GENETIC CONTROL OF HETEROCARYOSIS IN NEUROSPORA CRASSA[1]

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BEADLE and COONRADT (1944) showed that when two different biochemical mutants of *Neurospora crassa*, of the same mating type, were combined on a minimal medium unable to support the growth of either mutant, a heterocaryon with wild type growth rate was formed. The ability to form a heterocaryon between two mutant strains has been taken to indicate that the mutant genes involved are not allelic, although the failure to form a heterocaryon does not necessarily mean that the genes are allelic. The inability to form a heterocaryon may also be due to the impossibility of obtaining a suitable nuclear ratio through the dominance relationships of the genes concerned, or to the action of other genes as reported by GARNJOBST (1953) for inositolless and riboflavinless strains or as described below in a genetically more complex situation involving strains requiring pantothenic acid or lysine.

METHODS

The basal medium was the standard Neurospora minimal (BEADLE and TATUM 1945). All cultures were grown at 25°C. Special growth tubes as described by RYAN, BEADLE and TATUM (1945) were used for growing heterocaryons. Spore suspensions of the strains to be tested for heterocaryon formation were made in sterile distilled water from cultures grown on Horowitz complete medium (HOROWITZ 1947) for 6 days. Loopfuls of each of the two suspensions were then placed together on the surface of the agar in the growth tube. The general procedures for dissection of asci, determining the mating type of cultures, maintaining stock cultures, sterilization of media, etc., were the same as those used by BEADLE and TATUM (1945). Crosses were made on the medium described by WESTERGAARD and MITCHELL (1947), with the addition of appropriate supplements for the mutants concerned.

The mutants used were:—5531 (pantothenicless) TATUM and BEADLE (1942), unable to synthesize pantothenic acid; 1633 (aminobenzoicless) ZIMMER (1946), requiring p-amino-benzoic acid; and 4545 (lysineless)[4] DOERMANN (1946), requiring the amino acid lysine.

EXPERIMENTAL

When the experiments of BEADLE and COONRADT were repeated with reisolates of the same mutants, these being obtained from crosses of the original mutant strains

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to various wild types, heterocaryons were not formed as expected. This suggested that the factors controlling the formation of heterocaryons were more complex than previously thought. After preliminary experiments, the mutants 5531 (pantothenicless) and 4545 (lysineless) were selected to further investigate this problem. Only heterocaryons between strains of the same mating type have been investigated in this work.

5531A was crossed to the EMERSON wild type, E5297a, and 132 pantothenicless random spore isolates from this cross were tested for their ability to form heterocaryons with the lysineless tester strains 4545A and 4545a. Of these cultures 110 failed to form a heterocaryon with the tester strains. Of the 22 strains forming heterocaryons only 11 gave a heterocaryon with wild type growth rate. Following other crosses and after more isolates were tested it was possible to recognize six different types of heterocaryotic growth within this system. These various types were quite constant and classification of any particular growth was readily made into one or the other of the classes. The types may be described as follows (see fig. 1).

Type I

Growth begins within a few hours of inoculation and soon reaches the wild type growth rate (4.4 mm./hr.). The mycelium has the same appearance as wild type and the mycelial frontier is sharp and well defined.

Type II

The mycelial and growth characteristics of this type of heterocaryon are identical with those of type I. However growth does not begin until after a delay of 35–90 hours following inoculation. The commencement of growth is well defined and not gradual.

