HETEROCARYOSIS IN *NEUROSPORA CRASSA*

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ABSTRACT

It has been shown that heterocaryon formation between certain biochemical mutants of Neurospora crassa is controlled by a number of genes apart from the biochemical mutant genes concerned. Genetic control of heterocaryosis has been shown for several different combinations of mutants. A detailed investigation of the heterocaryon formed between a mutant requiring pantothenic acid and one requiring lysine has demonstrated four and possibly five genes to be concerned in the process of heterocaryon formation. These genes may not only prevent the formation of a heterocaryon but also modify the type of heterocaryotic growth. The characters so affected are the time at which heterocaryotic growth commences, the ability to maintain heterocaryotic growth and the vigor of the growth. The possible mode of action of such genes has been discussed.

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INTRODUCTION AND SURVEY OF LITERATURE.

In the fifty years since genetics became a recognized field of scientific experimentation, investigations on the nature of the hereditary mechanism have progressed to the point where the gene as the unit of inheritance is well established. Two important phases in the study of genes are firstly the identification and characterization of genes affecting particular characters of organisms, and secondly the mechanism by which the genes produce their specific effects. Studies on gene action have been greatly advanced since the introduction of microorganisms as objects of genetic study, for it is mainly in these studies that the influence of genetic mechanisms on metabolic reactions has been shown and with this a closer approach made to the nature of gene action.

The biochemical genetics of microorganisms was greatly accelerated through the use of the fungus Neurospora for work in this field. This has been adequately summarized in a number of reviews over the last 10 years (1, 2, 3, 4), and these clearly indicate the suitability of Neurospora for investigations on gene action. Because it is haploid, Neurospora does not permit study of such genetic phenomena as dominance, heterozygosis, and heterosis by the same methods used for diploid organisms. However, the condition in which two or more types of nuclei are present in a single cell, a situation known as heterocaryosis, does provide a special way of studying these phenomena. The study of heterocaryosis is therefore of general importance in genetics.

The term heterocaryosis was introduced by Hansen and

Smith (5) to cover situations in which two or more genetically different types of nuclei are found in the same cell or in the same cytoplasm. In an Ascomycete like Neurospora, there are no discrete cells as such in the mycelium. The hyphae do have transverse septa but each of these contains an aperture permitting cytoplasm and nuclei to flow from one part of the mycelium to another. The number of nuclei per "cell" varies but is probably of the order of one to ten.

Heterocaryosis in fungi may arise in one of two ways. In the growth resulting from a sexually produced spore or from a uninucleate conidiospore all nuclei are derived from a single haploid nucleus and unless mutation occurs should all be of the same genetic constitution. But if in the growth of the mycelium one or more nuclei undergoes mutation, the progeny of that nucleus will be genetically different from the rest of the nuclei and the mycelium will become heterocaryotic. The second method by which heterocaryosis may arise is following fusion between hyphae of two different mycelia and subsequent exchange of genetically different nuclei. The exact nature of the process leading to such exchange of nuclei is still unknown. Fusion between neighboring hyphae is frequently observed in many fungi, but it is not known whether nuclear exchange invariably follows such fusion, to what degree there is exchange of cytoplasm, whether any nuclear exchange that does take place is reciprocal or unilateral or what are the factors that control nuclear exchange. That exchange can and does take place has been clearly shown in a number of instances.

That there may be genetic factors influencing the frequencies with which hyphal fusions take place is suggested by Lindegren's evidence (6) that different frequencies of fusions occur with various combinations of different strains of Penicillium notatum.

The problem of the variability of fungi isolated from nature is one that has intrigued and perplexed mycologists and plant pathologists. An important step towards an understanding of this variability was made by Hansen and Smith (5). By isolating single spore cultures of Botrytis cinearea they showed the existence of a number of dissimilar morphological strains, varying chiefly in their ability to conidiate. When single spore isolations were made through several generations some of the strains remained constant but others broke up into further variants. It was found that one particular line gave rise to two homotypes and other heterotypes. If spores from the two homotypes were mixed together the resulting culture was a heterotype that subsequently gave rise to the two homotypes. To this behavior they gave the name "dual phenomenon". Their work represents the first analysis of a heterocaryotic mycelium. It was subsequently shown by Hansen (7) that the dual phenomenon was of wide distribution occurring in many other fungi isolated from nature. Thus the dual phenomenon merely represents a heterocaryon between conidial and aconidial forms of the same fungus.

Further understanding of the nature of heterocaryosis

came with Dodge's work on Neurospora, a fungus that has since proved to be highly suited for a study of this phenomenon. Dodge (8) studied the migration of nuclei through the points of anastomoses between two heterothallic races of opposite mating type of the normally homothallic Neurospora tetrasperma. Working with three heterothallic strains of this species Dodge (9) demonstrated the formation of heterocaryons between pairs of strains. These could be of different or of the same mating types. The three original strains were characterized by slow growth or reduced conidial formation. In all heterocaryons the growth was more vigorous than that of the component strains. Dodge gave the name "heterocaryotic vigor" to this phenomenon and pointed out the resemblance of this to certain cases of heterosis in higher plants [v. Robbins (10)]. Dodge and his coworkers (11) subsequently showed that the factors causing the reduced growth were genetic in nature, segregating and recombining in subsequent crosses, and they suggested that the strains might lack certain synthetic abilities as compared to wild type strains. When these strains were combined in a heterocaryon, with both nuclear types together in a common cytoplasm, there was a complementary effect, with subsequent increase in vigor. Thus here was a clear example of how heterocaryosis could modify the physiological status of the fungus and indicated a way in which intracellular studies on metabolism could be made.

With the discovery by Beadle and Tatum (12) that mutants

having specific growth requirements could be easily produced in N. crassa it became possible to set up artificial heterocaryons by combining such mutants. The work of Beadle and Coonradt (13) and Coonradt (14) thus extended and amplified the results obtained by Hansen and Smith and by Dodge. The phenomenon of heterocaryosis was placed on a predictable experimental footing by using experimental conditions which created a strong selectivity for the heterocaryotic growth over the growth of the component strains. Biochemical mutants of N. crassa produced by irradiation and with known deficiencies in the ability to synthesize amino acids and vitamins were inoculated in pairs on a medium which would not support the growth of either strain. If the mutants are recessive to the alternative wild type there is a complementary effect of one nucleus on the other and the heterocaryotic mycelium is able to grow and to continue growing. This type of growth has come to be known as a "balanced heterocaryon". That heterocaryosis does occur and that growth is not the result of syntrophic association without fusion may be demonstrated by the isolation of hyphal tips from a growing mycelium, which are then crossed to wild type strains. If from the progeny of such a cross ascospores of both mutant types comprising the heterocaryon are isolated, it is evident that the hyphal tips must have contained both nuclear types and hence that the mycelium was heterocaryotic.

Beadle and Coonradt concluded that if a heterocaryon composed of two mutant strains, each of which fails to grow at

the normal rate, grows as fast as wild type then each of the mutant genes is recessive to the wild type condition. This was found to be the case with 11 biochemical mutants tested, the mutants representing requirements for either amino acids or vitamins. Morphological mutants with reduced growth rate but with no known nutritional deficiency were also used and it was found that some combinations of these mutants did not give normal growth rates although all three of the mutants concerned were recessive in combination with one of the biochemical strains, pantothenicless. This may be explained as follows. In the hyphae of a heterocaryotic mycelium, in the absence of selection, there is presumably no fixed ratio of one type of nucleus to the other. However, with a heterocaryon grown on minimal medium between two components each of which has a submaximal growth rate there will be strong selection of hyphae which contain both types of nuclei. The relative recessiveness of the particular genes concerned will determine the ratio of nuclei capable of giving wild type growth. For example, with some mutants it may be necessary to have 60% or more of one type of nucleus for optimal growth. If the optimal requirement for the other type of nucleus is 40% or less then it is likely that maximal growth will result due to selection of hyphae with a nuclear ratio satisfying these conditions. On the other hand if the requirement for the second type of nucleus is more than 40%, it is obvious that the optimal nuclear ratio can never be achieved and growth will always be less than the wild type rate.

It is clear that the dominance relationships of the particular allelic pairs concerned are one of the factors controlling the range of nuclear ratios within which growth will occur in a heterocaryon. If the mutant alleles are strongly recessive there will be a wide range of nuclear ratios compatible with a wild type growth rate. If both are weakly recessive then the wild type growth rate may never be attained.

In duplicate tests of three heterocaryons having wild type growth rate the ratios of nuclei of one mutant to those of the other were found by Beadle and Coonradt to show the following variation; 1:17.6 and 1:1.6; 1:3 and 1:1.5; 1:2.2 and 5:1. Thus the mutants concerned in these three cases were evidently fairly recessive and a wide range of nuclear ratios compatible with wild type growth was possible. With the situation where the two mutants are partially recessive and the ratio for wild type growth cannot be attained, it is probable that a ratio will be attained which will give the fastest growth possible under the circumstances. There should be little variation from this ratio. This has been confirmed experimentally by Pontecorvo (15) using Aspergillus oryzae. With morphological mutants of this fungus which had lower growth rates than normal it was found that certain combinations gave heterocaryons which although having an increased growth rate over the component strains did not attain full wild type growth. When the nuclear ratio of such a heterocaryon was measured values obtained for four separate trials were as follows: 1:2.7; 1:2.9;

1:3.1 and 1:2.6. These values are uniform enough to lend support to the theory proposed by Beadle and Coonradt.

The measurement of nuclear ratios is subject to serious technical difficulties. Beadle and Coonradt measured the ratio in N. crassa by allowing the heterocaryon to form conidia, which are then used to fertilize protoperithecia of a wild type strain. The resulting ascospore progeny then consists of both mutant types and wild types. Random ascospores are isolated and identified and the ratio of the two mutant spore types is a measure of the nuclear ratio of the heterocaryon. With Aspergillus or Penicillium, or with microconidial strains of Neurospora, where uninucleate conidia only are produced, the conidia may be plated out on differential media when biochemical mutants are used or single germinating conidia picked off separately and identified where morphological mutants are used. However it is not known what relation the nuclear ratio of the hyphal tips, which are under the strongest selective force, bears to the nuclear ratio of the mycelium some distance back from the growing front. Nuclear ratios must be measured on structures "once removed" from the actively growing mycelial front, i.e. through conidia. In such cases the assumption must be made that the nuclear ratio of the conidia is a measure of the nuclear ratio of the heterocaryon as a whole.

The measurement of nuclear ratios would be greatly facilitated if one had a means of visibly identifying different types of nuclei. This has been attempted with Penicillium notatum

where in gigas forms produced by camphor treatment the nuclei are somewhat larger than the normal form (16). However, it is not clear whether the differences are sufficient for critical experimental work. What is required is some nuclear character expressed as a certain response to a nuclear stain, or perhaps discernable under phase microscopy. Thus estimation of the nuclear ratio could be made by direct observation of the living mycelium.

Pontecorvo (15) has discussed the various factors controlling nuclear ratios of heterocaryons particularly with regard to intracellular factors. He has suggested that various types of nuclei may divide at different rates, the rate being dependent on the ratio of one nuclear type to the other. The probable mode of action of such a mechanism would be through the production of metabolites by the various nuclei. If the specific growth factors are able to affect the division of the various types of nuclei then it is quite possible a similar effect could be obtained when the specific substances are supplied externally.

Ryan and Lederberg have given definite proof that external conditions may shift nuclear ratios in a heterocaryon (17) [see also Ryan (18)]. A strain of N. crassa requiring leucine occasionally shows reversion to leucine independence. This is due to back mutation of the gene for leucineless. When this occurs a heterocaryon is formed consisting of a mixture of

leucineless and reverted nuclei. It was found that if this heterocaryon was grown on a medium containing leucine, the leucineless nuclei were selected for and the strain became leucineless once more. In the absence of leucine the reverted nuclei usually outgrew the mutant nuclei. In some instances, however, where the heterocaryon was grown on media devoid of leucine wild type growth occurs at first followed by a slowing down and complete stoppage. Apparently the process of selection is not completely predictable. On the basis of this result it might be informative to measure the nuclear ratio of a balanced heterocaryon between two biochemical mutants on both unsupplemented medium and on a medium with suboptimal additions of the specific growth substances.

Pontecorvo (loc. cit) points out an interesting amplification of the theory of balanced heterocaryons. In the heterocaryon between two types of nuclei designated Ab and aB, where A and B are both weakly dominant, no ratio of nuclei will give maximal growth because both types of nuclei must be present in more than 50% of the total nuclei for this to occur. If, however, we have three types of nuclei AbC, aBC, and ABc forming heterocaryons two by two which for the same reason as before do not show full growth, then the three component heterocaryon may well show full growth, for in such a heterocaryon the alleles A, B, and C may be present in up to 66.6% of the nuclei. It follows that in a four component heterocaryon of this type each

of the four alleles may be present in proportions up to 75%. Pontecorvo suggests that multiple component balanced heterocaryons may have greater adaptability than those made up of only two components. However, there do not seem to be any reports in the literature of any heterocaryons of this type ever having been made. If biochemical mutants were used for making up such a heterocaryon, the choice of mutant would be important because of possible interactions and inhibitions in the metabolic reactions concerned. That such interactions can take place in heterocaryons has been shown by Emerson with heterocaryons involving a sulfonamide mutant (19). Because of the complex nature of this mutant a brief description of its characteristics is in order (20, 21, 22).

