A NEW INCOMPATIBILITY LOCUS IN *NEUROSPORA CRASSA*[1]

JAMES F. WILSON AND LAURA GARNJOBST

The University of North Carolina at Greensboro, and The Rockefeller University, New York City

Received December 3, 1965

OF the factors known which prevent formation of unisexual heterocaryons, perhaps the most specific are the *C* and *D* genotype differences (GARNJOBST 1953; 1955). A difference in the alleles at either or both of these unlinked loci results in cell death shortly after hyphal fusion of the two strains (GARNJOBST and WILSON 1956). In these investigations of the *C* and *D* genes and their alleles *c* and *d*, correlation was complete between failure to form successful heterocaryons and occurrence of the lethal reactions of the mixed protoplasms. This type of cell death, called the incompatibility reaction, could be readily recognized microscopically by visible changes in the cytoplasm and differentiated from cell death caused by injury. Further studies have shown that the incompatibility reaction involves cytoplasmic constituents, probably proteins (WILSON, GARNJOBST and TATUM 1961).

The present study is concerned with the differences in heterocaryon genotype between (1) strains derived from the standard St. Lawrence wild types (74*A* and 73*a*) and (2) strains derived from Lindegren stock (1*A* and 25*a*) known to contain the heterocaryon genes *C* and *D*, as shown in previous work (GARNJOBST and WILSON 1956). Compatibility tests by microscopic observation of hyphal fusions between unisexual pairs of strains were employed and found to be practicable between wild types, wild types and mutants, and mutants. The results of these observations, tests for heterocaryon formation, and crosses described prove that the strains derived from the standard St. Lawrence wild types and the Lindegren *CD* strains have one heterocaryon gene (*C*) in common and differ in two genes, one (*d*) previously encountered and one new gene (*e*).

MATERIALS AND METHODS

Transfers of the wild-type strains of unknown heterocaryon genotype, ST74*A*4, 74–OR8–1*a*, and 74–OR23–1*A*, were obtained from DR. PATRICIA ST. LAWRENCE, who also supplied 20 isolates from a sib-cross which were approximately nine generations inbred by backcrossing to ST74*A*. The first backcrosses were carried out by DR. MARY CASE and DR. F. J. DE SERRES (CASE, BROCKMAN and DE SERRES 1965), and extended by DR. ST. LAWRENCE to an additional six generations, beginning with 74–OR8–1*a* × ST74*A*. The parents of the sib-isolates were from the last backcross.

A transfer of ST74*A* was supplied by DR. D. D. PERKINS. The strain known as ST74*A*4 is the same as ST74*A* (ST. LAWRENCE, personal communication) except that conidia of ST74*A* were plated and a conidial isolate selected. This isolate eliminated a spontaneous mutation which had given the original culture a "peach-like" character (D. NEWMEYER, personal communication).

[1] This work was supported in part by Research Grants G 18880 from the National Science Foundation, and CA 03610 from the Public Health Service.

For convenience in this paper, ST74*A* and ST74*A*4 will be referred to as "74*A*" and "74*A*4," the OR strains as "OR*a*" and OR*A*," and the sib-isolates as "b_9." [ST designates standard (of ST. LAWRENCE) and OR designates Oak Ridge (of DE SERRES) (PERKINS, personal communication).]

Strains used as testers for heterocaryon formation were the same as, or derivatives of, the standard inositol- (37401, *inos*), riboflavin- (Y30539, *rib-2*), and nicotinic acid- or tryptophan- (39401, *nt*) deficient strains previously described (GARNJOBST 1953; 1955). Each mutant tester strain is represented by four isolates of the heterocaryon genotypes *CD*, *cD*, *Cd*, or *cd*, in each mating type (sex). Other strains, including new testers, will be described in connection with the cross or experiment in which they were used.

Crosses were made on the synthetic medium of WESTERGAARD and MITCHELL (1947), supplemented as required. Tests for unisexual heterocaryon formation were made on slants of minimal agar medium (VOGEL 1956) with 2% sucrose. The results are reported as heterocaryon positive (het^+) or heterocaryon negative (het^-). Heterocaryon positive strains have the same heterocaryon genotype, whereas the heterocaryon negative pairs have unlike heterocaryon genotypes (GARNJOBST 1955). The terms compatible and incompatible are reserved herein for the results obtained by microscopic observation of interstrain hyphal fusions.