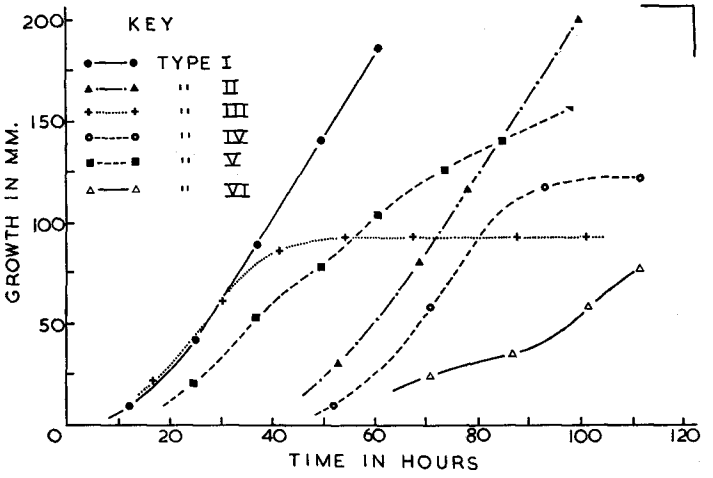

FIGURE 1. The types of heterocaryotic growth observed between different reisolates of 5531 and the tester 4545 strains.

Type III

Growth commences soon after inoculation and with the wild type growth rate, but comes to an abrupt halt after a period of growth varying from 30–60 hours. At the point where growth ceases the mycelium assumes a characteristic highly branched appearance and the immediate edge of the mycelium has a different appearance to the main body of the hyphae, being thinner and closely appressed to the medium. The actual time at which growth ceases is variable even when replicates of the same strains are inoculated. Hyphal tips taken from the region where growth has ceased grow on pantothenic acid supplemented media, but not on media containing lysine. In addition, if pantothenic acid is added to the region where growth has ceased the mycelium is stimulated to grow but no growth occurs if lysine is added in this way. If left for eight to ten days this type of heterocaryon may recommence growth and later stop again. Hyphal tips taken from the actively growing phase of such heterocaryons continue to grow for 24–48 hours after transfer but then stop. Thus this type of heterocaryon appears to be in an unstable state with the pantothenicless nuclei being in excess of the lysineless nuclei.

Type IV

This has the same general growth characteristics as type III with the exception that growth does not begin until 35–90 hours after inoculation.

Type V

Growth begins 12–24 hours after inoculation. The mycelium is much less vigorous than the wild type, is thinner and somewhat appressed to the medium. The mycelial frontier is not well defined. Sometimes the growth becomes progressively thinner, slows down and may eventually cease altogether. The maximum growth rate attained never exceeds 3 mm./hr. and growth is often not linear.

Type VI

The growth characteristics of this type are the same as for type V but like types II and IV growth does not begin until 35–90 hours after inoculation.

Type VII

For uniformity in the scheme, this type includes those strains which do not give any heterocaryotic growth with the tester strains.

Proof of the heterocaryotic condition

The method of BEADLE and COONRADT (1944) was used to prove the heterocaryotic condition and this was readily established for heterocaryon types I, II, V and VI. However it was not possible to use this method for types III and IV. Hyphal tips from these two types show only limited growth, insufficient to enable the heterocaryon to be crossed to wild type. However the hyphal tips from these two types show more growth on minimal medium than that obtained when hyphal tips from either mutant

TABLE 1

Results of intercrossing various heterocaryon types

Heterocaryon types crossed	Total no. asci tested	Results
I x I	15	Progeny all type I.
I x II	18	Progeny consisting of types I and II segregating 1:1 in the ascus.
I x III	16	Progeny consisting of types I and III segregating 1:1 in the ascus.
I x IV	17	Both parental types I and IV and recombinant types II and III recovered in progeny.
I x V	37	Types I and V only recovered, segregating 1:1 in the ascus.
I x VII	23	Types I, II, III, IV, V, VI, VII recovered.
VII x VII	18	All progeny type VII.
III x IV	11	Types III and IV segregating 1:1 in the ascus.

are transferred to minimal medium and this is taken as evidence for the presence of both mutant nuclei in these types of heterocaryon.

From the growth characteristics of the various heterocaryon types a number of features can be recognized which are associated with the formation and growth of a heterocaryon. (1) The establishment of a heterocaryon, (2) the time lag in the initiation of growth, (3) the ability to maintain growth, (4) the growth rate and habit. It has been possible to show that these various characteristics are under genetic control. When the various heterocaryon types are crossed to one another segregation for these types occurs in the progeny. The results of the various crosses are shown in table 1.