The mutant grows well at 25°C but on solid unsupplemented media at 35°C growth is extremely slow. Growth may be restored to the wild type rate at this latter temperature by addition of sulfonamides to the medium. However, when grown at 35° in the absence of sulfonamide a sudden change to wild type growth will often occur and it was found that this is due to a mutation resulting in the production of a suppressor gene. This mutation differs somewhat from the usual suppressor gene because in homocaryotic strains which carry both the suppressor gene and the sulfonamide requiring gene wild type growth does not result. In the heterocaryon there are present both nuclear types, but it appears that the suppressor is more effective when only a fraction rather than all nuclei carry it. The biochemical nature of the mutation resulting in the requirement for sulfonamide is particularly complex. It appears that the sulfonamide

requirement results from a deleterious reaction that takes place in the mutant strain but not in wild type and that this reaction is one requiring both homocysteine and p-aminobenzoic acid. The beneficial effect of sulfonamide results because this substance inhibits the deleterious reaction. Homocysteine is presumed to be used as a substrate in this reaction and p-aminobenzoic acts as a catalyst. The reaction either results in the destruction of threonine, or creates an increased requirement for this substance, for it was found that growth of the mutant is restored to wild type if threonine alone is added to the medium. Thus the sulfonamide eliminates this deleterious reaction by blocking the access to p-aminobenzoic acid.

The deleterious reaction can also be eliminated by genetic means. If the synthesis of homocysteine is blocked by making the double mutant sulfonamide requiring-homocysteineless, there is no longer a requirement for threonine and sulfonamide but the homocysteineless mutant leads to a complete deficiency of homocysteine and hence methionine, so that growth occurs with the double mutant when enough methionine is added for growth, but not enough to permit the deleterious reaction to go on. Another way of restoring growth in the absence of sulfonamide is to make the double mutant sulfonamide-requiring-p-aminobenzoic acidless. Here again there results a deficiency of aminobenzoic acid, and if the right amount is added normal growth results. It is apparent that a complex series of interactions are occurring in the system.

Emerson pointed out that one way of obtaining a situation where the right amount of each of the various metabolites is available

for normal growth would be through heterocaryons, where adjustment of the nuclear ratios can occur with resulting adjustments in the amount of the various metabolites produced. It was found experimentally that the heterocaryon between the sulfonamide-requiring strain and the double mutant sulfonamide requiring-aminobenzoicless grew vigorously (23). Here there is evidently an adjustment between the double mutant nucleus which requires aminobenzoic acid and the sulfonamide nucleus which produces an excess of this substance. It was found that the nuclear ratio of this heterocaryon strongly favored the double mutant. Presumably as a result of this it was found that sometimes these heterocaryons suddenly stop and after a period start again. This behavior is to be expected for the sulfonamide requiring strain produces about 100 times as much aminobenzoic acid as it required for essential reactions.

Attempts to form a heterocaryon between the double mutant sulfonamide requiring-cysteineless and sulfonamide requiring proved unsuccessful, and it is possible that a nuclear ratio which will produce the optimum amount of threonine and methionine cannot occur. Emerson's studies provide excellent examples of how heterocaryons may be used in the study of gene action particularly in relation to specific metabolic processes. It is probable that the situation where a heterocaryon may be formed between nuclei differing in only one gene is limited to those cases where specific interaction of metabolites occurs.

Emerson has pointed out (24) that the type of heterocaryon

described in the examples above represents a case of one gene heterosis, for the two types of nuclei differ by only one pair of alleles. This may be contrasted with the type of heterocaryon described by Beadle and Coonradt where two pairs of alleles are concerned. Here the heterosis is due to the mutually complementary nature of the nuclei involved. Each nucleus carries the favorable dominant allele of the corresponding mutant allele occurring in the other type of nucleus. Emerson suggests that cases of one gene heterosis probably occur rather frequently in Neurospora and cites a number of possible examples. Cases of one gene heterosis are known in other organisms, for example that reported by Jones (25) for inbred lines of corn. One gene heterosis may be of importance in the phenomenon of over dominance (26). In such cases it appears that the immediate gene products of the heterozygote are more suited for optimal growth than either homozygote. This seems to be the case for the sulfonamide requiring/sulfonamide requiring supressor heterocaryon. The importance of this case lies in the fact that the increased vigor may be explained in precise chemical terms.

There has been some difference of opinion as to whether bisexual heterocaryons could be formed in heterothallic species, such as N. sitophila or N. crassa. Lindegren (27) reported that certain cultures of N. crassa isolated from natural sources were bisexual self sterile heterocaryons as indicated by the fact that they would cross to strains of either mating type but were not capable of self fertilization. Lindegren claimed that these strains carried self sterility factors that prevented selfing but that in the presence of

another strain the action of these factors was overcome, probably by hormones. He suggested that this condition was widespread in cultures of N. crassa obtained from nature. He supposed that bisexual heterocaryons arose in nature by the anastomosis of hyphae containing 'A' nuclei with hyphae containing 'a' nuclei, and that under certain conditions natural selection would act in favor of self sterile heterocaryons, but he offered no evidence to support this view. Beadle (personal communication) made what appeared to be a self sterile heterocaryon between two nicotinicless strains, 3416 and 4540 of opposite mating type. This grew vigorously, did not form fruiting bodies and was fertile with both 'A' and 'a' strains. Attempts to reconstruct this heterocaryon were unsuccessful. It is possible that the bisexual heterocaryons found by Lindegren were derived from bisexual ascospores which may occasionally be formed in N. crassa (28) and which give rise to self fertile cultures. However the presence of sterility factors may have prevented self fertilization. Dodge (29) has suggested ways in which such self sterility factors might act.

Dodge was able to produce heterocaryons with heterothallic strains of the normally homothallic N. tetrasperma whether he combined two lines of the same or of opposite mating types (9). However, he was unable to show the existence of a bisexual heterocaryon in N. sitophila (8). Kohler (30) who worked extensively on hyphal anastomoses found no evidence that a bisexual mycelium in heterothallic species of Neurospora could be obtained by mixing unisexual conidia of both mating types. Sansome (31, 32) also failed to obtain evidence

of vegetative heterocaryons involving 'A' and 'a' nuclei in N. crassa. Beadle and Coonradt (13) found pronounced differences between heterocaryons formed between biochemical mutants of N. crassa of like and opposite mating type. They suggested that the lower growth rate of bisexual combinations is due to the direction of the energies of the fungus towards sexual reproduction rather than vegetative growth. There is evidently quite a different response between homothallic and heterothallic species of Neurospora to the presence of nuclei of both mating types in the cytoplasm. Both Sansome and Dodge have concluded that the association of nuclei of different mating types in heterothallic species occurs at the time of sexual reproduction and not by the fusion of vegetative hyphae.

In contrast to the work reported above, Gross (33) has recently claimed to have obtained heterocaryons between biochemical mutants of opposite mating type in N. crassa. Lysineless and pantothenicless strains of opposite mating type were combined on minimal media and growth of a heterocaryotic mycelium resulted. Proof of heterocaryosis was made by the isolation of hyphal tips, fifty percent of which subsequently grew on minimal. The resulting cultures crossed freely with both 'A' and 'a' tester strains and were also self-fertile. The growth rate of such heterocaryons was found to be slow and irregular. It was shown that the ratio of homocaryotic conidia for pantothenicless - lysineless heterocaryons between strains of the same mating type is from 2 - 8 pantothenicless conidia to one lysineless. In the heterocaryon lysine 'a' - pantothenicless 'A' the ratio

of pantothenicless conidia to lysineless conidia was 20 - 80 /1 and for the pantothenicless 'a' - lysineless 'A', 400 - 1200 /1. From this Gross draws the conclusion that nuclear ratios may be determined by factors other than the particular mutants under consideration, although whether he attributes the difference in nuclear ratios as being directly connected with the mating type locus is not stated. It is probable that this is not the case, and this could no doubt be confirmed by repeating these experiments with a number of different strains of like genotype with respect to the particular mutants but derived from different genetic stocks. From this work then it is reasonable to draw the conclusion that heterocaryons can be formed between strains of opposite mating type of N. crassa providing there is a strong enough selective force. In addition it is logical to assume that strains of this fungus may differ in their ability to form bisexual heterocaryons.

Work on heterocaryosis in other fungi has in general confirmed the results obtained with Neurospora. Heterocaryosis has been shown to occur in Penicillium, both in strains isolated from nature (34, 35) and between artificially produced mutants (36). Pontecorvo has developed a technique for the formal genetics of Aspergillus nidulans, a homothallic species, making use of balanced heterocaryons, which may readily be formed by combining biochemical mutants of this fungus (37, 38, 39). The relation of heterocaryosis in Penicillium notatum to penicillin production has been studied (6, 40).

Heterocaryosis has provided a useful tool for investigation of various genetic phenomena. By crossing heterocaryons of mutants

of N. crassa to wild types, Sansome (41) showed that the nuclei of the ascogenous hyphae in one peritheciumare sometimes derived from several initial combining pairs, a result independently confirmed by Grant (42). Atwood has described a method whereby heterocaryosis may be used for the isolation of special types of biochemical mutants (43). Atwood (44) and Atwood and Norman (45) have used heterocaryons between biochemical mutants of N. crassa for irradiation studies.

Heterocaryosis thus provides a means of studying some genetic phenomena to an extent generally not possible in higher organisms. Studies on heterocaryons have led to a fuller understanding of heterosis. Two hypotheses have been formulated to explain heterosis. The first which Crow (26) refers to as the dominance theory attributes the increased vigor of the heterozygosity to the masking of detrimental recessive alleles by the dominant beneficial alleles. This type of hybrid vigor is illustrated in the balanced heterocaryon between biochemical mutants. The second hypothesis assumes that the heterozygote is superior to either homozygote, a model for this explanation being the sulfonamide mutant described by Emerson and referred to above. A strict comparison between heterocaryotic vigor and heterosis is not possible. In a diploid heterozygous for a particular gene both alleles occur in the same nucleus and in a definite dosage ratio. In heterocaryons the alleles are present in different nuclei and the relative frequency of the genes depends on the nuclear ratio. The nuclear condition is constant for all cells, except gametes, for a diploid organism but the nuclear ratios of heterocaryons may vary considerably

in various parts of the thallus. However, heterocaryosis is the physiological equivalent of diploidy in a haploid organism and demonstrates the same phenomena correlated with this condition, such as dominance and heterosis.

It is justifiable to suppose that any genetic factor causing modification of the biochemical mutant genes concerned in the heterocaryon will produce effects on the heterocaryotic growth. It is well known amongst Neurospora workers that irregular results are occasionally obtained with attempts to form heterocaryons but this phenomenon has received little direct attention. It is the purpose of this work to demonstrate how heterocaryotic growth may be modified by genes other than these responsible for the deficiencies in biochemical synthesis.

STATEMENT OF PROBLEM

It will be shown here that a number of different genes affect the formation of heterocaryons between biochemical mutants in Neurospora crassa. An analysis has been made of one particular case, that of the heterocaryon formed between a mutant requiring pantothenic acid, and one requiring lysine. Only heterocaryons between strains of the same mating type have been considered. One of the mutants, pantothenicless, was crossed to various other strains, and the progeny tested against the strain of like mating type of two given lysine strains, the latter acting as tester strains. With the general experimental hypothesis that there are genes controlling the formation of heterocaryons, it is obvious that such genes will affect both partners of the heterocaryon and that the choice of tester strains will be of great importance. To make analysis

simple they should be identical with each other in these genes. It was not possible to determine the isogenicity of the two strains used, but as far as can be deduced from the results obtained, it appears that these two strains are similar in the genes controlling the formation of the particular heterocaryon under test. It will be shown later that different results are obtained if different tester strains are used.

Four genes have been shown to be involved in the formation of this particular heterocaryon. In one test there is evidence that at least one more may be concerned. These genes have been identified by their effects on heterocaryotic growth, and by following their segregation and recombination in crosses.

The preliminary experiments on which the idea of genetic control of heterocaryosis was based came from attempts to repeat the work of Beadle and Coonradt with different isolates of the same mutants used in their studies. For example, these workers found that a heterocaryon could be formed between the pantothenicless mutant 5531A and the lysineless mutant 4545A. A strain of 5531A thought to be the same as that used by Beadle and Coonradt was obtained and tested against various reisolates of 4545A. Some of these latter strains resulted from crosses to the Abbott wild type 12a and others to the Emerson wild type 5297a. When six such reisolates were tested against the pantothenicless strain 5531A for heterocaryon formation using the same techniques used by previous workers, no heterocaryotic growth was obtained. A strain of 4545A, reported to be the same as that used by Beadle and

Coonradt was obtained* and on testing this was found to form a heterocaryon with 5531. Similarly the mutant 1633, requiring p-aminobenzoic acid, while able to form a heterocaryon with 5531A and 4545A failed to do so with the reisolates of 4545A. On testing it was shown that 4545A and the various isolates of this mutant had identical responses to lysine and although no critical experiments were done it appeared that as far as the lysineless character was concerned, all these strains were alike. It was assumed, therefore, that other factors, probably genetic, were acting to prevent the formation of a heterocaryon and it was decided to further investigate this problem. It was hoped that not only might some information be obtained with regard to heterocaryon formation and heterocaryotic growth, but that further means of studying the actual nature of the metabolic derangement in biochemical mutants might be revealed. Although the investigation did not proceed quite this far there are indications that heterocaryosis would provide a potentially useful tool for such studies.

These mutants, 5531 and 4545 were found to be very suitable for the subsequent detailed analysis. Crosses in which the pantothenicless gene is carried by both parents are fertile to some degree permitting use of the backcross technique. In addition neither mutant backmutates readily and both have well defined biochemical requirements. Neither shows any growth on unsupplemented medium.

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* I wish to thank Dr. Laura Garnjobst of Stanford University for making this strain available to me.

MATERIALS AND METHODS

Mutants used

1. Pantothenicless 5531. Requires pantothenic acid for growth. Tatum and Beadle (46), Wagner (47), Wagner and Guirard (48) and Wagner and Haddox (49).
2. Aminobenzoicless 1633. Unable to synthesize p-aminobenzoic acid. Beadle and Tatum (12) and Zimmer (50).
3. Lysineless 4545. Requires the amino acid lysine. Doermann (51) and Good (52).