The compatibility tests were made by the microculture growth technique used for microsurgery (WILSON 1961). Colonies of the strains to be tested were grown in Petri plates for about 18 hours at 30°C. Small agar blocks were cut from these large colonies at the mycelial frontier and placed mycelium side down on 22 × 30 mm coverslips, previously coated with minimal agar medium supplemented when necessary. These preparations were incubated in a moist chamber at either 20° or 30°C until the hyphae of the two strains had intermingled for one or two hours. Each preparation was then placed on a de Fronbrune oil chamber (Aloe Company) filled with a 15% sucrose solution to keep the hyphae from swelling and bursting.

The preparations were examined with a standard brightfield microscope. Since cells killed by the incompatibility reaction are more dense optically than living hyphae, it is possible to recognize them with 150× magnification. When no incompatibility reactions were observed, the microcultures were reincubated at 30°C for one-half hour to establish protoplasmic flow as the hyphae close to the outside of the coverslip began to grow. It was then generally possible to trace the flow back through the interstrain fusions, proving that the strains were compatible. When necessary, the experiment was repeated until interstrain flow of protoplasm was observed.

RESULTS

Compatibility tests of wild types: 74*A*, 74*A*4, and OR*A* were rigorously tested with one another by observation of interstrain hyphal fusions and found to be compatible. Thus, by inference, these strains have the same heterocaryon genotype. Because of the nine generations of inbreeding, the heterocaryon genotype of the b_9 isolates, *a* and *A*, were assumed to be the same as 74*A*4 and OR*A*, and probably OR*a* as well. This assumption was tested by observation of hyphal fusions between these strains and the b_9 isolates of the same sex. Surprisingly, four of the nine *a* isolates were incompatible with OR*a* and seven of the 11 *A* isolates were incompatible with 74*A*4 and OR*A*. This difference was investigated by further microscopic tests, first between pairs of the b_9 isolates.

The results showed that all isolates which were incompatible with OR*a* or 74*A*4 (or OR*A*) were mutually compatible, and that all isolates compatible with the OR strains and 74*A*4 also were compatible among themselves. This finding suggested that the observed incompatibility was due to a single gene difference.

Representatives of the two groups of the b_9 isolates, *i.e.*, those that were com-

patible and those that were incompatible with the OR strains and 74*A*4, were tested with the standard inositol-requiring tester strains of the heterocaryon genotypes *CD*, *cD*, *Cd*, and *cd*. These b_9 isolates were found to be incompatible with all four types, an indication that, in addition to differing from each other by perhaps one gene, both groups differed from the known heterocaryon genotypes by at least one other gene.

To obtain additional information, members of the two groups of b_9 strains were crossed with each other in the following combinations: compatible *a* × compatible *A*; incompatible *a* × compatible *A*; incompatible *A* × compatible *a*; and incompatible *a* × incompatible *A*. Ordered ascospore isolations (10 or 12 asci) were made from each cross, and hyphal fusions between intra- and inter-ascus combinations of the progeny from each cross were observed microscopically. The results (Table 1) showed that all progeny from crosses of compatible × compatible were compatible in all combinations tested. Progeny from crosses of incompatible × incompatible were likewise mutually compatible. However, progeny from crosses of compatible × incompatible were of two classes, some mutually compatible, and some mutually incompatible. These findings suggested strongly that there was only a single incompatibility gene difference (later shown to be *D*) among the b_9 isolates.

Results of crosses of b_9 isolates (wild types) with a CD *strain:* To compare the heterocaryon genotypes of the b_9 isolates and the known *CD* strains, crosses were made between representatives of the b_9 and a *CD* strain. These representatives were selected from ascus 23 (incompatible *a* × compatible *A* cross, Table 1) because this ascus contained two pairs of ascospore isolates (23-1,2*A* and 23-5,6*a*) compatible with OR*A* and 74*A*4 or OR*a*, and two pairs (23-3,4*A* and 23-7,8*a*) incompatible with these strains, as tested microscopically. One of each type was crossed with a known *CD*, *inos*, *A* strain. Since only the *inos* progeny could be tested for heterocaryon formation with the *rib-2* tester strains, the ascospores were isolated at random. Forty *inos* progeny, also taken at random from each cross, were tested.