On the basis of the data summarised in table 1 and on other evidence to be presented below, the following hypothesis for the genetic control of heterocaryons in *N. crassa* has been formulated. The formation of a heterocaryon between strains of the pantothenicless mutant 5531 and the lysineless mutant 4545 is under the control of genetic factors. These genes control not only whether or not a heterocaryon will form, but the growth characteristics of the heterocaryon, should it form. Four genes have been identified as controlling these characteristics. This represents the minimum required to fit most of the present data, and it is likely that additional genes affect the process. These four genes can be described in terms of their effects as follows:—

Gene W (*alternative allele W'*). Heterocaryotic growth can only occur in the presence of the *W* allele of this gene. However allele *W* alone is not sufficient and an interaction between this allele and alleles *X* and *Y* of the gene pairs *X*/*X'*, *Y*/*Y'* (described below) occurs, so that allele *W* must be present with either of alleles *X* or *Y* for a heterocaryon to form.

Gene X (*alternative allele X'*). Allele *X* of this gene exerts an influence on the growth rate and general vigour of the heterocaryotic mycelium. Segregation of this gene and its alternative allele *X'* has been demonstrated. Pantothenicless strains giving

type V heterocaryotic growth with the tester strains differ from those giving type I by the presence of allele X' in the former strains and in crosses of these two types a 1:1 segregation of the parental types occurs in the ascal progeny.

Gene Y (alternative allele Y'). Allele Y of this gene is concerned with the maintenance of the heterocaryotic condition. Pantothenicless strains giving type III heterocaryons with the tester strains differ from those giving type I growth by having the alternative allele Y' and crosses between these two types result in only the parental types occurring in the progeny, segregating 1:1 in the ascus.

Gene Z (alternative allele Z'). The presence of the Z allele of this gene enables any heterocaryon formed to commence growing soon after inoculation but in the presence of the alternative allele Z' growth will be delayed, as in types II, IV, and VI.

It is not known which of the above alleles of these various genes is the dominant form. The only test for dominance in Neurospora is the heterocaryon test so that the dominance or recessiveness of genes affecting the process of heterocaryon formation cannot be determined.

On the basis of this scheme genotypes, with respect to the four genes, of pantothenicless strains giving the various heterocaryon types can be formulated as follows:

I	$W\ X\ Y\ Z$
II	$W\ X\ Y\ Z'$
III	$W\ X\ Y'Z$
IV	$W\ X\ Y'Z'$
V	$W\ X'Y\ Z$
VI	$W\ X'Y\ Z'$
VII	W' – – –
	$-\ X'Y'-$

As previously mentioned the genetic constitution of the tester strain will undoubtedly be of importance. To investigate this would require an analysis of the lysineless strains in much the same way as that made for the pantothenicless strains. This is probably not possible because crosses in which both parents carry the lysineless gene are highly infertile, and so the analysis would have to be made through crosses to wild types and this would be both difficult and tedious. Clearly, the genotypes assigned above are valid only for the particular tester strains used.

In order that the segregation and phenotypic effects of the various genes postulated to affect heterocaryon formation may be studied it is necessary that the alternative alleles to the active alleles be introduced into the strains to be analysed. As 5531 evidently carried all the genes necessary to form a type I heterocaryon the first step in the genetic analysis is to cross this strain to another which could be expected to carry the alternative alleles of the various genes. As described above, the wild type EMERSON wild type E5297a was selected for this purpose and crossed to 5531A. The analysis of such a cross is limited by the fact that half the progeny are wild type and thus cannot be tested for heterocaryon formation. If both parents carry the pantothenicless allele then all spore pairs of the ascus may be examined for the ability to form a heterocaryon. If one parent of the cross is of type I, such as 5531, it is obvious then that the segregation of the maximum number of genes affecting heterocaryon can be obtained by selecting a type VII (i.e. a pantothenicless strain unable to form a

heterocaryon with the tester strain) as the other parent. Accordingly a strain of this type, 449-8a, was selected from the progeny of the cross 5531A x 5297a and then crossed to 5531A. The hypothesis described above is based on the subsequent analysis of progeny resulting from this cross.