Cultural Methods

As shown by Beadle and Coonradt (loc. cit) when two biochemical mutants are inoculated together on a medium which will not support the growth of either, any subsequent heterocaryotic growth is under a strong selective influence. The basal medium used in this work has been the so-called 'Fries #3 minimal'. This consists of Fries #3 salt solution, with the addition of 2% sucrose, and 0.005 mg. biotin per litre, solidified with 2% Difco agar, and containing no amino acid or other vitamin supplements. All cultures were grown at 25°C.

In earlier tests heterocaryons were grown on slants in 6" test tubes but in later ones growth tubes, as described by Ryan, Beadle and Tatum (53), were used. Such tubes provide a more delicate means of measuring growth than the test tubes. They are of Pyrex glass, tubular, with an internal diameter of 13 mm. The ends of the tubes

are turned up at an angle of 45° about 6 cm. from the end, and the effective growing surface varies in the different sized tubes from 220 mm. to 450 mm. No effect of the different sized tubes could be found on any of the results to be described. The amount of agar added to each tube varies with the size, but with the 220 mm. tubes it is 10 ml. The amount of agar was always kept constant in different trials and dispensed from an automatic pipetting machine.

Measurements of the advancing mycelial front were made at intervals of from twelve to eighteen hours and marked with a grease pencil on the lower half of the tube. Later these marks were measured and the growth rate of the mycelium calculated.

Spore suspensions of the strains to be tested for heterocaryon formation were made in sterile distilled water from cultures grown on Horowitz complete medium (54) for six days. Loopfulls of each of the two suspensions were then placed together on the surface of the agar in the growth or test tube. The general procedures for dissection of asci, determining the mating type of cultures, maintaining stock cultures, sterilization etc. were the same as those used by Beadle and Tatum (55).

Crosses were made on the medium described by Westergaard and Mitchell (56) with the addition of appropriate supplements. The mutants used cross readily with wild type. In crosses in which both strains carry the same mutant allele, there is considerable variability in the fertility depending on the particular mutant used. With pantothenicless strains it is usually possible to get fertile crosses, although the

viability of the ascospores is never very high. With lysineless a few perithecia are formed, but very few viable ascospores and with aminobenzoicless no perithecia are formed at all. Horowitz and Leupold (57) quote unpublished work of Gershowitz who showed that in crosses of this type with the methionine mutants, if twice the optimal requirement of methionine is supplied to the crossing medium sterility is considerably reduced. This general procedure was tried with the three mutants used in this work. Crosses were set up on Westergaard-Mitchell medium between strains each carrying the same mutant allele, and the medium supplemented with from 1 to 20 times the optimal requirement of the specific substrate. With pantothenicless no effect of addition of the substrate was found, with lysineless sterility became more pronounced at higher concentrations of lysine, and with aminobenzoicless no perithecial formation occurred at any level of substrate addition. All these crosses were made with both simultaneous inoculation and reciprocal protoperithecial inoculation and it appears as though the phenomenon described by Gershowitz does not hold with the three mutants used in this work.

The system of nomenclature of cultures has been to number the asci consecutively and to number the spores of each ascus from one to eight, i.e. 560 - 5 represents the fifth spore of the five hundred and sixtieth ascus dissected. For convenience the odd number spore of each spore pair has been taken as the representative of the spore pair.

EXPERIMENTAL

After several hundred strains obtained from the progeny of various crosses of the pantothenicless mutant had been tested against the tester strains in ordinary six inch test tubes it was obvious that a number of different growth responses were being observed. It has been hoped that the progeny could be classified into two groups; Those strains able to form a heterocaryon with the tester strains, and those unable to do this. It was at this stage that the change to growth tubes was made and all tests previously made in test tubes were repeated in growth tubes. Under these conditions a number of heterocaryotic growth types occur between various strains of the pantothenicless mutant and the lysineless tester strains.

Types of heterocaryotic growth

Previous to these results only two types of heterocaryotic growth have been described for heterocaryons between biochemical mutants of N. crassa. Beadle and Coonradt describe these two types. The first is the growth resulting when mutants of like mating type are paired. Here the growth is very similar to the growth of wild type Neurospora; growth starts rapidly, and progresses at a growth rate of approximately 4 mm./hr. at 25°C. The second type observed was that of heterocaryons between strains of opposite mating type and in this case the growth rate varied from 1 mm./hr. to 3.8 mm./hr., with the difference that the growth rate is rarely linear, as occurs with the heterocaryons of like mating type; the growth is slow in starting and the general vegetative vigor of the cultures is low.

In the experiments reported here other variations in the type of heterocaryotic growth have been obtained. Types of growth varying in the vigor of the mycelial growth, the time at which growth commences and the ability to continue heterocaryotic growth have been found. In general it has been found possible to classify these responses into six types of heterocaryotic growth (see Fig. 1). These various types are remarkably constant and classification of any particular growth is easily made into one or the other of the classes. A description of the growth habit of the various types of heterocaryotic growth follows.

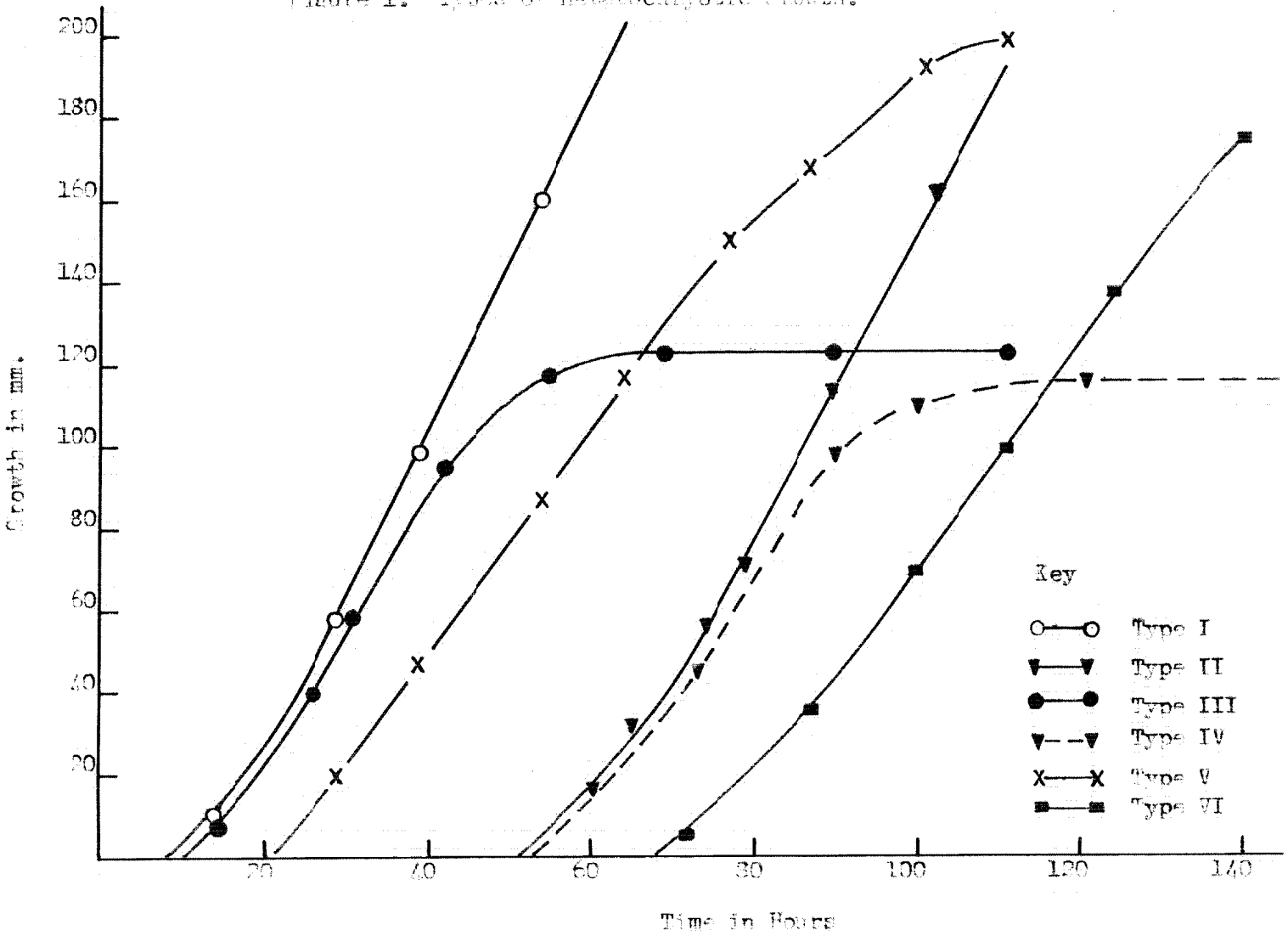
Type I

Growth begins within a few hours of inoculation and rapidly assumes a wild type rate. The mycelium is vigorous and eventually fills the whole growth tube. This type is identical with the growth obtained with wild type strains, and is the type of growth described by Beadle and Coonradt for heterocaryons between biochemical mutants of like mating type. The growth rate varies between 3.8 and 4.5 mm./hr. Hyphal tips taken from the growing front continue growing vigorously on minimal medium after a delay of a day or so. The mycelial frontier in growth tubes is sharp and well defined.

Type II

The growth characteristics and appearance 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 beginning of growth is well defined and not gradual. The growth

Figure 1. Types of heterocaryotic growth.



rate again varies between 3.6 and 4.5 mm./hr.

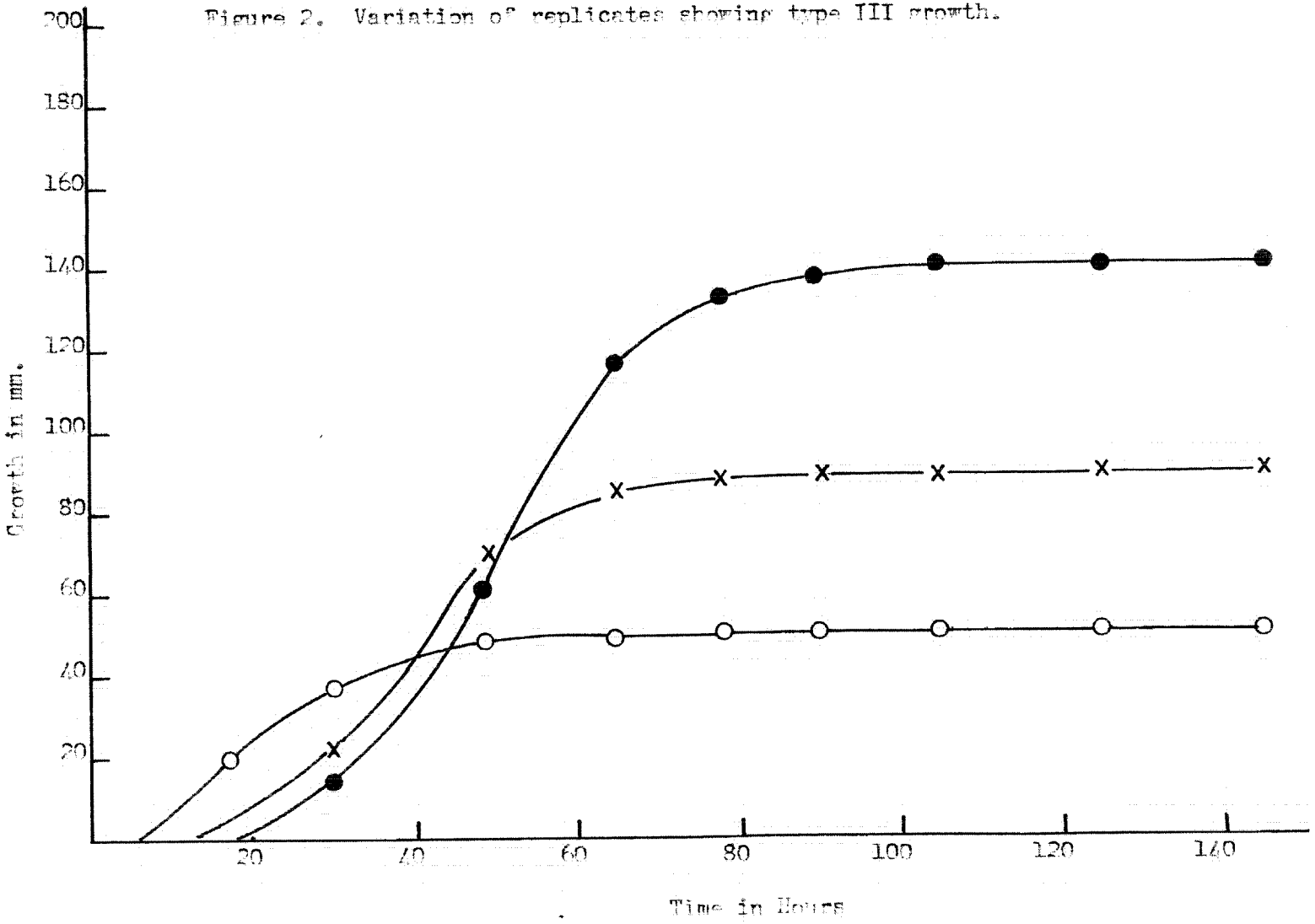
Type III

Growth commences soon after inoculation but comes to an abrupt halt after a period of growth varying from 30 - 60 hours. The growth rate before stopping is that of wild type. The point at which growth ceases cannot be predicted, so that exact information on the rapidity with which growth ceases cannot be easily obtained. However, from the growth curves obtained it appears there is some slowing up of the growth before actual cessation. The appearance of the mycelium of the growth before stopping is identical with that of wild, but 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. Hyphal tips from the actively growing phase of the heterocaryotic growth continue growing for 24 - 48 hours after transfer but then stop, becoming highly branched with numerous short branches. The growth from such hyphal tips is much more than that obtained from hyphal tips of either mutant transplanted onto minimal. The actual point at which growth ceases is extremely variable even between replicates of the same strain. Fig. 2 shows the growth curves of a number of replicates of a combination of strains giving this type of growth. Further experiments to obtain more information about this type of growth are described in a subsequent section.