The results of Cross 1 (Table 2) show that 23-5*a* contains the *d* allele and probably differs from the tester used by not more than one other heterocaryon gene, assuming the *inos* progeny were representative of both the wild-type and

TABLE 1

Inheritance of heterocaryon incompatibility among progeny of intercrosses of b_9 isolates

Cross*	Intra-ascus combinations		Inter-ascus combinations	
	No. tested	No. incompatible	No. tested	No. incompatible
$1a^+ \times 11A+$	16	0	16	0
$3a^- \times 12A-$	14	0	6	0
$1a^+ \times 12A-$	12	11	12	7
$3a^- \times 11A+$	8	6	12	6

* b_9 strains used as parents. "+" refers to b_9 progeny compatible with OR*a* or 74*A*4 and OR*A*; "−" refers to b_9 progeny incompatible with these strains.

TABLE 2

Heterocaryon tests of b_9 inos *derivatives with the four standard* rib-2 *tester strains, and inferred genotypes* of parents and progeny*

Cross†	No. of progeny				
	CD(E)	*cD(E)*	*Cd(E)*	*cd(E)*	*(CDe, Cde)*‡
1. 23–5*a*, *inos*$^+$ × *CD(E), A, inos*$^-$ *(Cde)*	12	0	8	0	19 *(CDe, Cde)* (1 lost)
2. 23–8*a*, *inos*$^+$ × *CD(E), A, inos*$^-$ *(CDe)*	20	0	0	0	20 *(CDe)*

* The inferred genotypes are enclosed in parentheses.
† The *a* parent in each cross was obtained from the $3a^- \times 11A^+$ cross listed in Table 1.
‡ Heterocaryon negative with all four tester strains.

the *inos* progeny of the cross. Only one gene appears to have segregated in Cross 2, wherein all progeny are *D*. The difference, then, between 23-8*a* and the *CD*, *inos*, *A* parent seems to be a new gene concerned with heterocaryon formation. This gene was named *e*. Accordingly, all the *rib-2*, *nt*, and *inos* standard testers were assumed to contain the alternate allele (*CDE*, *cDE*, *CdE*, and *cdE*). If gene *e* were present also in 23-5*a* and among the isolates of the cross which were *het*$^-$ with all four testers, a tentative conclusion could be drawn that two genes were segregating independently in Cross 1, as shown in Table 2.

Representatives of the het$^-$ *a* progeny in Cross 1 were tested microscopically with 23-5*a* and with 23-8*a*. Each *a* isolate was found to be compatible with one of the testers but not with the other. The same results were obtained when the het$^-$ *A* isolates were tested with 23-1*A* and with 23-4*A*, indicating that the two classes (*CDe* and *Cde*) were indeed present among the het$^-$ isolates of Cross 1, and that 23-5*a* is *Cde*.

Strain 23-5*a* was already known to be compatible with OR*a*, therefore OR*a* also is *Cde*. Since the same pattern of inheritance was observed in the *A* as in the *a* isolates of Cross 1, and since all progeny of the b_9 isolates compatible with 74*A*4 and the OR strains were themselves compatible, it follows that 74*A*4 and OR*A* also are *Cde*. Sample microscopic testing with OR*a* of those isolates from Cross 1 and from Cross 2 compatible with 23-8*a* (*CDe*) confirmed this conclusion in that all tested were incompatible.

Segregation of the heterocaryon genes: Further information regarding segregation of the three genes was obtained from ordered ascospore isolations from a series of crosses: 3, 4, 5, and 6. The *CDe* strains used in this series were 141*a*, *inos* and 159*A*, *inos* from Cross 2 (Table 2).