From the progeny of the cross 449-8a x 5531 all heterocaryon types I through VII were recovered. On the basis of the four gene hypothesis 28 types of asci with respect to the seven different types of heterocaryons are possible. Of 23 asci analysed, 12 of these types, and no others, were obtained. The segregation of the four genes was demonstrated by taking asci showing various segregations of heterocaryon types and making crosses between the various ascospore pairs within each ascus. Two such asci were analysed in this way, asci 560 and 562, both from the cross 449-8a x 5531A.

Analysis of ascus 560

The segregation of the ability to form a heterocaryon in ascus 560 is shown in table 2. All crosses possible between members of the ascus and crosses to the parents were made. The progeny were then tested against the standard 4545 tester strain of like mating type. The results of the various crosses were as follows:—

560-1 x 560-3.—Only segregation for W/W' can be predicted for this cross. Twenty asci were analysed with the results seen in table 3. As only phenotypes III, IV, and VII occur in this cross it is likely that it is heterozygous for W/W' and homozygous for both X and Y'. As types III and IV occur it must be heterozygous for Z/Z'. There is a 1:1 segregation for the ability to form a heterocaryon. On the basis of this cross we can assign the genotype $W'XY'Z$ to 560-3 and so 560-7 must be of genotype $W'X'YZ'$. This can be confirmed by the other crosses.

560-1 x 560-7.—On the basis of the previous cross, this cross should be heterozygous for W/W', X/X', Y/Y' and homozygous for Z'. Thus only types II, IV, VI and VII should appear in the progeny, and this was found to be the case (see table 4). The results from this cross are consistent with the previously postulated genotypes for the spore pairs of ascus 560.

It has previously been postulated that genes W, X and Y interact so that for a heterocaryon to form, gene W and either or both of genes X and Y must be present. The cross supplies evidence for this view. Of the 19 asci analysed from this cross, 10 show segregation for one pair able to form a heterocaryon and three pairs unable to do this, and three asci show segregation of four pairs unable to form a heterocaryon. As the cross is heterozygous for W/W', then two ascospore pairs in each ascus will be of type VII and be unable to form a heterocaryon. When a third or fourth ascospore pair also fail to form a heterocaryon the genotype of these members must contain the W allele and hence failure to form a heterocaryon must be due either to a fifth gene controlling the ability to form heterocaryons or to an interaction between alleles W, X and Y. As no evidence has been found to indicate a fifth gene with this postulated effect and the results can be completely accounted for by assuming the interaction, this has been the explanation adopted. This cross also supplies evidence that the allelic pair Z/Z' does not effect the actual formation of a heterocaryon but only the time at which heterocaryotic growth begins. The formation of heterocaryons or the failure to do so can be completely explained in terms of genes W, X and Y.

TABLES 2 TO 11

Genetic analyses of two asci from the cross 449-8a (pantothenicless, type VII) x 5531-A (panthothenicless, type I)

Numbers of ascospore pairs producing different types of heterocaryons when combined with standard lysineless strains (4545A and 4545a) of like mating type

Cross	Number asci	Type I	Type II	Type III	Type IV	Type V	Type VI	Type VII
		Postulated genotype						
		$\frac{W\ X}{Y\ Z}$	$\frac{W\ X}{Y\ Z'}$	$\frac{W\ X}{Y'\ Z}$	$\frac{W\ X}{Y'\ Z'}$	$\frac{W\ X'}{Y\ Z}$	$\frac{W\ X'}{Y\ Z'}$	$\frac{W'\text{—}**}{\text{-}X'Y'\text{-}}$
449-8a x 5531-A					1	1		2
Table 2	1				(560-1a)	(560-5a)		(560-3A, 560-7A)
560-1a x 560-3A	2			2				2
Table 3	13			1	1			2
	5				2			2
560-1a x 560-7A	4		1		1			2
Table 4	1		1				1	2
	3				1		1	2
	3		1					3
	3				1			3
	2						1	3
	3							4
560-5a x 560-7A	4					2		2
Table 5	3						2	2
	12					1	1	2
560-3A x 560-5a	1	2						2
Table 6	1	1		1				2
	2	1				1		2
	2	1					1*	2
	3			1		1		2
	5			1			1*	2
	1					1	1*	2
560-1a x 5531-A	2	2			2			
Table 7	7		2	2				
	8	1	1	1	1			
449-8a x 5331-A	1	1				1		2
Table 8		(562-7a)				(562-5A)		(562-1a, 562-3A)