Type IV

The description of the growth of type III heterocaryons

Figure 2. Variation of replicates showing type III growth.



applies to type IV with the exception that growth does not commence until 35 - 90 hours after inoculation. Here again there is the same variation in the amount of growth occurring before the mycelium stops growing and in addition to this is the variation in the time at which growth will begin (see Fig. 3).

Type V

Growth begins 12 - 24 hours after inoculation. The mycelium is much less vigorous than in the previous cases, it is thinner and somewhat appressed to the medium. Considerable variation occurs in the actual amount of mycelial growth even between replicates. Generally the mycelial frontier is not very well defined. Sometimes the growth becomes progressively thinner, slows down and may eventually cease altogether. The maximum growth attained in this type of growth never exceeds 3 mm./hr. and is often not linear. This type of growth shows some similarities to that described by Beadle and Coonradt for growth between biochemical mutants of opposite mating type.

Type VI

The general characteristics of this type are the same as those described for type V, but as with types II and IV, growth does not begin until 35 - 90 hours after inoculation. The time of starting is usually towards the upper limit of this range, but as type V sometimes shows a tendency towards late starting, it is occasionally difficult to decide whether one is observing an early starting type VI or a late starting type V. However, this occurrence is rare and has presented little difficulty.

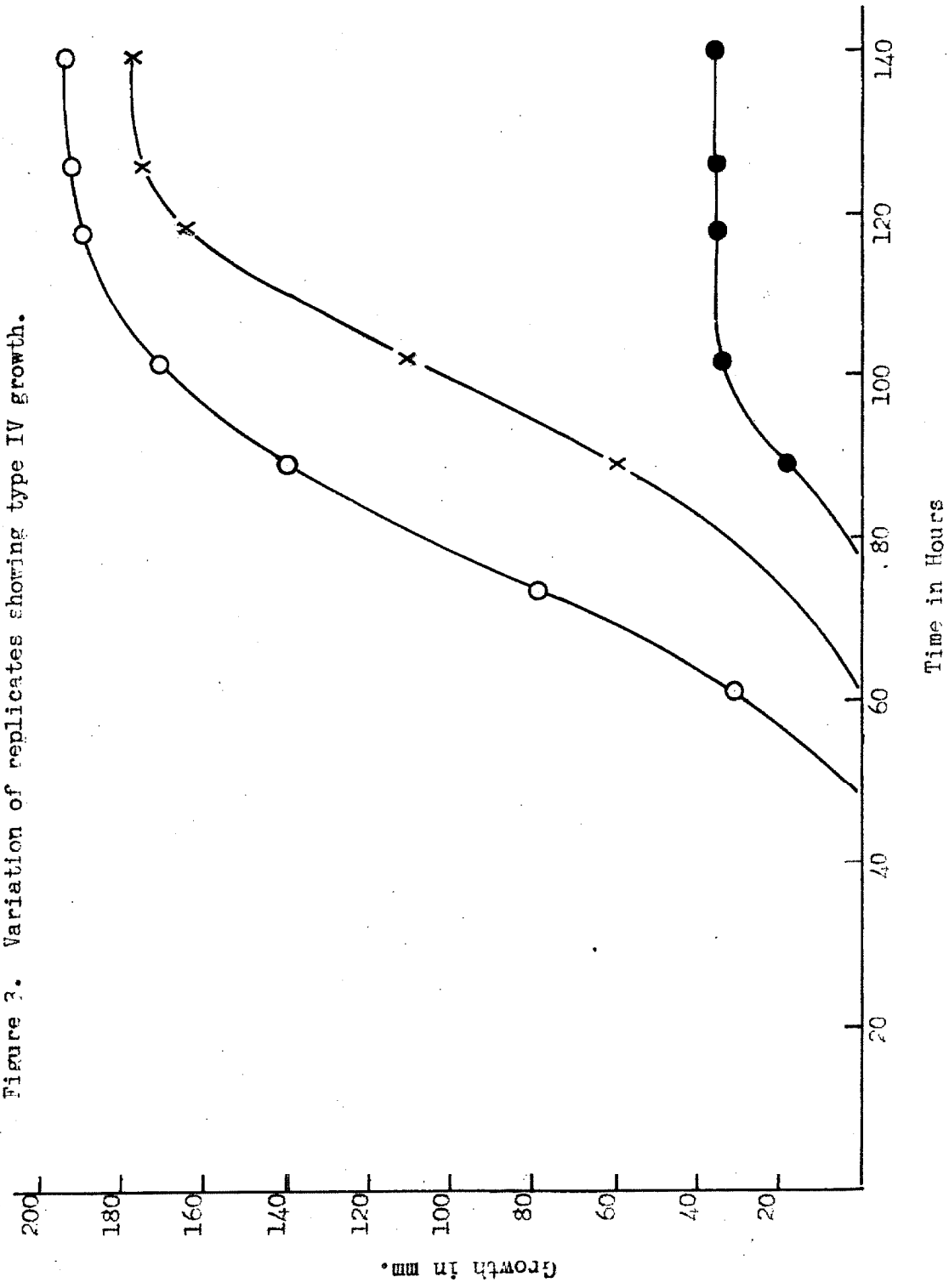


Figure 3. Variation of replicates showing type IV growth.

Type VII

Here no heterocaryotic growth occurs at all. Slight growth may occur at the site of the inoculum after several days. This probably results from autolysis of some of the conidia with liberation of growth substances which then become available to the remaining viable conidia. Such growth never progresses more than a centimetre or two from the point of inoculation.

From the growth characteristics of the various heterocaryons a number of features associated with the formation and growth of a heterocaryon can be recognized, viz.

1. The ability to form a heterocaryon.
2. The time lag in initiation of growth of the heterocaryon.
3. The ability to continue growing as a heterocaryon.
4. The growth rate and habit of the heterocaryon.

From the results of certain crosses it appears that not only is the formation of a heterocaryon subject to genetic control but also the various types of heterocaryotic growth may be a result of genetic modification. All types of growth were observed in heterocaryons with pantothenic strains of either mating type and hence with both tester strains. These types of growth do not result from any observable growth characteristics of the particular parent strains themselves. The two lysineless strains grow at wild type growth rate when grown on media containing adequate amounts of lysine. Isolates of the pantothenicless mutant representing the different types of heterocaryotic growth gave wild type growth when grown alone on media supple-

mented with pantothenic acid.

Proof of the heterocaryotic condition

It is possible to establish beyond reasonable doubt that the heterocaryotic condition is in fact present in supposed heterocaryons and that growth is not due to syntrophy. Beadle and Coonradt have described the most reliable method of proving the heterocaryotic condition (13). Mycelium is taken from the advancing front of the growing heterocaryon and transferred to an agar plate containing unsupplemented medium solidified with 4% agar. The mycelium will continue growing and with the aid of a sharpened platinum spatula hyphal tips are cut from the advancing hyphae, and transferred to tubes containing minimal medium. After a lag period of 24 hours or so growth will continue, and within a few days conidia will form. If these conidia are then used to fertilize protoperithecia of a wild type, the resulting perithecia will have two types of ascospore progeny, mutant and wild, and if both mutant types of nuclei were originally present in the hyphal tip then the conidia used for fertilization will contain both types, which will then be represented in the ascospore progeny. By spreading random spores on the surface of a minimal plate, heat treating to induce germination, and allowing them to germinate, it is relatively easy to distinguish the mutant from the wild spores on the basis of their germination pattern. The mutant spores are then picked off and transferred to a medium which will support the growth of both mutant types. Each individual culture can then be tested for growth on one or the other specific substrates required for the mutants concerned, and

classified into one or the other type. If both types of mutants are found in the progeny then the original culture from which hyphal tips were taken must have been heterocaryotic.

Where uninucleate conidia are available they may be used for a proof of the heterocaryotic condition. Hyphal tips are isolated in the same way as before, and when they have produced conidia these may be plated out on selective media, or single germinating conidia picked off, transferred and individually tested. This method has been used by Sansome with 'fluffy' strains of N. crassa (58) and by Pontecorvo and his coworkers with Penicillium and Aspergillus.

The method used in the present work has been that of Beadle and Coonradt and by this the heterocaryotic condition of types I, II, V and VI has been proved. However, it was not possible to use this method for types III and IV. It will be recalled that hyphal tips of these two types show only limited growth and this is insufficient for them to be crossed to wild type. As the strains used produce multinucleate macroconidia the other method described above could not be used. It has therefore not been possible to prove definitely that types III and IV are heterocaryons. The growth obtained from the hyphal tips of these types is much more than that obtained with such tips from either mutant when transferred to unsupplemented media and this is taken as evidence for the presence of both nuclear types in the one mycelium.

Experiments on the growth characteristics of types III and IV

Various additional experiments were carried out to determine

the nature of the heterocaryotic growth associated with types III and IV. It will be recalled that in these types growth begins at the wild type rate but subsequently comes to an abrupt halt. Inoculation of a combination giving the type III response was made in a petri dish containing minimal medium. The resulting growth was not circular as normally occurs with Neurospora grown under these conditions, but irregular and sectorial indicating different parts of the mycelium were growing at different rates. Conidia taken from the heterocaryotic growth were plated onto supplemented sorbose medium and it was found that the pantothenicless nuclei showed a great preponderance over the lysineless nuclei. No heterocaryotic conidia were found as indicated by the lack of colony formation when conidia were plated out on minimal sorbose medium, but this could be another manifestation of the inability of the heterocaryon to continue growing.

The edge of the mycelium where growth has ceased was found to have a different appearance from the main body of hyphae. If hyphal tips are taken from this region they are found to grow on media supplemented with pantothenic acid, but not on either lysine or minimal media. Similarly if pantothenic acid in solution is added to the arrested front of this type of growth the mycelium is stimulated to grow, but no growth occurs when lysine is added in this way. From this it can be concluded that the pantothenicless nuclei predominate and take over the frontier and this presumably accounts for the cessation of growth.

Thus this heterocaryon appears to be in an unstable state with one type of nucleus, the pantothenicless, predominating over the lysineless nuclei. Various conditions may act to upset the hetero-

caryotic condition so that only the pantothenicless nuclei remain in the growing front and hence growth ceases.

The genetic basis of heterocaryon types

If the various types of heterocaryons are a result of genetic modification it is obvious that on crossing the various types to each other, some idea of the genetic mechanism involved can be obtained by testing the progeny against tester strains. This has been done in a number of cases. In all these crosses both parents carry the pantothenicless gene, so it is possible to analyse all spore pairs of each ascus, testing the spores of each mating type against the lysine tester strain of like mating type. The results from a number of crosses of this type are given in Table 1.

On the basis of the data summarized in Table 1 and other evidence to be presented later the following hypothesis 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 data. Probably additional genes affect the process. These four genes are 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. Allele W by itself is

TABLE 1

Results of testing progeny from crosses between pantothenic strains which give various heterocaryon types when combined with standard lysine tester strains.

Cross	No. asci analysed		Results
I x I		15	Progeny all type I
I x II	#1 #2	9 9	Progeny consisting of types I and II, segregating 1:1 in the ascus.
I x III	#1 #2	6 10	Progeny consisting of types I and III segregating 1:1 in in the ascus
I x IV		17	Both parental types I and IV recovered in the progeny, but in addition recombinant types II and III
I x V	#1 #2	20 17	Types I and V segregating 1:1 in the ascus
VII x VII	#1 #2	18 17	All progeny type VII
III x IV		11	Types III and IV only in the progeny segregating 1:1 in the ascus

not sufficient and must be present in combination with either or both X and Y described below. The evidence for the existence of this gene is somewhat circumstantial and not as definite as with the three other genes. It has not yet been possible to make a cross in which this gene alone of the four identified is segregating with its alternative allele. Crosses in which it and one other of the four genes are segregating have been analyzed.

Gene X Allele X of this gene exerts an influence on the growth rate and general vigor of the heterocaryotic mycelium. Segregation of this gene and its alternative allele X' has been demonstrated. Pantothenic strains giving type V growth differ from those giving type I by the presence of the alternative allele in the former strains, so that in crosses of these two types a 1:1 segregation of the parental types occurs in the ascal progeny.

Gene Y Allele Y of this gene is concerned with the maintenance of the heterocaryotic condition. Pantothenic strains giving type III heterocaryotic growth 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 The presence of the Z allele of this gene enables any heterocaryotic growth formed to start growing soon after inoculation, and 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 (vide Beadle and Coonradt loc. cit), so that the dominance or recessiveness of genes affecting the process of heterocaryon formation cannot be determined.

To elaborate this scheme further genotypes of the various heterocaryon types as assigned on the basis of the above hypothesis are listed below.

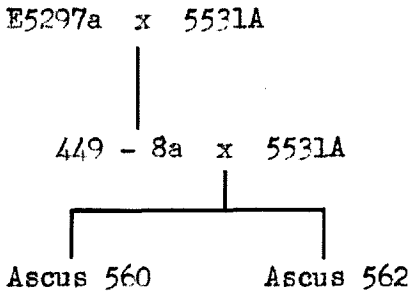
Heterocaryon type	Genotype
I	W X Y Z
II	W X Y Z'
III	W X Y' Z
IV	W X Y'Z'
V	W X'Y Z
VI	W X'Y Z'
VII	W'---; --X'Y'--

As mentioned before the genetic constitution of the tester strain will undoubtedly be of importance. To investigate this would, of course, require an analysis of the lysine strains of much the same nature as that made for the pantothenicless strains. Unfortunately this is probably not possible because crosses in which both parents carry the lysineless gene are not fertile. This means the analysis would have to be made with crosses to wild type 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 analyzed. With the assumption that 5531 carried all the genes necessary to form a type I heterocaryon, which may be considered as the 'normal' form, the first step in the genetic analysis is to cross this strain to another which could be expected to carry alternative alleles of the various genes. The wild type Emerson 5297a was selected for this purpose and crossed to 5531. The progeny from this cross were found to show, with some limitations, the various phenotypic modification described above and the indications were that a number of genes were probably concerned in the process of heterocaryon formation. 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. Much more information can be obtained if all the progeny of the cross can be examined for the character under analysis. This may be done if both parents of the cross carry the pantothenicless allele. If one parent of the cross is of type I, such as 5531, it is obvious that we can probably obtain the segregation of a maximum number of genes by selecting a type VII as the other parent. A strain of the type, 449 - 8 was selected from the progeny of the cross 5531 x 5297a, and crossed to 5531. The genetic history of the various strains used in the analysis is shown in Fig. 4.