The ten asci isolated from Cross 3 (*CDe, inos, A* × *CDE, inos, a*) were analyzed by testing the four ascospore pairs of each ascus with the standard *CDE*, *rib-2* testers, *a* or *A*. A 1:1 segregation of het$^+$ to het$^-$ was obtained, as expected. The het$^+$ pairs, therefore, are *CDE*. The asci could not be tested further until appropriate *CDe*, *a* and *A*, testers were available. These were obtained from Cross 4 (*CDE, nt, a* × *CDe, inos, A*) by selecting *nt* isolates which formed heterocaryons

with the *CDe, inos* strains, *a* or *A* from Cross 2. Since only the het⁻ pairs in the ten asci from Cross 3 formed heterocaryons with the new *nt* tester strains, these pairs must be *CDe*.

Twelve asci from Cross 5 (*CDe, nt, A* from Cross 4 × *cdE, nt, a*), in which three heterocaryon genes would be expected to segregate, were analyzed by testing each ascus pair for heterocaryon formation with the four standard *rib-2* testers and two *inos* strains, *CDe* from Cross 2 and *Cde* from Cross 1, *a* or *A* sex. For some asci the genotypes of one or two *het*⁻ pairs were deduced from the known pairs present.

Inositolless strains of the same heterocaryon genotypes as the *nt* isolates from Cross 5 were obtained from Cross 6 (*CDe, inos, A* × *cdE, inos, a*) (Table 3). The same testers were used as for Cross 5 but *CDe, nt* and *Cde, nt* strains were substituted for the *inos* strains. In addition, the het⁻ pairs were tested for heterocaryon formation with *cDe, nt* and *cde, nt* strains (deduced genotypes) from Cross 5.

The results of the analysis of asci from Cross 5 and from Cross 6 show that the three heterocaryon genes were segregating independently. Also, it was clear that each pair of an ascus promptly formed a successful heterocaryon with only one tester. Similar results were obtained on testing the progeny from crosses involving combinations of the genes *C* and *D*, and their alleles *c* and *d* (GARNJOBST 1955). In the het⁻ combination *Cde* + *cde*, as in the combination *Cd* + *cd* previously reported (GARNJOBST 1955), delayed growth response sometimes occurred.

Compatibility tests of strains containing three heterocaryon genes: The eight possible combinations of the three genes were obtained in each of the nutrition-

TABLE 3

Ascus segregation of c, d, *and* e *(Cross 6)*
Parent genotypes: *CDe, inos, A* × *cdE, inos, a*

No. of asci*	Ascus segregation					
	From centromere†			Between genes‡		
	c	*d*	*e*	*c & e*	*c & d*	*d & e*
1	1st	1st	1st	PD**	NPD	NPD
5	1st	2nd	2nd	T	T	T
1	2nd	2nd	2nd	PD	PD	PD
1	2nd	2nd	2nd	NPD	T	T
1	1st	2nd	2nd	T	T	NPD
1	1st	2nd	1st	NPD	T	T
2	1st	2nd	2nd	T	T	PD
2	1st	2nd	1st	PD	T	T
1	2nd	2nd	2nd	T	NPD	T
1	2nd	2nd	1st	T	T	T
2	2nd	1st	2nd	T	T	T

* Total number of asci 18; 93.1% germination of ascospores, each pair/ascus represented.
† Centromere distances obtained: *c*, 16.6; *d*, 41.6; *e*, 36.1; sex, 11.1. The centromere distances are uncorrected for multiple crossovers.
‡ Recombination frequencies: *c & e*, 44.4%; *c & d*, 52.7%; *d & e*, 47.2%.
** PD = Parental ditype segregation; NPD = nonparental ditype segregation; T = tetratype segregation.

ally complementary strains *inos* and *nt*, as described. Thirty-six different pair combinations of the heterocaryon genes are thus possible between the two nutritionally deficient strains. Of these pairs, (1) eight are alike in heterocaryon genotype and (2) 28 are unlike. The early growth of all possible pair combinations of each group has been observed microscopically. These observations indicate that the interstrain hyphal fusions of group (1) are cytoplasmically compatible, whereas those of group (2) produce the incompatibility reaction. Therefore the correlation, previously reported for the *c* and *d* loci, between failure to form a successful heterocaryon and occurrence of the lethal incompatibilty reaction also appears to hold true for unlike combinations of all the genes at the three loci.