TABLES 2-11—*continued*

Cross	Number asci	Type						
		I	II	III	IV	V	VI	VII
		Postulated genotype						
		$\frac{W\ X}{Y\ Z}$	$\frac{W\ X}{Y\ Z'}$	$\frac{W\ X}{Y'\ Z}$	$\frac{W\ X}{Y'\ Z'}$	$\frac{W\ X'}{Y\ Z}$	$\frac{W\ X'}{Y\ Z'}$	$\frac{W'—**}{-X'\ Y'-}$
562-3A x 562-7a	1	1	1					2
Table 9	1	1			1			2
	1	1				1		2
	1			2				2
	2			1	1			2
	1			1		1		2
	1			1			1	2
	2		1					3
	2			1				3
	1				1			3
	1					1		3
	1						1	3
562-1a x 5531-A	2	1	1					2
Table 10	3	1		1				2
	1		2					2
	4		1	1				2
	1			2				2
	3			1	1			2
562-5A x 449-8a	1					2		2
W'X'Y'Z'	3					1	1	2
Table 11	2						2	2
	2					1		3
	3						1	3
	1							4

* Type VI unexpected segregant.

** Type VII phenotype can be: *W'XYZ*, *W'XYZ'*, *W'XY'Z*, *W'XY'Z'*, *W'X'YZ*, *W'X'YZ'*, *W'X'Y'Z*, *W'X'Y'Z'*, *WX'Y'Z*, or *WX'Y'Z'*.

560-5 x 560-7.—On the basis of the previously assigned genotypes, W/W' and Z/Z' are heterozygous and alleles X' and Y are homozygous in this cross. Thus only types V, VI and VII should be obtained in the progeny, and a 1:1 segregation of ability to form a heterocaryon should occur in the ascus. This was the result obtained (table 5).

560-3 x 560-5.—This cross is heterozygous for allelic pairs W/W', X/X' and Y/Y' and homozygous for Z. Thus only types I, III, V and VII should appear in the progeny. This expectation was not borne out experimentally and a number of asci

showed segregation for type VI. The various segregations observed are shown in table 6.

The presence of all the expected heterocaryon types in this and other crosses confirms the four gene hypothesis of heterocaryon formation. The occurrence of type VI amongst the progeny can be accounted for by postulating an additional gene, the action of which is similar to that of the allelic pair Z/Z', but acting specifically on type V heterocaryons. The allele of the gene causing delayed growth can only be detected in the presence of strains giving the type V response. The cross is evidently heterozygous for this gene. If the allele causing delayed growth is carried by 560-3 then the cross to 560-1 would not allow its expression because the progeny of this cross does not include any strains giving type V heterocaryotic growth with the tester strains. If this allele is carried by 560-5 then in the cross 560-5 x 560-7 its presence would not be detected as the cross is heterozygous for alleles Z/Z' and type VI heterocaryons are expected in the progeny of this cross. Thus the complete segregation of this postulated gene could not be followed. Effects attributable to this gene were not detected in any other cross, so the presence and effects of this fifth gene must remain presumptive.

560-1 x 553-1.—This cross is heterozygous for both Y/Y' and Z/Z' and homozygous for W and X, and the observed segregation for types I, II, III, and IV only and in the ascal configurations expected is additional confirmation of the four gene hypothesis (see table 7).

560-5 x 5531.—Here the only allelic pair segregating is X/X' and the 17 asci analysed showed a 1:1 segregation for heterocaryon types I and V when combined with the tester strains.

560-3 x 449-8.—This cross is homozygous for W'. As expected therefore all progeny were of type VII, i.e. unable to form a heterocaryon with the tester strain.