Figure 4. Genetic history of strains used.



The hypothesis described above is based on the subsequent analysis of progeny resulting from the cross 449 - 8 x 5531. The crosses mentioned above where the various types of heterocaryon formation are shown to differ from type I by one or more genes were carried out by crossing progeny of the cross 449 - 8 x 5531 to either 5531 or to other progeny of this generation. The results of the crosses 5531A x 5297a and 5531 x 449 - 8 will be described in detail after the presentation of data showing the segregation of the postulated genes.

The crosses outlined in Table 1 will now be discussed in more detail and their relation to the experimental hypothesis shown.

Type I x Type I

562 - 7 x 5531A

Fourteen asci were analyzed for the ability to form heterocaryons with the standard tester strains. The results are given in Table 2. From these data it is seen that the progeny are all of Type I. This cross is therefore homozygous for the factors giving

TABLE 2

562 - 7 (type I) x 5531 (type I)

Ascus and spore no.	Mating type	Heterocaryon type	Ascus and spore no.	Mating type	Heterocaryon type
1131-1	A	I	1138-1	A	I
-3	A	I	-3	a	I
-5	a	I	-5	A	I
-7	a	I	-7	a	I
1132-1	A	I	1139-1	a	I
-3	a	I	-3	a	I
-5	A	I	-5	A	I
-7	a	I	-7	A	I
1133-1	A	I	1140-1	a	I
-3	A	I	-3	a	I
-5	a	I	-5	A	I
-7	a	I	-7	A	I
1134-1	A	I	1141-1	a	I
-3	A	I	-3	A	I
-5	a	I	-5	a	I
-7	a	I	-7	A	I
1135-1	a	I	1143-1	A	I
-3	a	I	-3	A	I
-5	A	I	-5	a	I
-7	A	I	-7	a	I
1136-1	a	I	1144-1	a	I
-3	a	I	-3	a	I
-5	A	I	-5	A	I
-7	A	I	-7	A	I
1137-1	A	I	1145-1	a	I
-3	a	I	-3	a	I
-5	A	I	-5	A	I
-7	a	I	-7	A	I

the type I response. The cross gives no information regarding the segregation of genes affecting the observed phenotypic variations of the heterocaryotic growth. The crosses below give this information.

Type I x Type II

Two crosses of this sort were analyzed, 844 - 7 x 5531, and 723 - 4 x 5531, where both 844 - 7 and 723 - 4 show the type II response when combined with 4545a and 5531 gives the type I response with 4545A. Nine asci were analyzed from each cross with the results given in Tables 3 and 4.

In both crosses types I and II segregate 1:1 in the ascus indicating a one gene difference between these types. Relating this result to the experimental hypothesis it is apparent that the allelic pair Z/Z' is segregating in this cross.

Type III x Type IV

824 - 1 (type III) x 707 - 3 (type IV)

Types III and IV differ only in the time at which heterocaryotic growth commences, bearing the same relation to each other as do types I and II. As the latter have been shown to differ by one gene, then it is likely that the former types will also show this difference. The results of analyzing 10 asci from this cross are given in Table 5.

As expected, the 'lateness' character segregates as though controlled by one gene in the same way as the cross between types I and II. Thus types III and IV differ with respect to gene Z.

TABLE 3

844 - 7 (type II) x 5531 (type I)

Ascus and spore no.	Mating type	Heterocaryon type	Ascus and spore no.	Mating type	Heterocaryon type
1161-1	a	II	1166-1	A	II
-3	a	I	-3	a	I
-5	A	I	-5	A	II
-7	A	II	-7	a	I
1162-1	A	I	1167-1	A	I
-3	A	II	-3	A	II
-5	a	I	-5	a	II
-7	a	II	-7	a	I
1163-1	a	I	1168-1	A	II
-3	a	I	-3	A	II
-5	A	II	-5	a	I
-7	A	II	-7	a	I
1164-1	a	I	1169-1	a	I
-3	a	II	-3	A	II
-5	A	II	-5	A	II
-7	A	I	-7	a	I
1165-1	a	I			
-3	a	II			
-5	A	II			
-7	A	I			

TABLE 4

723 - 4 (type II) x 5531 (type I)

Ascus and spore no.	Mating type	Heterocaryon type	Ascus and spore no.	Mating type	Heterocaryon type
1181-1	A	I	1187-1	a	I
-3	a	I	-3	A	I
-5	a	II	-5	A	II
-7	A	II	-7	a	II
1182-1	A	I	1188-1	a	I
-3	A	I	-3	a	II
-5	a	II	-5	A	I
-7	a	II	-7	A	II
1184-1	a	I	1189-1	A	II
-3	a	I	-3	a	I
-5	A	II	-5	a	I
-7	A	II	-7	A	II
1185-1	a	I	1190-1	A	II
-3	a	I	-3	A	II
-5	A	II	-5	a	I
-7	A	II	-7	a	I
1186-1	a	I			
-3	a	II			
-5	A	II			
-7	A	I			

TABLE 5

824 - 1 (type III) x 707 - 3 (type IV)

Ascus and spore no.	Mating type	Heterocaryon type	Ascus and spore no.	Mating type	Heterocaryon type
1201-1	A	III	1206-1	A	III
-3	A	III	-3	a	IV
-5	a	IV	-5	a	IV
-7	a	IV	-7	A	III
1202-1	a	IV	1207-1	a	IV
-3	a	III	-3	A	IV
-5	A	III	-5	a	III
-7	A	IV	-7	A	III
1203-1	a	III	1208-1	A	IV
-3	a	IV	-3	a	III
-5	A	III	-5	a	III
-7	A	IV	-7	A	IV
1204-1	a	IV	1209-1	a	III
-3	A	III	-3	a	III
-5	a	IV	-5	A	IV
-7	A	III	-7	A	IV
1205-1	A	III	1210-1	a	IV
-3	A	IV	-3	a	III
-5	a	IV	-5	A	III
-7	a	III	-7	A	IV

Type I x Type III

Two crosses of this sort were analyzed, 906 - 5 x 5531 and 564 - 5 x 5531 where both 906 - 5 and 564 - 5 give the type III reaction and 5531 is of type I. The results of the crosses are given in Tables 6 and 7.

Types I and III are seen to segregate 1:1 in the ascus showing a one gene difference between these two types. Again, relating this result to the experimental hypothesis, types I and III differ in respect to the allelic pair Y/Y' again supporting the hypothesis.

Type I x Type V

Two crosses between these two types have been analyzed, 560 - 5 x 5531 (20 asci) and 562 - 5 x 562 - 7, where both 560 - 5 and 562 - 5 are type V and 5531 and 562 - 7 are type I. The results of these crosses are shown in Tables 8 and 9.

In addition to the above asci a number of random spores of both mating types from one of these crosses was also analyzed. These data are given in Table 10.

The results show that a one gene difference occurs between types I and V. As stated in the hypothesis the allelic pair X/X' affects the heterocaryotic vigor of the growth and this allelic pair is segregating in this cross.

Type IV x Type I

560 - 1 (type IV) x 5531 (type I)

Type IV which is late starting and subsequently stops, differs in two characters from the 'normal' Type I. As it has already

TABLE 6

906 - 5 (type III) x 5531 (type I)

Ascus and spore no.	Mating type	Heterocaryon type	Ascus and spore no.	Mating type	Heterocaryon type
1241-1	A	III	1244-1	A	III
-3	A	III	-3	A	III
-5	a	I	-5	a	I
-7	a	I	-7	a	I
1242-1	A	III	1245-1	A	III
-3	a	I	-3	A	I
-5	A	I	-5	a	I
-7	a	III	-7	a	III
1243-1	A	I	1246-1	a	I
-3	A	III	-3	a	I
-5	a	III	-5	A	III
-7	a	I	-7	A	III

TABLE 7

564 - 5 (type III) x 5531 (type I)

Ascus and spore no.	Mating type	Heterocaryon type	Ascus and spore no.	Mating type	Heterocaryon type
1281-1	A	I	1288-1	A	III
-3	a	I	-3	A	I
-5	A	III	-5	a	I
-7	a	III	-7	a	III
1282-1	a	III	1289-1	a	III
-3	A	III	-3	A	III
-5	a	I	-5	a	I
-7	A	I	-7	A	I
1284-1	a	I	1290-1	a	III
-3	A	III	-3	A	I
-5	A	III	-5	A	III
-7	a	I	-7	a	I
1285-1	A	III	1291-1	A	I
-3	A	III	-3	A	I
-5	a	I	-5	a	III
-7	a	I	-7	a	III
1286-1	a	I	1292-1	A	I
-3	A	III	-3	a	I
-5	a	III	-5	a	III
-7	A	I	-7	A	III
1287-1	a	III			
-3	a	III			
-5	A	I			
-7	A	I			

TABLE 8

560 - 5 (type V) x 5531 (type I)

Ascus and spore no.	Mating type	Heterocaryon type	Ascus and spore no.	Mating type	Heterocaryon type
741-1	a	V	748-1	a	V
-3	A	V	-3	a	V
-5	a	I	-5	A	I
-7	A	I	-7	A	I
742-1	a	V	749-1	A	I
-3	A	V	-3	A	I
-5	A	I	-5	a	V
-7	a	I	-7	a	V
743-1	A	I	750-1	A	V
-3	a	V	-3	A	I
-5	A	I	-5	a	V
-7	a	V	-7	a	I
744-1	A	V	751-1	A	V
-3	A	V	-3	A	I
-5	a	I	-5	a	I
-7	a	I	-7	a	V
745-1	a	I	752-1	a	V
-3	a	V	-3	A	V
-5	A	I	-5	A	I
-7	A	V	-7	a	I
746-1	A	I	753-1	A	V
-3	A	V	-3	A	I
-5	a	V	-5	a	V
-7	a	I	-7	a	I
747-1	A	V	754-1	a	V
-3	a	V	-3	a	V
-5	A	I	-5	A	I
-7	a	I	-7	A	I

TABLE 8 (cont.)

Ascus and spore no.	Mating type	Heterocaryon type	Ascus and spore no.	Mating type	Heterocaryon type
755-1	A	I	758-1	A	I
-3	A	V	-3	a	V
-5	a	I	-5	a	I
-7	a	V	-7	A	V
756-1	A	V	759-1	A	I
-3	A	I	-3	A	I
-5	a	V	-5	a	V
-7	a	I	-7	a	V
757-1	A	I	760-1	a	V
-3	A	V	-3	A	V
-5	a	I	-5	A	I
-7	a	V	-7	a	I

TABLE 9

562 - 5 (type V) x 562 - 7 (type I)

Ascus and spore no.	Mating type	Heterocaryon type	Ascus and spore no.	Mating type	Heterocaryon type
1021-1	A	I	1028-1	a	V
-3	A	I	-3	a	I
-5	a	V	-5	A	I
-7	a	V	-7	A	V
1022-1	A	I	1029-1	A	I
-3	A	I	-3	A	V
-5	a	V	-5	a	I
-7	a	V	-7	a	V
1023-1	a	V	1030-1	A	I
-3	a	V	-3	a	V
-5	A	I	-5	A	I
-7	A	I	-7	a	V
1024-1	A	I	1031-1	a	I
-3	A	V	-3	a	I
-5	a	V	-5	A	V
-7	a	I	-7	A	V
1025-1	A	V	1032-1	a	I
-3	a	V	-3	a	V
-5	a	I	-5	A	V
-7	A	I	-7	A	I
1026-1	a	V	1033-1	A	I
-3	a	V	-3	A	I
-5	A	I	-5	a	V
-7	A	I	-7	a	V
1027-1	A	I	1034-1	a	V
-3	A	I	-3	a	V
-5	a	V	-5	A	I
-7	a	V	-7	A	I

TABLE 9 (cont.)

Ascus and spore no.	Mating type	Heterocaryon type	Ascus and spore no.	Mating type	Heterocaryon type
1035-1	A	V	1037-1	a	V
-3	A	I	-3	A	I
-5	a	V	-5	a	V
-7	a	I	-7	A	I
1036-1	a	V			
-3	A	I			
-5	A	I			
-7	a	V			

TABLE 10

Types of heterocaryon formation occurring in random spore progeny of cross 560 - 5 (type V) x 5531 (type I).

Heterocaryon type	Mating type		Total
	a	A	
I	28	15	43
II	--	--	--
III	--	--	--
IV	--	--	--
V	19	10	29
VI	--	--	--
VII	--	--	--
	47	25	72

been shown that type II (late starting) and type III (arrested growth) differ from type I with respect to a single gene in each case, we would expect type IV to be the 'double mutant' and differ from type I in both genes. In a cross between type I and type IV in the absence of complete linkage we would therefore expect to find not only the parental types I and IV but also the recombinant types II and III. Seventeen asci were analyzed from the above cross and the results are given in Table 11.

