly dominant to *i*. Several attempts have been made to determine the exact proportion of *i* nuclei needed to prevent the increase in *I* nuclei during heterokaryotic growth, but this proportion has yet to be pinpointed. That is, it has not been demonstrated, for example, that a heterokaryon with 70 percent *i* nuclei will have a stable nuclear ratio whereas in the same experiment one with slightly less than 70 percent will show an increase during growth of *I* nuclei. Although frequencies of *i* nuclei approaching, but less than, 70 percent are undoubtedly formed, the rapid increase in *I* nuclei probably prohibits detection of such ratios. Nevertheless, a knowledge of the approximate

proportion of *i* nuclei needed for stable nuclear proportions during growth has been extremely useful not only in interpreting the growth behavior of heterokaryotic conidia, but also in explaining the exceptional types. Conidial-derived *I-i* heterokaryotic cultures with the normal wild-type coloration are routinely found in small proportions in certain *I-i* heterokaryons but are very rare in others. When present, they are almost invariably found to have a high enough proportion of *i* nuclei to insure normal growth and color. When heterokaryotic conidia from such cultures are isolated, they again give rise to cultures, the majority of which have a high proportion of *I* nuclei. Regardless of the explanation of the origin of such cultures, the important thing is that this phenotype usually is unstable.

The study of *I* and *i* in heterokaryotic combinations has not only demonstrated the importance of nuclear proportions in the expression of nuclear competition resulting in incompatibility, but also has emphasized the difficulties encountered in attempting to characterize compatibility and incompatibility. If heterokaryotic compatibility is provisionally defined as the ability of different nuclei to coexist in harmony in the same cytoplasm, then a compatible heterokaryon would be phenotypically recognized as one capable of growing at a constant rate comparable to the fastest growing nuclear component. Any heterokaryotic culture deviating from this norm would be considered incompatible. Nuclear competition resulting in homozygosity would thus be considered an incompatible reaction. The interaction of *I* and *i* demonstrates the difficulties inherent in such a definition since such a combination would appear to be either compatible or incompatible depending on the nuclear ratio present. A similar problem can exist in any forced heterokaryon which might exhibit a submaximal growth rate because of nuclear disproportion rather than from any inharmonious interaction between the nuclei. These two extremes emphasize the necessity of adequate testing to determine the phenotypic potentials of any heterokaryotic combination. Although trying to fit the behavior of these allelic genes into a preconceived notion of compatibility is largely a matter of semantics, certain ambiguous results in our studies also emphasize the difficulty of trying to make all of our observations conform to current thinking regarding the mechanism of genetic control of compatibility. The best evidence available on this point is the work of GARNJOBST (1955) on the *het*$^-$ factors, *C* and *D*, and their alleles. Her studies revealed that homozygosity of these genes is necessary for the formation of a stable, compatible heterokaryon. Many other cases of incompatibility (see particularly HOLLOWAY 1955) also have been shown to involve heterozygosity of certain genes. Apparently it has, therefore, been generally assumed that homozygosity of genes controlling heterokaryon compatibility is a prerequisite for normal heterokaryotic growth. This generalization is obviously true as far as it goes because in the absence of heterozygosity there are no heterokaryons. However, what kinds of genes affect heterokaryotic compatibility and must be homozygous remains a question. Continued study may show that heterozygosity of almost any gene, in the proper genetic background, has the potential of affecting compatibility. For example, in the present studies we have observed that two

different *i* isolates give the expected phenotypes with a common *i* and *I* tester, but with a different *I* tester one of the *i* strains gives the expected phenotype and the other does not. This suggests that the residual genotype of the *I* and *i* strains may either enhance or prevent the full expression of these genes. These observations and others more pertinent noted below suggest that some genes may be responsible for incompatibility in some combinations but have no effect in other combinations. That is, compatibility may involve not only interaction between alleles, but also interaction between nonalleles.

Returning to the question of the kind of genes that affect compatibility, the study of RYAN and LEDERBERG (1946) demonstrates that a gene which functions in the synthesis of an essential amino acid may also affect incompatibility. They showed that on leucine-limiting medium a nonadaptive increase of a leucine-requiring nucleus took place when it was in heterokaryotic combination with a wild-type strain which had arisen by back mutation of the leucine mutant. Thus they demonstrated a nonadaptive increase in one nuclear type which presumably differed from the other nuclear component by a single gene. Hence this incompatibility is attributable to heterozygosity of a single gene not ordinarily thought of as influencing compatibility. On the other hand, RYAN (1946) reported that the nonadaptive increase of the leucineless mutant failed to take place when the wild-type component of the heterokaryon did not arise by reversion of the leucine mutant. Since these two wild-type strains appear to be different in their residual genotypes and at the leucine locus as well, several interpretations of the results are possible. One is that the intra-allelic interaction between the nutritional mutant and the respective isoallelic wild-types may result in compatibility or incompatibility, depending upon the particular allele involved. A second is that allelic genes which usually interact to give an incompatible phenotype might also interact with certain nonallelic genes to give a compatible phenotype or *vice versa*. The results also suggest that certain ambiguous cases of noncomplementation in intra-allelic heterokaryons may be caused by a similar type of incompatibility. DR. VAL WOODWARD (personal communication) has recently shown us some very interesting unpublished data in support of such a notion. His results have shown that (1) nonallelic mutation changes the complementation pattern, (2) the new mutation is specific to a given pair of alleles (in a series of over 50 heteroalleles), and (3) the pattern is the same as that of nonallelic compatibility.