Linkage of c: The *d* locus is 25 units distal to fluffy (*fl*) in linkage group II, R (GARNJOBST 1953). It is also clear that *c* segregated independently of *d*, and that *c* is not linked to *inos* (group V, R) or to *nt* (group VII, R). In the first study no attempt was made to locate *c*. However, with more markers available the possibility of locating *c* has now been reexamined, as follows:

The strains containing marker genes used in this analysis were all first crossed with the inositolless strain 37401, *CDE* heterocaryon genotype. Since the heterocaryon genotype of some of the marker strains was unknown, double mutants were selected having the *CDE* genotype, as tested with the standard *rib-2* testers. The *cDE* strains crossed with these doubly marked strains were inositolless, in order that no wild-type pairs would be expected to appear in any ascus.

The *c* locus is about 26 units from the centromere (GARNJOBST 1953), so the first marker genes selected were those listed in Table 4. Linkage group II was omitted because *c* had previously been shown to segregate independently of *d* (GARNJOBST 1953). Since none of the recombination frequencies obtained from crosses with these markers, and sex (I, L), indicate linkage (Table 4), other left arm markers were used in further crosses.

The first left arm marker tested (except sex) was ropy-3 (R2354, *ro-3*) in group II, to which *c* is closely linked (5.0% recombination). In 20 asci, the dis-

TABLE 4

Results of crosses to locate c

Marker symbol* (isolation No.)	Linkage group	Distance of marker from centromere†	No. of asci	Percent recombination with *c*
Right arm‡				
al-2 (15300)	I	31.0	12	58.3
tyr-1 (Y6994)	III	32.0	9	55.5
pan-1 (5531)	IV	31.5	12	45.8
tryp-2 (S4266)	VI	13.5	11	54.5
Left arm				
sex	I	6.5	52	39.4
ro-3 (R2354)	II	18.0	20	5.0

* Symbols are abbreviations for albino, tyrosine, pantothenic acid, tryptophan, and ropy.
† Taken from BARRATT *et al.* (1954), except *ro-3*, from PERKINS *et al.* (1962).
‡ *fl* (II, R), *inos* (V, R), and *nt* (VII, R), tested previously.

TABLE 5

Ascus segregation in cross establishing linkage of c *and* ro-3
Parent genotypes: *CDE, inos, ro-3, a* × *cDE, inos, A*

	Ascus segregation					
	From centromere†			Between genes‡		
No. of asci*	*ro-3*	*c*	sex	*c* & *ro-3*	*c* & sex	*ro-3* & sex
6	2nd	2nd	1st	PD§	T	T
6	1st	1st	1st	PD	PD	PD
1	1st	2nd	1st	T	T	PD
3	1st	1st	1st	PD	NPD	NPD
1	2nd	2nd	2nd	T	T	T
1	2nd	2nd	2nd	PD	PD	PD
1	1st	1st	2nd	PD	T	T
1	1st	2nd	1st	PD	PD	PD

* Total number of asci 18; 98.0% germination of ascospores, each pair/ascus represented.
† Centromere distances obtained: *ro-3*, 20.0; *c*, 25.0; sex, 7.5. The centromere distances are uncorrected for multiple crossovers.
‡ Recombination frequencies obtained: *c* & *ro-3*, 5.0%; *c* & sex, 37.5%; *ro-3* & sex, 35.0%.
§ PD = parental ditype segregation; NPD = nonparental segregation; T = tetratype segregation.

tance from the centromere for *c* is 25.0 units, and for *ro-3*, 20.0 units (Table 5). These results show good agreement with centromere distances previously obtained: 26.0 for *c* (GARNJOBST 1953) and 18.0 for *ro-3* (PERKINS, GLASSEY and BLOOM 1962). The *c* locus, therefore, appears to be slightly distal to *ro-3* in group II, left arm.