TABLE 11

560 - 1 (type IV) x 5531 (type I)

Ascus and spore no.	Mating type	Heterocaryon type	Ascus and spore no.	Mating type	Heterocaryon type
721-1	A	III	729-1	A	II
-3	a	II	-3	A	II
-5	A	II	-5	a	III
-7	A	III	-7	a	III
722-1	a	III	730-1	A	III
-3	a	IV	-3	A	II
-5	A	II	-5	a	III
-7	A	I	-7	a	II
723-1	A	III	731-1	A	II
-3	a	II	-3	a	IV
-5	a	II	-5	A	III
-7	A	III	-7	a	I
724-1	A	I	733-1	a	II
-3	A	II	-3	a	I
-5	a	III	-5	A	IV
-7	a	IV	-7	A	III
725-1	a	III	734-1	a	II
-3	A	IV	-3	A	III
-5	A	II	-5	A	I
-7	a	I	-7	a	IV
726-1	a	I	735-1	a	III
-3	A	IV	-3	a	II
-5	A	II	-5	A	III
-7	a	III	-7	A	II
727-1	A	II	737-1	A	II
-3	a	II	-3	a	I
-5	a	III	-5	a	III
-7	A	III	-7	A	IV
728-1	a	I	738-1	A	III
-3	A	I	-3	A	II
-5	A	IV	-5	a	II
-7	a	IV	-7	a	III

TABLE 9 (cont.)

Ascus and spore no.	Mating type	Heterocaryon type	Ascus and spore no.	Mating type	Heterocaryon type
740-1	A	I			
-3	A	IV			
-5	a	IV			
-7	a	I			

SUMMARY

Phenotypic Segregation	No. observed
I, I, IV, IV	2
II, II, III, III	7
I, II, III, IV	8
	17

A number of random spores were also analyzed from this cross and the results are given in Table 12.

TABLE 12

Types of heterocaryons found in random spore progeny of cross 560 - 1 (type IV) x 5531 (type I).

Heterocaryon type	Mating type		Total
	a	A	
I	8	7	15
II	10	11	21
III	6	5	11
IV	2	7	9
V	--	--	--
VI	--	--	--
VII	--	--	--
	26	30	56

Thus we may conclude that type IV differs from type I with respect to two genes, controlling the time of initiation of heterocaryotic growth and the maintenance of growth. These genes were designated Z and Y respectively in the experimental hypothesis. Evidently the cross is homozygous for gene X, and with the assumption that gene W is required for heterocaryon formation this cross is

between genotypes W X Y Z (type I) and W X Y' Z' (type IV). If this be the case there are only three possible phenotypic types of segregation as follows.

Genotype	Phenotype	Genotype	Phenotype	Genotype	Phenotype
W X Y Z	I	W X Y Z'	II	W X Y Z	I
W X Y Z	I	W X Y Z'	II	W X Y Z'	II
W X Y'Z'	IV	W X Y'Z	III	W X Y'Z	III
W X Y'Z'	IV	W X Y'Z	III	W X Y'Z'	IV

Only these types of segregation were observed as seen in Table 11, a result consistent with the hypothesis.

All these crosses therefore give evidence supporting the experimental hypothesis. The identification of three of the four postulated genes has been made by demonstrating their segregation with an alternative allele. The evidence for the fourth gene W will be presented in a subsequent section and for the present it is necessary to assume that the allele W is required for heterocaryon formation as postulated previously. The genotypes given on p. 39 for the various phenotypes have been confirmed with regard to genes X, Y and Z.

Further evidence for genes controlling heterocaryon formation

It has been shown above that types II, III and V differ from type I in genes Z, Y and X respectively. It was mentioned earlier that the alternative alleles of these genes had been introduced by the E5297a parent in the cross 5531A x E5297a, and evidently 449 - 8 carried these alleles into the cross 5531A x 449 - 8a. The evidence for this will now be examined more closely. The results on testing

progeny from the former cross are shown in Table 13. As described before random spores rather than asci were tested in the progeny of this cross.

TABLE 13

5531A x 5297a

Heterocaryon type	Mating type		Total
	a	A	
I	3	0	3
II	1	5	6
III	0	0	0
IV	3	4	7
V	0	0	0
VI	2	4	6
VII	50	60	110
	59	73	132

It has already been shown that 5531A carries alleles X, Y and Z and as types II, IV and VI occur in the progeny of this cross it may be concluded that the alternative alleles of these genes are segregating in the cross 5531 x 5297a, the alternative alleles having been introduced by the parent 5297a.

We may set out the theoretical expectations of such a cross

with a number of assigned genotypes for E5297a, on the basis of the four gene hypothesis described previously. With the additional assumption that 5531 carries allele W we may predict the range of phenotypic heterocaryon types on the basis of the number of genes segregating, assuming random segregation of all four genes. When only one, two or three genes are segregating it will be assumed that the remaining genes are homozygous for their active allele (i.e. W, X, Y, or Z) (see Table 14).

TABLE 14

Expected range of phenotypes from the cross 5531A x E5297a with various genes segregating.

Genes segregating	Phenotypes expected
1. W/W'	I, VII
2. X/X'	I, V
3. Y/Y'	I, III
4. Z/Z'	I, II
5. W/W', X/X'	I, V, VII
6. W/W', Y/Y'	I, III, VII
7. W/W', Z/Z'	I, II, VII
8. X/X', Y/Y'	I, III, V, VII
9. X/X', Z/Z'	I, V, VI
10. Y/Y', Z/Z'	I, III, IV
11. W/W', X/X', Y/Y'	I, III, V, VII
12. W/W', X/X', Z/Z'	I, V, VI, VII
13. W/W', Y/Y', Z/Z'	I, II, III, IV, VII
14. X/X', Y/Y', Z/Z'	I, II, III, IV, V, VI, VII
15. W/W', X/X', Y/Y', Z/Z'	I, II, III, IV, V, VI, VII

On comparison of these theoretical segregations with these data it is seen that none fit the observed results. If only genes X, Y, and Z are segregating at random, then the ratio of positive type heterocaryons (I, II, III, IV, V, VI) to the negative type VII will be 6:2. As these data from the cross 5531A x F5297a give a ratio of positive type heterocaryons to negatives of 22:110 the conclusion may be drawn that more genes than these three are segregating in the cross.

As only random spores were analyzed in the progeny of this cross the amount of information which can be obtained is limited. However, if whole asci can be examined for heterocaryon formation we can obviously obtain more precise information about the number of genes segregating. By crossing another pantothenicless strain to 5531A all progeny are pantothenicless and the analysis of complete asci can be made. The cross 449 - 8a x 5531A was made, where 449 - 8 is pantothenicless, and 23 asci analyzed. All types I through VII were found amongst the progeny. If the assumption is made that the four postulated genes are segregating in this cross there are 28 kinds of asci with reference to the phenotypes occurring in the ascus. Included in the table is a typical genotype segregation for each phenotype segregation. There are of course in many cases other variant segregations of these genes which will give these same phenotypic segregations but they are omitted for the sake of brevity. Also included in the table are the various numbers of each type of segregation observed amongst the asci analyzed.

TABLE 15

Kinds of asci with reference to phenotypes. Segregation in the cross 5531A x 449 - 8a.

Heterocaryon type Segregation	Genotype	No. obs.	Heterocaryon type Segregation	Genotype	No. obs.
I	W X Y Z		II	W X Y Z'	
I	W X Y Z	0	III	W X Y'Z	1
VII	W'X'Y'Z'		VII	W'X'Y'Z'	
VII	W'X'Y'Z'		VII	W'X'Y Z	
I	W X Y Z		II	W X Y Z'	
II	W X Y Z'	0	IV	W X Y'Z'	0
VII	W'X'Y'Z'		VII	W'X'Y'Z	
VII	W'X'Y'Z		VII	W'X'Y Z	
I	W X Y Z		II	W X Y Z'	
III	W X Y'Z	1	V	W X'Y Z	2
VII	W'X'Y'Z'		VII	W'X'Y'Z'	
VII	W'X'Y Z'		VII	W'X Y'Z	
I	W X Y Z		II	W X Y Z'	
IV	W X Y'Z'	1	VI	W X'Y Z'	0
VII	W'X'Y'Z'		VII	W'X'Y'Z	
VII	W'X'Y Z		VII	W'X Y'Z	
I	W X Y Z		III	W X Y'Z	
V	W X'Y Z	3	III	W X Y'Z	0
VII	W'X'Y'Z'		VII	W'X'Y Z'	
VII	W'X Y'Z'		VII	W'X'Y Z'	
I	W X Y Z		III	W X Y'Z	
VI	W X'Y Z'	1	IV	W X Y'Z'	0
VII	W'X'Y'Z'		VII	W'X'Y Z	
VII	W'X Y'Z		VII	W'X'Y Z'	
II	W X Y Z'		III	W X Y'Z	
II	W X Y Z'	0	V	W X'Y Z	4
VII	W'X'Y'Z		VII	W'X'Y'Z'	
VII	W'X'Y'Z		VII	W'X Y Z'	

TABLE 15 (cont.)

Heterocaryon type Segregation	Genotype	No. obs.	Heterocaryon type Segregation	Genotype	No. obs.
III	W X Y'Z		I	W X Y Z	
VI	W X'Y Z'	1	VII	W'X'Y'Z'	3
VII	W'X'Y'Z'		VII	W X'Y'Z'	
VII	W'X Y Z		VII	W'X Y Z	
IV	W X Y'Z'		II	W X Y Z'	
IV	W X Y'Z'	0	VII	W X'Y'Z'	0
VII	W'X'Y Z		VII	W'X Y Z	
VII	W'X'Y Z		VII	W'X'Y'Z	
IV	W X Y'Z'		III	W X Y'Z	
V	W X'Y Z	2	VII	W X'Y'Z'	0
VII	W'X'Y'Z'		VII	W'X Y Z	
VII	W'X Y Z		VII	W'X'Y Z'	
IV	W X Y'Z'		IV	W X Y'Z'	
VI	W X'Y Z'	0	VII	W X'Y'Z'	2
VII	W'X'Y'Z		VII	W'X'Y Z	
VII	W'X Y Z		VII	W'X Y Z	
V	W X'Y Z		V	W X'Y Z	
V	W X'Y Z	0	VII	W X'Y'Z'	2
VII	W'X Y'Z'		VII	W'X Y'Z'	
VII	W'X Y'Z'		VII	W'X Y Z	
V	W X'Y Z		VI	W X'Y Z'	
VI	W X'Y Z'	0	VII	W X'Y'Z'	0
VII	W'X Y'Z'		VII	W'X Y'Z	
VII	W'X Y'Z		VII	W'X Y Z	
VI	W X'Y Z'		VII	W X'Y'Z	
VI	W X'Y Z'	0	VII	W X'Y'Z'	0
VII	W'X Y'Z		VII	W'X Y Z'	
VII	W'X Y'Z		VII	W'X Y Z	

A small number of random spores were also analyzed for this cross and the results are given in Table 16.

TABLE 16

5531A x 449 - 8a (random spores, mating type 'a')

Heterocaryon type	No. observed
I	1
II	1
III	1
IV	4
V	2
VI	3
VII	20
	32

Here the ratio of strains able to form a heterocaryon to those giving a negative reaction is 12:20 which is the same as the expected ratio on the basis that the four postulated genes are segregating at random in this cross.

On examining the various types of segregations of asci obtained in Table 15 it is seen that a wide range of the possible segregations has been obtained. The results are compatible with the assumption that only the four genes postulated are segregating. Additional evidence for these four genes may be obtained if intercrosses are made

between the progeny of the cross 449 - 8a x 5531A and more information can be obtained if the spore pairs of one ascus are crossed to one another and also to the parents. This procedure has been carried out and has provided valuable confirmatory evidence for the proposed hypothesis.

Two asci have been analyzed in this way, Asci 560 and 562. The genetic history of these asci has been given earlier in Fig. 4. The extent of the analysis is shown in Table 17.

TABLE 17

Analysis of asci 560 and 562 for genes affecting heterocaryon formation.

Cross	No. asci analyzed	No. random spores tested
560-1a x 560-3A	20	24
560-1a x 560-7A	19	99
560-1a x 5531A	17	55
560-3A x 560-5a	15	85
560-3A x 449-8a	18	—
560-5a x 560-7A	19	58
560-5a x 5531A	17	72
560-7A x 449-8a	17	—
562-1a x 562-3A	11	—
562-1a x 562-5A	--	--
562-1a x 5531A	14	--
562-3A x 562-7a	15	--
562-3A x 449-8a	--	--
562-5A x 562-7a	17	--
562-7a x 5531A	14	--
562-5A x 449-8a	12	--

Analysis of ascus 560

The method of analysis was to make the various crosses listed in Table 17, isolate whole asci and random spores, and after determining the mating type of the various isolates to test them against the standard 4545 tester strain of like mating type. Ascus 560 when tested in this manner itself gave the following reactions.

560 - 1a Type IV

- 3A Type VII

- 5a Type V

- 7A Type VII

The crosses 560-5a x 5531A and 560-1a x 5531A were described above in the section on crosses between types. On the basis of the hypothesis we can now assign genotypes to certain members of this ascus.

560 - 1 W X Y'Z'

560 - 3 W'- - -

560 - 5 W X'Y Z

560 - 7 W'- - -

The various crosses will now be taken in turn and discussed individually.

560 - 1 x 560 - 3

Genotypically all that can be said of this cross is that it is between genotypes W X Y'Z' and W'- - - so that only W-W' segregation can be predicted for this cross. Twenty asci were analyzed with the following results (Table 18).

TABLE 18

560 - 1 x 560 - 3

Phenotypic segregation	No. asci
III, III, VII, VII	2
III, IV, VII, VII	13
IV, IV, VII, VII	5
	20

Twenty-four random spores were also tested from this cross with the following results (Table 19).

TABLE 19

560 - 1 x 560 - 3. Random spores.