560-7 x 449-8.—All progeny of this cross failed to form heterocaryons with the tester strains. From the genotypes of the parents, this cross is homozygous for both W' and X', so this was the expected result.

Analysis of ascus 562

An analysis similar to that made for ascus 560 was carried out with ascus 562. While the analysis was not so detailed it is sufficient to show the segregation of the four genes already postulated. When members of ascus 562 were tested against the tester strains for heterocaryon formation the responses shown in table 8 were obtained. It is seen that genotypes may be assigned on the basis of these results in a much more complete manner than was possible with ascus 560. This distribution can be completed and confirmed by making various crosses with the parents and between the various spore members. The results of these crosses will now be considered in turn.

562-1 x 562-3.—Eleven asci were analysed from this cross. All the progeny failed to form heterocaryons with the tester strains. This result is consistent with the assigned genotypes for 562-1 and 562-3. This cross gives no information about the segregation of alleles X/X', but this is supplied by several of the other crosses.

562-1 x 562-5.—The ascospores from this cross showed extremely poor germina-

tion and although over forty asci were dissected none gave germination in all four spore pairs thus preventing the analysis of this cross.

562-3 x 562-7.—Fifteen asci were analysed from this cross with the results given in table 9. It is seen that all heterocaryon types I through VII occur in the progeny, and hence all four genes must be heterozygous in this cross. As 562-7 is type I and hence of genotype *W X Y Z*, then 562-3 must have the genotype *W'X'Y'Z'*. Here again we have additional evidence for the interaction between genes *W*, *X* and *Y* to give the type of ascus in which more than two spore pairs show the type VII response. From the results of this cross we now assign genotypes to all members of this ascus, 562-1 *W'XY'Z'*, 562-3 *W'X'Y'Z'*, 562-5 *WX'YZ*, 562-7 *WXYZ*. Additional evidence for this segregation comes from the following crosses.

562-1 x 5531.—Fourteen asci were analysed from this cross with the results given in table 10. From the results of the cross 562-3 x 562-7 we have assigned the genotype (*W'XY'Z'*) to 562-1. Hence in the cross of this latter strain to 5531, which has the genotype *WXYZ*, we expect to find only types I, II, III, IV and VII. This was found to be the case and the results of this cross confirm the genotypes assigned on the basis of the previous cross.

562-5 x 449-8.—This cross completed the analysis of ascus 562. The results from this cross are given in table 11. This cross is between genotypes *WX'YZ* and *W'X'Y'Z'* and the expected segregation for types V, VI, and VII only is realized. This cross supplies additional confirmatory evidence for the interaction occurring between *W*, *X*, and *Y* in the formation of a heterocaryon.

No further evidence was found in the analysis of ascus 562 for the additional gene previously postulated to act specifically on the type V heterocaryon to produce the type VI heterocaryon.

The results of the analysis of both asci 560 and 562 thus confirm the experimental hypothesis for the genetic control of heterocaryon formation and growth. Preliminary experiments with other mutants suggest that the results obtained with the lysineless-pantothenicless heterocaryon are of general application in the formation of heterocaryons between biochemical mutants but these tests were not carried far enough to indicate the number of genes involved nor their relationship, if any, to genes *W*, *X*, *Y* and *Z*.

Influence of the tester strain on the type of heterocaryotic growth

It has been shown above that the genotype of one partner in the combination of two strains to form a heterocaryon will determine the nature of the heterocaryotic growth resulting or may even prevent heterocaryon formation. It is thus likely that the genotype of the second strain will also exert a similar influence. Thus four genes carried by the pantothenicless partner have been shown to influence heterocaryon formation so that it is quite reasonable to expect that perhaps a similar number is carried by the lysineless component. It would be also expected that such genes may have a complementary action. Although the exact nature of the genetic control exerted by the lysine strain is not known experiments have been carried out which demonstrate that the genotype of the lysine strain does affect the subsequent heterocaryotic growth.