TABLE 6

Results of crosses to locate e*

Locus symbol† (isolation No.)	Linkage group	Distance of marker from centromere‡	No. of asci	Percent recombination with *e*
Right arm				
al-2 (15300)	I	31.0	11,41	40.9,46.3
fl (fluffy)	II	41.0	21	42.8
leu-1 (D221)	III	10.5	20	55.0
tyr-1 (Y6994)	III	32.0	9	44.4
pan-1 (5531)	IV	28.0	11	40.9
inos (37401)	V	31.5	18	41.7
lys-1 (33933)	V	near Centromere	22	34.0
tryp-2 (S4266)	VI	13.5	33	39.3
me-7 (4894)	VII	near Centromere	23	43.5
nt (39401)	VII	21–32	18	30.6
Left arm				
sex	I	14.0	11,41,17	50.0,54.5,50.0
ro-3 (R2354)	II	18.0	17	55.9
lys-5 (StL7)	VI	11.0	21	57.1
chol-2 (47904)	VI	36.0	20	52.5
nic-3 (Y31881)	VII	21.0	41	28.0

* Centromere distance of *e*, 35.2 (288 asci).
† Symbols not previously explained are abbreviations of leucine, lysine, methionine, choline, and nicotinic acid.
‡ Centromere distances taken from BARRATT *et al.* (1954), except the markers in left arm, from PERKINS *et al.* (1962).

Linkage of e: Twenty-six asci isolated from two earlier crosses in which *e* had segregated gave a centromere distance for *e* of 30 to 39 units, so the marker strains used to detect linkage in crosses with the *CDe, inos* isolates 141*a* or 149*A* were those listed in Table 6. Although *e* and *d* had segregated independently, *fl* was included because of the possibility that *e* might show linkage to *fl*. The heterocaryon genotype of all right and left arm marker strains was *CDE*, and each strain contained one marker gene and *inos*. Two exceptions were the crosses *CDe, nt, A* × *cdE, nt, a*, and *CDe, inos, A* × *CDE, nt, a*, but in the latter cross the heterocaryon genotypes of the wild-type pairs obtained were deduced from the types shown by heterocaryon tests to be present in these asci. Table 6 also lists the recombination frequencies obtained in asci in which the marker genes and the new heterocaryon gene *e* had segregated. As shown, the three lowest percentages were obtained with *lys-1* (34.0%), *nt* (30.6%), and *nic-3* (28.0%).

The *lys-1* locus is near the centromere in group V, but in which arm is still uncertain (STRICKLAND, PERKINS and VEATCH 1959). It was already known from the results of Cross 4 (*CDe, inos, A* × *CDE, nt, a*) that *e* is not linked to *inos* (right arm), but it seemed possible from the 35-unit centromere distance that *e* might be in the left arm of group V. However, no other gene has been reported in this arm, so further tests could not be made.

nt is located about 21–32 units from the centromere in the right arm of group VII (BARRATT *et al.* 1954) and if *e* were in this arm, linkage would have been close in the asci obtained from Cross 4 (*CDe, inos, A* × *CDE, nt, a*). This was not the case; the recombination frequency found was 30.6% (Table 6).

That *e* might be present in the left arm of group VII also seemed a possibility. This could be tested using *nic-3*, 21.0 units left of *me-7*, which is very close to the centromere (PERKINS *et al.* 1962). Genes *e* and *nic-3* are indeed linked (12 parental ditype: 0 nonparental ditype asci [$P < 0.005$]). The centromere distance for *e* in 23 asci from the cross *CDe, inos, a* × *CDE, inos, me-7, A* is 34.8, and in 41 asci from the cross *CDe, inos, a* × *CDE, inos, nic-3, A* this distance is 37.8. As shown in Table 7, the centromere distance for *nic-3* in the 41 asci is 21.9, which is in good agreement with that previously found (PERKINS *et al.* 1962). According to these results the calculated distance between *e* and *nic-3* is 15.9 units. The recombination frequency obtained, however, is 28% (Table 7). The difference of 12.1 units probably means that *e* actually is farther out from the centromere than 37.8 units.