Heterocaryon type	Mating type a
I	-
II	-
III	6
IV	5
V	-
VI	-
VII	13
	24

Only three phenotypes occur in the progeny of this cross, III, IV and VII. The cross must evidently be homozygous for gene Y'

and heterozygous for gene Z. Here we have a 1:1 segregation of the ability to form a heterocaryon indicating that this ability may be controlled by one gene. This cross therefore supplies evidence for the gene W previously postulated and the cross must be heterozygous for the gene. As no types V or VI were isolated it is probable that the cross is homozygous for gene X. Thus we may deduce that the cross is between genotypes W X Y'Z' (type IV, 560 - 1) and W'X Y'Z (type VII, 560 - 3). On this basis we may assign the following genotypes to ascus 560 with respect to these four genes affecting heterocaryon formation.

560-1 W X Y'Z'

560-3 W'X Y'Z

560-5 W X'Y Z

560-7 W'X'Y Z'

This result may now be confirmed by the various other crosses.

560 - 1 x 560 - 7

On the basis of the previous cross, this cross is between genotypes W X Y'Z' and W'X'Y Z', so only types II, IV, VI and VII should occur in the progeny, and also there will be only certain ways in which these types can segregate in the ascus. These are listed in Table 20 with the various number of asci found for each. No other types of segregation were observed.

TABLE 20

560 - 1 x 560 - 7

Phenotypic segregation	No. asci
II, II, VII, VII	-
II, IV, VII, VII	4
IV, IV, VII, VII	-
II, VI, VII, VII	1
IV, VI, VII, VII	3
VI, VI, VII, VII	-
II, VII, VII, VII	3
IV, VII, VII, VII	3
VI, VII, VII, VII	2
VII, VII, VII, VII	3
	19

A number of random spores were also analyzed from this cross with the results given in Table 21.

TABLE 21

560 - 1 (type IV) x 560 - 7 (type VII)

Heterocaryon type	Mating type		Total
	a	A	
I	-	-	--
II	9	6	15
III	-	-	--
IV	8	3	11
V	-	-	--
VI	1	3	4
VII	33	36	69
	51	48	99

The results from this cross agree with those of the previous one and are consistent with the postulated genotypes for 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. This present cross supplies evidence consistent with this view. Of the 19 asci analyzed from this cross 10 show segregation for one positive type heterocaryon (either type II, IV or VI) and three negative (type VII), and three asci show segregation of four negatives. The cross is between genotypes W X Y'Z' and W'X'Y Z'. The presence of the W' allele will ensure

that two ascospore pairs in every ascus will be negative. When a third or a fourth ascospore pair are also negative 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 such that in the absence of both X and Y (i.e. in the presence of both X' and Y') a heterocaryon is not formed even if allele W be present. As no evidence has been found to indicate a fifth gene with this postulated effect and the results can be satisfactorily accounted for by assuming the interaction, this has been the explanation adopted. For example the phenotype segregation II, VII, VII, VII results from the following genotype segregation:

W X Y Z'

W X'Y'Z'

W'- - -

W'- - -

This cross also supplies evidence that the allelic pair Z/Z' does not affect the formation of heterocaryons per se but only affects the time of initiation of the heterocaryotic growth. In this cross allele Z' is homozygous while alleles W/W', X/X' and Y/Y' are all heterozygous. The formation of heterocaryons or the failure to do so can be completely explained in terms of the genes W, X and Y without involving Z.

560 - 5 x 560 - 7

This cross is between the assumed genotypes W X'Y Z and

W'X'Y Z'. The only types segregating should be types V, VI and VII and in only the three phenotypic ascus configurations given in Table 22. The results of the cross are given in Table 22.

TABLE 22

560 - 5 x 560 - 7

Phenotype segregation	No. asci
V, V, VII, VII	4
VI, VI, VII, VII	3
V, VI, VII, VII	12
	19

Thus only the expected phenotypes and ascal configurations were found confirming the results obtained with the previous crosses. Random spores tested from the progeny of this cross gave results consistent with the above (Table 23).

TABLE 23

560 - 5 x 560 - 7. Random spores.

Heterocaryon type	Mating type		Total
	a	A	
I	-	-	-
II	-	-	-
III	-	-	-
IV	-	-	-
V	5	6	11
VI	1	9	10
VII	22	15	37
	28	30	58

This cross supplies additional evidence for the action of the allelic pair W/W'. Only W/W' and Z/Z' are segregating in this cross and as gene Z has no effect on the ability to form a heterocaryon a clear 1:1 segregation of progeny with and without this ability as shown in these data leads to the conclusion that this character is under the control of one gene, thus definitely implicating gene W in this connection.

560 - 3 x 449 - 8

This cross is supposedly between genotypes W'X Y'Z and W'X'Y'Z'. As a consequence only negative progeny are to be expected.

Eighteen asci were analyzed from this cross and all proved to be of type VII as expected.

560 - 7 x 449 - 8

This cross is assumed to be similar to the previous one. The genotypes of the parents are W'X'Y Z' and W'X'Y'Z', hence only type VII progeny are expected. In 17 asci analyzed this was found to be the case.

560 - 3 x 560 - 5

This cross is postulated to be between the genotypes W'X Y'Z and W X'Y Z; it follows that only types I, III, V and VII should appear among the progeny. This expectation was not born out experimentally, and a number of asci showed segregation for type VI, the delayed type V. The various segregations observed, and the results of analyzing 85 random spores from this cross are shown in Tables 24 and 25.

TABLE 24

560 - 3 (type VII) x 560 - 5 (type V)

Phenotypic segregations	No. asci
I, I, VII, VII	1
I, III, VII, VII	1
I, V, VII, VII	2
I, VI, VII, VII*	2
III, III, VII, VII	-
III, V, VII, VII	3
III, VI, VII, VII*	5
V, V, VII, VII	-
V, VI, VII, VII*	1
I, VII, VII, VII	-
III, VII, VII, VII	-
V, VII, VII, VII	-
	15

* Segregations not expected on the basis of assumed genotypes.

TABLE 25

560 - 3 x 560 - 5. Random spores.

Heterocaryon type	Mating type		Total
	a	A	
I	12	3	15
II	--	-	--
III	11	3	14
IV	--	-	--
V	7	1	8
VI	4	3	7
VII	36	5	41
	71	14	85

To check the possibility of a mistake in setting up and recording the results from the above cross, it was repeated with 560 - 6, the ascospore partner of 560 - 5. Random spores were isolated and tested with the same results, however. These results were incorporated with those from the cross 560 - 5 x 560 - 3. There appears in the progeny of this cross a type of heterocaryon not expected on the basis of the hypothesis. In contrast to the appearance of type VI, a type differing from type V only in being late starting, the corresponding late types of types I and III do not occur. Thus

it appears that the results of this cross cannot be explained on the simple four gene hypothesis. If it is assumed that an additional gene is segregating in this cross which interacts with the genotype W X'Y Z (type V) to give phenotype VI, but which has no similar effect on types I or III, the results are formally accounted for. If this modification of the original hypothesis is correct the assumed fifth gene must be carried by one other spore pair of ascus 560 and might be expected to express itself in other crosses. The only crosses in which its action could be detected are the one under discussion, 560 - 5 x 5531, and 560 - 5 x 560 - 7. If the detectable allele of this gene is carried by 560 - 5 we would expect type VI to occur in the progeny of the cross 560 - 5 x 5531. Since such progeny did not occur it follows that the allele in question was brought into the cross 560 - 3 x 560 - 5 by the 560 - 3 parent. If so, the same allele should be carried by another spore pair in the ascus, either 560 - 1 or 560 - 7. Strain 560 - 7 was crossed to three others, 560 - 1, 560 - 5 and 449 - 8, but in none of these crosses is type V expected, on the four gene hypothesis, to occur in the absence of type VI. Therefore the postulated fifth gene could not be detected. If the detectable allele of the assumed fifth gene were carried by 560 - 1 it could not be demonstrated, for in none of the crosses involving this strain does type V appear in the progeny. If there is an additional gene which acts specifically on type V to make it type VI, it is not possible to determine its segregation in ascus 560 from the crosses studied.

Although these data are consistent with the five gene hypothesis the evidence for the fifth gene consists of the results of a single cross and must therefore be regarded as suggestive only.

Analysis of ascus 562

An analysis similar to that made for ascus 560 has been carried out with ascus 562. While the analysis was not as 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 following responses were obtained.

	Heterocaryon type	Assumed genotype
562 - 1	VII	W'?Y'Z'
- 3	VII	W'?Y'Z'
- 5	V	W X Y Z
- 7	I	W X Y Z

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 analyzed from this cross. All the progeny were found to be type VII (negative). This result is consistent with the assigned genotypes for 562 - 1 and 562 - 3. However, this cross gives no information about the segregation of alleles X/X'.

562 - 1 x 562 - 5

The ascospores from this cross showed extremely poor germination and although over forty asci were dissected none gave germination in all four spore pairs. Analysis of this cross was therefore not carried out.

562 - 3 x 562 - 7

Fifteen asci were analyzed from this cross with the results given in Table 26.

TABLE 26

562 - 3 x 562 - 7

Phenotypic segregation	No. asci
I, II, VII, VII	1
I, IV, VII, VII	1
I, V, VII, VII	1
III, III, VII, VII	1
III, IV, VII, VII	2
III, V, VII, VII	1
III, VI, VII, VII	1
II, VII, VII, VII	2
III, VII, VII, VII	2
IV, VII, VII, VII	1
V, VII, VII, VII	1
VI, VII, VII, VII	1
	15

It is seen that all heterocaryon types I through VII occur in the progeny, and hence all four genes must be segregating with their alternative alleles. 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'. This cross is then the same as that between 449 - 8 and 5531A given previously. 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 give type VII. From this cross it is obvious that the segregation of the four genes in ascus 562 is as follows.

562 - 1	W'X Y'Z'
- 3	W'X'Y'Z'
- 5	W X'Y Z
- 7	W X Y Z

Additional evidence for this segregation comes from the following crosses.

562 - 1 x 5531

Fourteen asci were analyzed from this cross with the results given in Table 27.

TABLE 27

562 - 1 x 5531

Phenotypic segregations	No. asci
I, I, VII, VII	-
I, II, VII, VII	2
I, III, VII, VII	3
I, IV, VII, VII	-
II, II, VII, VII	1
II, III, VII, VII	4
II, IV, VII, VII	-
III, III, VII, VII	1
III, IV, VII, VII	3
IV, IV, VII, VII	-
	14

From the results of the cross 562 - 3 x 562 - 7 we would expect the genotype of 562 - 1 to be W'X Y'Z'. Hence in the cross of this latter strain to 5531 which has the genotype W X Y Z 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 provide agreement with those found for the previous cross. Only fourteen asci were analyzed, so the absence of types V and VI from the progeny of this cross cannot be taken as conclusive evidence of the assumed genotype for this strain as these types may have been missed by sampling errors. The results

obtained with this cross do provide additional evidence for the segregation of gene W. It is seen that all asci analyzed segregate 1:1 for the ability to form a heterocaryon showing a one gene difference for this character.

562 - 5 x 449 - 8

One additional cross was made to complete the analysis of ascus 562. The results from this cross are given in Table 28.

TABLE 28

562 - 5 x 449 - 8

Phenotypic segregations	No. asci
V, V, VII, VII	1
V, VI, VII, VII	3
VI, VI, VII, VII	2
V, VII, VII, VII	2
VI, VII, VII, VII	3
VII, VII, VII, VII	1
	12

This cross is between genotypes W X'Y Z and W'X'Y'Z' and the expected segregations for types V, VI and VII only is realized. This cross supplies additional confirmatory evidence for the interaction between W, X and Y. It is seen that a number of asci were observed in which three or four negative types occur among the four

spore pairs, and as described previously it may be concluded that either or both of alleles X and Y must be present in addition to allele W for a heterocaryon to form.

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

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.

LINKAGE RELATIONSHIPS OF GENES CONTROLLING HETEROCARYOSIS

The number of asci in which segregation for specific genes can be determined is not sufficient to provide accurate measures of the centromere distances of the four assumed genes. One of the allelic pairs, X/X' appears to be sex linked. Relevant data are given in Table 29.

TABLE 29

XA x X'a

	No. asci
1. XA XA X'a X'a	12
2. Xa Xa X'A X'A	1
3. XA X'A X'a Xa	13
4. XA Xa X'A X'a	5
5. XA X'a XA X'a	5
6. XA X'a Xa X'A	1
7. Xa X'A Xa X'A	0
	37

The indicated recombination frequency is 28%. These data suggest that X is possibly on the opposite side of the centromere from the sex determining region. It should be noticed that the mating type locus showed 30% second division segregation. This discrepancy was observed with many other crosses and is discussed in the appendix.

Other examples of genetic control of heterocaryosis

The detailed analysis of one particular case of genetic control of heterocaryosis has been described above. It will now be shown this control can occur with other mutants combined to form heterocaryons. It is not known whether or not the phenomenon is of general application but as these mutants were chosen more or less at random it is reasonable to assume that this is the case. In the analysis of the cases to follow essentially the same techniques were used, with the exception that six inch test tubes instead of growth tubes were used for testing for heterocaryon formation.

These tests were made prior to the identification of the various types of heterocaryotic growth, and the results were scored for heterocaryon formation or its absence. However, it became apparent that there was an intermediate class (designated $\pm$ in these data below). This probably corresponds to types II, III, IV, V and VI in the system of classification subsequently adopted with the lysineless-pantothenicless heterocaryon.

Three mutants were used, 5531A pantothenicless, 1633A aminobenzoicless, and 4545A lysineless. These three mutants always form type I heterocaryons when combined with one another. The procedure

was to cross one of the mutants with wild type, and test random spores of mating type A from the progeny against the other two mutants which thus acted as tester strains. This has been done for all three mutants with each of three wild type strains, Lindegren 25a, Emerson 5297a and Abbott 12a. The results for the cross 5531A x 5297a are not included here as they have already been given above in detail. The results of the other crosses are given in Table 30 below.