TABLE 12

Influence of different lysineless tester strains on the formation of heterocaryon types with various pantothenicless strains

Pantothenic strain	Heterocaryon types formed					
	4545 A standard tester	Random spores from cross 4545A x 25a, mating type 'A'				
		L102	L104	L106	L109	L112
741-7A	I	II	II	I	IV	III
726-5A	II	II	III	II	II	II
731-5A	III	II	II	III	II	II
742-4A	IV	V	VI	IV	VII	V

A number of random spores were selected from the progeny of the cross 4545A x 25a (LINDEGREN wild type). They were combined with different pantothenicless strains of like mating type, the latter known to form heterocaryons of various types with the standard tester strains. The results are given in table 12.

These results indicate that the genetic hypothesis advanced for the control of heterocaryosis between these two mutants is true only when the tester strains used in the experiments are involved. Had other tester strains been used it is clear that the hypothesis would have to be modified or extended. It is of interest to note the comparatively large number of delayed starting types of heterocaryons (II, IV and VI) obtained in the above results. This supports the previous suggestion that the inheritance of this character is complex. Another interesting result from the above experiment is that apparently strain L106 carries the same genic complement with respect to the genes under consideration as does 4545A, because both give the same results with the four strains tested.

Apparently the two tester strains 4545a and 4545A are alike with respect to the principal genes controlling heterocaryosis. In all the crosses tested the range of types obtained amongst the progeny were the same for either mating type and no results suggested that 4545A and 4545a were not isogenic for the genes under consideration.

DISCUSSION

From the evidence presented above it may be concluded that certain genes control the formation of heterocaryons between biochemical mutants of *Neurospora crassa*.

Little can be said regarding the mode of action of genes affecting heterocaryosis. Although this aspect of the problem has not been investigated, certain suggestions can be made. The first situation to be considered is that of the formation of heterocaryons between some strains and not between others. Since the first stage in the formation of a heterocaryon is presumably the fusion of two germinating spores it should be possible to determine by appropriate micro-techniques whether failure to form a heterocaryon is due to an initial failure to fuse. In the formation of some heterocaryons it is apparent that fusion can and does take place quite soon after inoculation. With some type I heterocaryons growth is visible within a matter of hours after inoculation and the initiation of growth is just as rapid as with wild type strains.

Experiments were done in which two strains known not to form a heterocaryon were grown alone on agar plates supplemented with sub-optimal requirements of the specific growth requirements. Squares were then cut from the agar containing growing hyphae of each strain and placed in apposition at one end of a growth tube containing minimal medium. Thus the strains had every opportunity to fuse but in no case where this was tried did heterocaryotic growth result. Similar experiments in which the specific substrate was incorporated with the inoculum of spores failed to have any effect in initiating heterocaryotic growth.

In those heterocaryons which show delayed growth it is not known whether this is due to a delay in fusing or a delay following fusion. Inocula of such combinations examined under the microscope seem to show no more germination than that observed with either strain inoculated by itself so the former situation may be the case.

There is no reason to believe that the four (or possibly five) genes shown above to be concerned in the formation of the lysineless-pantothenicless heterocaryon represents the total number of genes capable of exerting such an effect. Furthermore it is not known whether these genes are specific in their action for the particular mutants concerned or whether they can affect other heterocaryotic systems. GARNJOBST (1953) has shown that the genes *het-1* and *het-2* can influence the heterocaryon forming ability of a number of biochemical mutants. Unfortunately it is not possible at present to relate these two genes to any of those described above for the pantothenicless-lysineless system.

SUMMARY

Heterocaryon formation between certain biochemical mutants of *Neurospora crassa* is controlled by a number of genes apart from the biochemical mutant genes concerned. Four genes, and possibly a fifth, have been shown to be concerned in the process of heterocaryon formation between a mutant requiring pantothenic acid and one requiring lysine. These genes may not only prevent the formation of a heterocaryon but may also modify the type of heterocaryotic growth. The growth characteristics so affected are the time at which heterocaryotic growth commences, the ability to maintain heterocaryotic growth, and the vigor of the growth.

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