That particular genes may affect compatibility in one genotype and not in another also can be inferred from the studies of DAVIS (1960 a,b). He found that gene *m* affects the ability of *pan-1* strains to grow on limiting amounts of pantothenate. In a heterokaryon between two nonpantothenate-requiring strains the gene *m* in a heterozygous or homozygous condition would presumably have no effect on compatibility but when *m* is present in only one strain of a heterokaryon homozygous for *pan-1*, it results in a nonadaptive increase of the m^+ *pan-1* component. Thus it is apparent that the gene *m* in a heterozygous condition would have a very different effect on the interaction of the two nuclear

components, depending on whether the two nuclei making up the heterokaryon were homozygous for *pan-1* or homozygous for the normal allele of *pan-1*.

Although these two isolated instances, along with certain observations of *I-i* interactions mentioned earlier, may not constitute convincing evidence in support of the contention that specific genetic factors may be compatible in one genotype but incompatible in another, considerable evidence appears to be irrefutable in this regard. HOLLOWAY (1953) has shown that three strains 5531A (*pan*), 1633A (*pab*), and 4545A (*lys*) form stable compatible heterokaryons in all possible combinations with one another. He then crossed the *pab* strain to wild-type Abbott 12a and selected 55 different *pab A* isolates. These were then tested for compatibility with the original *pan A* and *lys A* strains. Thirty-seven of the *pab* reisolates were compatible with *pan A* and only two were compatible with *lys A* even though *lys A* and *pan A* are compatible with one another. When *lys A* was crossed with Abbott 12a, 49 *lys A* reisolates were recovered. Only one was compatible with *pab A* and only one, with *pan A*. Twenty-eight reisolates of *pan A* were recovered from a cross to Abbott 12a; 13 were compatible with *pab A* and none, with *lys A*. Although these data were originally presented to support the contention that heterokaryotic compatibility was under genetic control, they are now even more meaningful in another regard. These results make it perfectly clear that two strains' being compatible with one another does not insure that a third strain compatible with the first will necessarily be compatible with the second. Such behavior may mean that compatibility is often strain specific and that heterozygosity of certain genes may have no effect on compatibility in one genotype, but in another such a condition will result in incompatibility.

[ADDENDUM: Since the findings of HOLLOWAY (1953) are especially pertinent to an understanding of the genetic control of compatibility, we have repeated the same type of experiments after our manuscript was submitted. Different strains have had to be used and what we consider to be more reliable techniques for detecting incompatible heterokaryotic combinations have been utilized. Our results are different from HOLLOWAY's for in rather large scale experiments we invariably have found that when two strains are compatible with one another, both are always either compatible or incompatible with a third strain; that is, two compatible strains have never reacted differently with the same tester strain.]

Since the interaction of *I* and *i* may result in incompatibility, their relationship to other genes affecting heterokaryon compatibility is of interest. All of our *I* and *i* strains of mating type *a* are incompatible with the *CD*, *Cd*, *cD* and *cd* testers kindly furnished by DR. LAURA GARNJOBST. Since their incompatibility phenotype is also different there in every reason to believe that different loci are involved. The nonadaptive increase of *I* under certain conditions is reminiscent of the unstable *Y-Y'* heterokaryon reported by HOLLOWAY (1953, 1955). We have been unable, however, to find any evidence that *Y-Y'* combinations show normal growth under any conditions and for this reason alone it must be assumed that this system is different from *I-i*, although it is conceivable that they could be the same.

SUMMARY

1. An allelic pair of genes, designated *I* and *i*, have been found whose only known phenotype is expressed when the genes are present in a heterokaryon in a heterozygous condition. When certain nuclear proportions are present in forced heterokaryons, *I* nuclei operationally inhibit the multiplication of *i* nuclei with the result that *I* nuclei undergo a nonadaptive increase leading to homozygosity of this nuclear component.

2. The data suggest that *I* is only weakly dominant to *i* because the nonadaptive increase in the *I* nuclear component takes place only when the proportion of *I* nuclei is approximately .30 or greater. When more than 70 percent of the nuclei are *i*, growth is normal for an indefinite period and no systematic change in the nuclear ratio is found. These relationships are found either in heterokaryons prepared with a variety of nuclear ratios, or in cultures derived from single heterokaryotic conidia.

3. At present the interaction of *I* and *i* can be described only in operational terms. The incompatibility of *I* and *i* apparently is not from a cytoplasmic difference, nor can the results be satisfactorily explained by an inherent difference in the rates of nuclear division of the two types. It has not been possible to demonstrate that *I* nuclei produce a substance which inhibits the division of *i* nuclei or that their interaction is from competition for a substrate conceivably needed for nuclear replication.

4. The behavior of *I* and *i* has been satisfactorily studied using *nic-1*, *nic-2*, *pan-1*, *lys-3*, and *tryp-1* in forced heterokaryons which suggests that the interaction of these alleles is independent of the nutritional mutants present. However, some *i-i* and *I-i* heterokaryons do not show the expected phenotypes, indicating that their interaction is not completely independent of the residual genotypes of all of the strains used.

5. Inheritance studies show that *i* segregates as a single genetic factor in crosses and is located either on linkage group I or II. *i* has been found in wild-types ST 74A and 74-OR-8-1a.

6. The interaction of genes effecting compatibility in heterokaryotic systems is discussed, along with their relationship to problems involved in the study of complementation phenomenon.

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