As shown in Table 6, all suitable markers in each linkage group were tested. Markers are not known for the three untested left arms (III, IV, and V), except fissure (*fi*) in IV (PERKINS *et al.* 1962). It may be concluded, however, that *e* is in linkage group VII, about 28.0 units distal to *nic-3*, 35.2 to 49.0 units from the centromere.

DISCUSSION

In the study of heterocaryosis in Neurospora, a distinction must be made between (1) the formation of a heterocaryon and (2) maintenance of the wild-type

TABLE 7

Ascus segregation in cross establishing linkage of e *and* nic-3
Parent gentoypes: *CDe, inos, a* × *CDE, inos, nic-3, al-2, A*

	Ascus segregation							
		From centromere†			Between genes‡			
No. of asci*	*e*	*al-2*	*nic-3*	sex	*nic & e*	*al & e*	sex & *e*	*al* & sex
1	2nd	2nd	1st	2nd	T§	T	T	T
3	2nd	2nd	2nd	1st	PD	T	T	T
3	2nd	1st	2nd	2nd	PD	T	T	T
2	2nd	1st	1st	1st	T	T	T	T
2	1st	1st	2nd	2nd	T	NPD	T	T
2	2nd	1st	2nd	1st	PD	T	T	PD
2	2nd	2nd	1st	1st	T	PD	T	T
1	2nd	2nd	2nd	1st	T	NPD	T	T
1	2nd	2nd	2nd	1st	T	T	T	T
1	1st	2nd	2nd	1st	T	PD	PD	T
3	2nd	1st	1st	2nd	T	T	T	T
2	2nd	2nd	1st	1st	T	T	T	T
1	2nd	2nd	2nd	1st	PD	PD	T	T
2	2nd	1st	1st	1st	PD	T	NPD	T
1	1st	1st	1st	1st	PD	PD	NPD	NPD
1	1st	1st	1st	1st	PD	NPD	PD	NPD
1	2nd	2nd	1st	2nd	T	NPD	NPD	PD
1	2nd	1st	2nd	2nd	PD	T	T	T
1	1st	1st	1st	2nd	PD	PD	T	T
2	1st	1st	2nd	1st	T	T	T	PD
1	1st	1st	2nd	2nd	T	T	T	T
1	2nd	2nd	2nd	2nd	PD	PD	T	T
1	2nd	1st	2nd	1st	T	NPD	T	T
1	2nd	1st	2nd	1st	T	PD	T	T
1	1st	1st	2nd	1st	T	T	T	T
1	1st	2nd	2nd	1st	PD	T	T	PD
1	1st	2nd	1st	2nd	T	PD	T	T
1	1st	2nd	1st	1st	PD	T	PD	T

* Total number of asci 41; 98.1% germination of ascospores; in six asci one pair represented by one germinant.
† Centromere distances obtained: *e*, 37.8; *nic-3*, 21.9; *al-2*, 20.7; sex, 10.7. The centromere distances are uncorrected for multiple crossovers.
‡ Recombination frequencies obtained: *e* & *nic-3*, 28.0%; *e* & *al-2*, 46.3%; *e* & sex, 54.9%; *al-2* & sex, 42.7%.
§ PD = parental ditype segregation; NPD = nonparental segregation; T = tetratype segregation.

rate of growth for a considerable length of time (GARNJOBST 1955). Both are valid approaches. At present the second appears to be complex and probably depends upon numerous factors, both in the particular genomes concerned and in the changing environment. PITTENGER (1964) deals with this latter aspect. The results presented indicate that the factors in certain pairs of strains which he described and considered "incompatible" are somehow altered during growth so that the pairs become compatible. No direct evidence was presented relating the genes concerned with the heterocaryon genotypes previously described (GARNJOBST and WILSON 1956). It seems unfortunate that the same terms—incompatibility and incompatibility reaction—have been applied to situations

which appear different. From the viewpoint of heterocaryon formation, it would be of interest and importance to know whether the pairs in question were initially cytoplasmically compatible or incompatible.