TABLE 30

5531A x 25a, 5531A x 12a, 1633A x 25a
1633A x 12a, 1633A x 5297a, 4545A x 25a
4545A x 12a, 4545A x 5297a,

Cross	5531 x 25a		5531 x 12a	
Heterocaryon formation	tester strain 1633A	4545A	tester strain 1633A	4545A
+	62	19	13	0
±	8	25	13	2
−	0	26	2	26
Total	70	70	28	28

TABLE 30 (cont.)

Cross	1633 x 25a		1633 x 5297a		1633 x 12a	
Heterocaryon formation	tester strain 5531A	4545A	tester strain 5531A	4545A	tester strain 5531A	4545A
+	35	15	14	5	37	2
±	5	10	6	1	3	1
−	6	21	24	38	15	52
Total	46	46	44	44	55	55

Cross	4545 x 25a		4545 x 5297a		4545 x 12a	
Heterocaryon formation	tester strain 1633A	5531A	tester strain 1633A	5531A	tester strain 1633A	5531A
+	13	11	2	4	1	1
±2	10	9	1	1	0	1
−	12	15	30	28	48	47
Total	35	35	33	33	49	49

It is apparent that the progeny of the crosses described above may differ from the mutant parent in their ability to form a heterocaryon with the other strains concerned. Assuming this to have a genetic basis the various wild types appear to differ with respect to genes affecting this process. It will be recalled that all these mutants are derived from the Lindegren wild type and it will be noted that of the three wild types used, a higher proportion of progeny from crosses involving the Lindegren wild type is able to form heterocaryons

than from either of the other two. Very few of the progeny of crosses involving Abbott 12a are able to form heterocaryons with the tester strains.

An attempt was made to see whether there was correlation in the ability of the progeny to form heterocaryons with both tester strains. For example in progeny of crosses involving the lysineless mutant 4545A there appears to be no correlation between the ability to form a heterocaryon with 1633A and the ability to do this with 5531A. In no case was any correlation of this type found.

These results suggest that genetic control of heterocaryosis may be of wide distribution in many mutants.

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 pantothenic 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 lysine component. It would be expected that such genes would 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 such a control does affect the subsequent heterocaryotic growth.

Ten random spore isolates of genotype lysineless mating type

'a' were selected from the progeny of the cross 4545A x 5297a. These were then tested for heterocaryon formation with 560 - 5 a strain giving type V growth with the tester strain 4545a. All the combinations of the pantothenicless 560 - 5 strain with the 10 random spore isolates gave the type VII response. Thus these 10 strains must differ with respect to genes controlling heterocaryon formation from the 4545a tester strain.

A number of random spores were selected from the progeny of the cross 4545A x 25a. They were combined with different pantothenic 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 31.

TABLE 31

4545A x 25a

Pantothenic strain	4545a standard tester	Random spores from cross 4545A x 25a, mating type 'a'			
		L101	L103	L107	L108
726 - 1	I	VII	VII	II	IV
726 - 7	III	VII	VII	VII	IV
731 - 4	IV	VII	IV	IV	VII
730 - 7	II	IV	II	III	VII
741 - 1	V	IV	II	II	III

TABLE 31 (cont.)

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

These results indicate that the genetic hypothesis advanced for the control of heterocaryosis between these two mutants is only applicable when the particular tester strains used in the main body of this work are considered. Had other tester strains been used it is clear that the hypothesis would have to have been modified or extended. It is of interest to note the comparatively large number of late types (II, IV and VI) in the above results. This supports the previous suggestion that the inheritance of this character is complex. An 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 in testing the progeny with the 'a' strain was the same as that found with the 'A' strain.

In the analysis of the progeny of the cross 1633A x 5297a some of the strains were tested in growth tubes. One isolate W 367 - 3 (paba 'A') gave a type I reaction when combined with 5531A but a type VII (negative) reaction with 4545A. Strain W 367 - 3 was then tested with eight mating type 'A' strains from the cross 5531 x 5297a all of which gave negative reactions with both 1633A and 4545A. Two other isolates from the cross 1633 x 5297a which gave negative reactions with both 5531A and 4545A were also included in the test. The three paba strains and the eight pantothenicless strains were then paired in all possible combinations. The results are shown in Table 32.

TABLE 32

1633 x 5297a and 5531 x 5297a

	from cross 1633 x 5297a		
Pantothenic isolates from cross 5531 x 5297a	367 - 3	368 - 3	369 - 5
441 - 7	I	VII	VII
443 - 3	III	VII	VII
444 - 4	V	VII	VII
445 - 2	V	VII	VII
446 - 1	V	VII	III
447 - 3	V	VI	VII
447 - 5	I	VII	VII
448 - 1	III	V	VII

These results suggest that some complementary action does

occur between the two heterocaryon components. For example, although strains 447 - 3 and 368 - 3 fail to form a heterocaryon with 1633 and 5531 respectively, they do form a heterocaryon of type VI with each other. A similar situation occurs with 369 - 5 and 446 - 1. It is also noteworthy that although the various heterocaryon types were based on heterocaryons between lysineless and pantothenicless strains, the same types of heterocaryons form between lysineless and aminobenzoicless and between aminobenzoicless and pantothenicless. This suggests that the observed responses are general ones and not specific ones due to the mutants concerned.

DISCUSSION

From the evidence presented above it may be concluded that certain genes control the formation of heterocaryons between biochemical mutants of Neurospora crassa. In one particular case investigated in detail, four such genes have been identified and their phenotypic effects demonstrated. There are indications, however, that more genes are involved in the process. The genes identified determine whether or not a heterocaryon will be formed between strains of the pantothenicless mutant 5531 and certain strains of the lysineless mutant 4545. They also may modify the type of heterocaryotic growth produced by affecting the time at which heterocaryotic growth commences, the ability to continue growing as a heterocaryon and the vigor of the heterocaryotic mycelium. These effects are produced by various combinations of the four genes identified. Of these genes, the allelic pair X/X' is carried in the sex chromosome. The others appear to segregate

independently of this gene and of each other. Linkage tests involving biochemical mutants are probably not feasible in view of the possible disturbance to the formation of heterocaryons through their introduction.

An interesting aspect of the work reported here concerns the mode of action of genes affecting heterocaryosis. Although this aspect of the problem has not been investigated, a number of possible mechanisms can be suggested. 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 microtechniques 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 have been done in which two strains known not to form heterocaryons with the usual techniques were grown alone on agar plates supplemented with suboptimal 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. If there is a failure to fuse it is possibly similar to that found with the 'aversion reaction' (59) occurring in some Ascomycetes and many Basidiomycetes and known to be genetically controlled.

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.

If fusion does take place the next step in the formation of a heterocaryon must be the exchange of nuclei. It is possible that 'incompatibility factors' may prevent or inhibit the association of certain types of nuclei in the same cytoplasm. However, it may be that the three phenotypical responses of failure to initiate heterocaryotic growth, instability of heterocaryotic growth and lowered heterocaryotic vigor may all be manifestations of the same genetic mechanisms. It was shown by Beadle and Coonradt that the mutant gene pantothenicless was relatively recessive. As an amplification of their theory of balanced heterocaryons they pointed out that with genes having partial dominance a nuclear ratio compatible with wild type growth may not be possible. It is possible that the genes responsible for modification of the heterocaryotic growth in the system analyzed here may be modifiers of dominance acting specifically on the pantothenic or lysine genes. Thus in the case of types V and VI it may be that the dominance of the wild type allele of the pantothenicless gene carried

by the lysineless nuclei is reduced to the point that the nuclear ratio required for wild type growth cannot be attained. This modification of dominance may be brought about through a reduction in efficiency in the production of pantothenic acid by this wild type allele. Another possibility is that the allele X' allows a deleterious reaction to proceed which requires increased pantothenic acid. In the case of combinations in which growth fails entirely the modification in dominance may be so extreme that all nuclear ratios are inadequate for growth.

With those pantothenic strains which give the type III or IV response it is obvious that a nuclear ratio able to give wild type growth rate is possible. However, dominance modification may lead to an extremely high proportion of one type of nucleus. This could come about through an increase in efficiency in the wild type allele of the pantothenicless nuclei permitting a few lysineless nuclei to supply the necessary pantothenic acid. Under these conditions the lysineless nuclei may become lost by accident or chance rearrangement, a condition that would lead to cessation of growth. It will be recalled that the mycelium at the point where growth ceases in such cases has a different appearance, and if hyphal tips are taken from this region they are found to grow on a medium supplemented with pantothenic acid but not on minimal medium or a medium supplemented with lysine. Also if pantothenic acid in solution is added to the point where growth has ceased, the mycelium will resume growing but no such effect occurs when lysine is added. Thus it appears that this fringe of mycelium is homocaryotic for pantothenicless. If tubes in which growth has

stopped are left for four or five days they will sometimes commence growing again and later again stop. Presumably in these cases reshuffling of the nuclei has taken place so that a few lysineless nuclei have become temporarily available.

Thus the action of the genes may not be in affecting heterocaryosis *per se*, but they may actually be modifying genes of the particular mutant genes concerned, with their effects such that they may only be detected in this heterocaryotic system. Perhaps by using refined techniques it may be possible to detect some action of these genes on other phases of the growth of these strains. It also suggests that genes controlling heterocaryosis are specific in action for the mutants concerned and not general in action. However, there are undoubtedly some genes which do have a general action. The allelic pair Z/Z' is probably of this type, for it will be recalled that their effect may be exerted on all three of the other phenotypic effects. Thus a reasonable conclusion seems to be that genes controlling heterocaryosis are of two kinds, those affecting the particular mutants concerned, and those having a general effect on all or most mutants.

Thus it can be seen how a study of heterocaryosis may lead to information on the mechanism of gene action. Perhaps a further study of these genes affecting heterocaryosis may increase our knowledge of the nature of dominance and recessiveness, as expressed in terms of biochemical reactions.

APPENDIX

The segregation of the mating type locus

It was necessary to analyze asci for the segregation of the mating type locus prior to testing their ability to form heterocaryons. The results of this analysis are given in Table 33.

The centromere distance of the mating type locus for N. crassa given by Houlahan, Beadle and Calhoun (60) is 5.80 units with 1811 asci derived from 88 crosses. Lindegren (61) obtained a value of 7.5 units based on 627 asci. The data here give a centromere distance of 15.5 units with 511 asci derived from 32 crosses.

If these data are segregated into two groups on the basis of whether or not E5297a, the Emerson wild type, is one of the parents or is an ancestor of one of the parents the following results are obtained.

Crosses involving E5297a.

29 crosses, 435 asci, centromere distance 17.25 units.

Crosses not involving E5297a.

3 crosses, 76 asci, centromere distance 6.5 units.

It therefore appears that E5297a has a marked effect in increasing the amount of crossing over on the sex chromosome of N. crassa. Whether this is a localized effect in the region of the mating type locus, applies to the whole of this chromosome, or involves crossing over in all the chromosomes is not known. The only loci for which sufficient data are available from this work to determine this point, the pantothenicless locus, the lysineless locus and the amino-

TABLE 33

Segregation of the mating type locus

Pantothenicless x pantothenicless	Segregation		Total
	I	II	
560 - 1 x 560 - 7	14	5	19
560 - 1 x 5531A	12	8	20
560 - 5 x 5531	13	7	20
560 - 3 x 560 - 5	6	8	14
560 - 5 x 560 - 7	16	3	19
560 - 1 x 560 - 3	11	9	20
560 - 3 x 449 - 8	12	5	17
562 - 2 x 5531	9	6	15
562 - 4 x 562 - 8	20	9	29
562 - 6 x 562 - 8	13	3	16
562 - 6 x 449 - 8	9	3	12
562 - 2 x 562 - 4	7	5	12
562 - 7 x 5531	10	4	14
844 - 7 x 5531	7	2	9
723 - 4 x 5531	6	2	8
824 - 1 x 707 - 3	6	5	11
	171	84	225

TABLE 33 (cont.)

Pantothenicless x wild	Segregation I	Segregation II	Total
5531 x 25a	51	8	59
5531 x 5297a	57	23	80
5531 x 20 - 3	3	1	4
	111	32	143

Aminobenzoicless x wild	Segregation I	Segregation II	Total
1633 x 5297a	5	4	9
1633 x 25a	9	1	10
1633 x 8a	1	5	6
	15	10	25

TABLE 33 (cont.)

Lysineless x wild	Segregation I	Segregation II	Total
4545 - 311 - 6A x 5297a	5	7	12
4545 - 311 - 6A x 12a	4	5	9
4545 - 311 - 6A x 25a	7	1	8
4545 - 311 - 6A x 8a	5	5	10
4545 - 311 - 3a x 7A	6	7	13
4545 - 239 - 5A x 25a	8	3	11
4545 - 239 - 5A x 5297a	7	2	9
4545A x 12a	6	1	7
4545A x 5297a	5	2	7
4545A x 7A	1	1	2
	54	34	88
Total	351	160	511

benzoicless locus, are all more than 25 units from the centromere and hence unsuited for determining modifications in crossover values.

All that can be done is to suggest several mechanisms by which crossing over could be increased or a greater proportion of crossover asci isolated.

As pointed out by Houlahan et al. (60) "...if failure of

spores to mature or to germinate is associated with particular genotypes or types of segregation then selection of complete asci for dissection and after germination, for classification will lead to errors which are not easily avoided." Perhaps this may be the case here and such a mechanism has resulted in a selection for those asci in which a crossover has occurred between the mating type locus and the centromere.

There may be chromosomal aberrations in other chromosomes which although reducing crossing over in these chromosomes, cause an increase in other chromosomes, in this case the sex chromosome. This type of situation is known in Drosophila.

Undoubtedly other mechanisms could be suggested to account for this result. Whatever the case this result obviously has an important relation to any work on the location of genes in Neurospora. It indicates that any values given for centromere distances in this organism may vary with the particular strains used.

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