The heterocaryon genes *C* and *D*, and their alleles *c* and *d*, have proved to be quite stable in the *inos*, *nt* and *rib-2* standard tester strains. These strains and those derived from them have been retested following lyophilization, and growth from ascospores and on repeated transfer. Although morphological mutations have occurred spontaneously, they have not affected heterocaryon formation, nor has any change in heterocaryon genotype been found. The presence of the *D* allele in some of the sib-cross isolates (b_9) may be an exception, if its presence is due to mutation. Genes modifying the effectiveness of allele *d* have been encountered in asci from crosses between strains of known and unknown heterocaryon genotype, but these have been avoided until the primary genes concerned with heterocaryon formation are more fully characterized.

One might suppose that the lethality of pairs of cytoplasmically incompatible strains would entirely prevent growth, as it does when very few cells are present. The delayed, erratic growth which takes place between some pairs of incompatible strains in heterocaryon tests with slightly larger inocula might be the result of numerous hyphal fusions and death of cells, thereby liberating growth substances which can then be used by the remaining cells. Microscopic observations of regenerative growth following an incompatibility reaction (GARNJOBST and WILSON 1956) lends some support to this possibility.

The observation of unidirectional killing on transfer of protoplasm by microinjection between certain incompatible pairs (WILSON *et al.* 1961) suggests another possibility for limited growth under suitable circumstances. Although survival of one of a pair of fused incompatible cells has not been observed, even when the protoplasmic flow was apparently unidirectional (GARNJOBST and WILSON 1956), such a flow from *cd* into *Cd* might result in a heterocaryon with limited growth potential.

SUMMARY

The heterocaryon compatibility genotypes of the wild-type strains ST74*A*, ST74*A*4, 74-OR8-1*a*, and 74-OR23-1*A* have been determined by microscopic observation of hyphal fusions in addition to standard heterocaryon growth tests. The heterocaryon genotype of these wild types was found to be *Cde*, and strains derived from Lindegren stock, *CDE*. The new gene, *e*, segregated independently of the previously described genes, *c*, and *d*, and resembled them in its effect on heterocaryon formation. The following linkage relations have now been established: *d* in the right arm of II (previously reported); *c* in the left arm of II, distal to *ro-3;* and *e* in the left arm of VII, distal to *nic-3*.

LITERATURE CITED

BARRATT, R. W., D. NEWMEYER, D. D. PERKINS, and L. GARNJOBST, 1954 Map construction in *Neurospora crassa*. Advan. Genet. **6**: 1–93.

CASE, M. E., H. E. BROCKMAN, and F. J. DE SERRES, 1965 Further information on the origin of the Yale and Oak Ridge wild-type strains of *Neurospora crassa*. Neurospora Newsl. **8**: 25–26.

GARNJOBST, L., 1953 Genetic control of heterocaryosis in *Neurospora crassa*. Am. J. Botany **40**: 607–613. —— 1955 Further analysis of genetic control of heterocaryosis in *Neurospora crassa*. Am. J. Botany **42**: 444–448.

GARNJOBST, L., and J. F. WILSON, 1956 Heterocaryosis and protoplasmic incompatibility in *Neurospora crassa*. Proc. Natl. Acad. Sci. U.S. **42**: 613–618.

PERKINS, D. D., M. GLASSEY, and B. A. BLOOM, 1962 New data on markers and rearrangements in Neurospora. Can. J. Genet. Cytol. **4**: 187–205.

PITTENGER, T. H., 1964 Spontaneous alterations of heterocaryon compatibility factors in Neurospora. Genetics **50**: 471–484.

STRICKLAND, W. N., D. D. PERKINS, and C. C. VEATCH, 1959 Linkage data for group V markers in Neurospora. Genetics **44**: 1220–1226.

VOGEL, J. H., 1956 A convenient growth medium for Neurospora. Microbial Genet. Bull. **13**: 42–43.

WESTERGAARD, M., and H. K. MITCHELL, 1947 Neurospora. V. A synthetic medium favoring sexual reproduction. Am. J. Botany **34**: 573–578.

WILSON, J. F., 1961 Micrurgical techniques for Neurospora. Am. J. Botany **48**: 46–51.

WILSON, J. F., L. GARNJOBST, and E. L. TATUM, 1961 Heterocaryon incompatibility in *Neurospora crassa*—Microinjection studies. Am. J. Botany **48**: 299–305.