

A PERICENTRIC INVERSION IN *NEUROSPORA*, WITH UNSTABLE DUPLICATION PROGENY¹

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DURING a study of the arginine-1 locus in *Neurospora crassa*, one mutant strain (H4250) was found to produce, on crossing to wild type, an unusual class of progeny. These progeny initially show very slow "inhibited" growth with a characteristic spidery morphology, and are darkly pigmented on complete medium; they are therefore called Dark Agars. After several days, they escape from the inhibition and grow like wild type; many of the resulting cultures behave as if both mating-type alleles are present. The present study shows that the Dark Agars are viable duplications produced by crossing over in a pericentric inversion. The initial inhibition appears to be due to heterozygosity for the mating-type locus, which is included in the duplication; the cultures formed after escape from inhibition are mixtures of somatic derivatives that are homozygous or hemizygous for mating type. Somatic segregation for other markers in the duplicated region is also found.

The H4250 inversion provides favorable material for studying somatic segregation, because the duplication covers 50 map units in a well marked region, and because there is strong selection for the products of somatic segregation, which can be recovered and purified by plating.

Short duplications in *Neurospora* have been obtained previously by ST. LAWRENCE (1959), BARRY (1960, 1961) and DE SERRES (1957), among the progeny of insertional translocations; markers in these duplications do not appear to segregate somatically. Recently, however, PERKINS (1966) has found a long insertional translocation in *Neurospora* which produces Dark Agar duplication progeny, which look very much like those produced by the H4250 inversion; his duplication strains also segregate somatically for mating type. Furthermore, BAINBRIDGE and ROPER (1966) have described a translocation in *Aspergillus nidulans* which produces unstable duplications, and a brief report by NGA and ROPER (1966) describes a different unstable duplication in the same organism.

The present paper is primarily concerned with proof and characterization of the inversion, location of the break points, description of the duplication, and evidence suggesting that the initial Dark Agar phenotype is due to mating-type heterozygosity; preliminary results bearing on the mechanism of somatic segregation are also given. A preliminary account has been published (NEWMAYER 1965).

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MATERIALS AND METHODS

Strains: The original aberration stock, H4250, was isolated as an arginine mutant by HUNGATE and MANNELL (1952), after S³⁵ treatment of the *pe^mfl* strain Y8743-21 (13-7)a. We have found no evidence of a similar aberration either in their starting material or in two other mutants which they isolated in the same series of experiments—H6196 (phenylalanine) and H3211 (citrulline). The arginine requirement is due to a mutation at the *arg-1* locus (NEWMAYER 1957, 1962, and unpublished data); the requirement and the aberration are closely linked but separable. In this paper the symbol *arg-1* (or simply *arg*) will be used to signify the H4250 allele whenever *arg-1* is in coupling with the aberration, and the standard allele B369 whenever *arg-1* is in the normal sequence.

Standard wild types were ST74A, STA-4, and later 74-OR23-1A and 74-OR8-1a (derived from ST74A and ST73a). Standard mating-type (*mt*) testers were the fluffy strains *fl*(P605)*a* and *fl^PA*. The following markers were used: *acr-3*, acriflavine-resistance (KH14); *ad-5*, adenine (Y152M40, also called *adJ7*); *ad-9*, adenine (Y154M37); *arg-3*, arginine or citrulline, here called *cit*, (30300); *aur*, aurescent (34508); *cyt-1*, cytochrome (C115); *eth-1*, ethionine-resistance (*r-eth-1* of METZENBERG, KAPPY, and PARSONS (1964)); *fr*, frost (B110); *leu-3*, leucine (R156); *me-6*, methionine (35809); *mt*, mating type (A/a), also called *sex*; *nic-1*, nicotinic (3416); *nic-2*, nicotinic (43002); *os*, osmotic (B135); *phen-1*, phenylalanine (H6196); *ser-3*, serine (47903); *so*, soft (B230s); *suc*, succinic (66702); *thi-1*, thiamine (56501); *un*(55701*t*) and *un*(46004*t*), unknown temperature-sensitive (two loci). References for all these strains are given by BACHMANN and STRICKLAND (1965) and/or BARRATT and OGATA (1966). Also used were *leu-4*, leucine [D133 and R359 (GROSS and GROSS 1961)] and a new morphological clock-like mutant (D317), isolated by DR. S. R. GROSS after ultraviolet treatment of *inos* (89601). Linkage testers were *alcoy* (PERKINS 1964) and LT2a (PERKINS 1959). All markers (except linkage testers and fluffy) are in linkage group I. Most markers were backcrossed to standard wild type two or three times before use.

Standard aberration (Ab) stocks were chosen to minimize the possibility of using derived aberrations which might not be structurally identical. Two similar non-Dark Agar progeny from the 7th backcross generation of H4250 to wild type 74A were chosen as *arg* Ab standards (*A* and *a*). The *arg* *Ab* *a* standard was then crossed to a well backcrossed *ad-5* stock to select an *arg*⁺ *Ab* *a* standard and the latter was crossed to the *arg* *Ab* *A* standard to obtain an *arg*⁺ *Ab* *A* standard. All four *Ab* standards appear structurally identical by the following criteria: (1) When crossed to normal sequence (standard wild-type or *fl* testers) they produced about 25% Dark Agars (DA's) and 25% white (defective) spores, and many of the asci had 6 black and 2 white spores; (the frequency of 6:2 asci varied, as would be expected from variations in cross-over frequency). (2) When crossed to each other, the *Ab* standards produced very few white spores and no DA's. Crosses of both types were highly fertile.

All aberration stocks carrying other markers were derived from one of the four standard *Ab* stocks. All such stocks were tested in crosses with both *Ab* and normal standards and shown to conform to the above criteria, except that the examination of asci was often omitted. All data given below were obtained with the standard and marked *Ab* stocks just described. The similarity of DA production and defective asci suggests that the aberration in the original H4250 stock is the same as that in the standard aberration stocks described here, but this has not been proved.

Gene order: The normal order of group I markers (see Tables 2 and 4) is based entirely on 3-point data. Much of it is documented in BARRATT and STRICKLAND (1962), PERKINS, GLASSEY and BLOOM (1962), PERKINS and MURRAY (1963, 1964), HOWE (1962), METZENBERG *et al.* (1964), NEWMAYER (1957) and HSU (1965). The sequence *mt-phen-1-ad-5* was established by H. B. HOWE (personal communication). Mutants *ser-3* and *morph*(D317) were first located in group I by D. D. PERKINS (personal communication). The exact order of *cyt-1*, *ser-3*, *morph*(D317), *acr-3*, *suc*, *eth-1*, and *un*(46006*t*) was determined by us and will be documented elsewhere.

Technical methods: Most genetic methods were essentially as described in PERKINS (1959). Mating type, fertility, and presence of aberration were scored routinely using fluffy testers in individual 75 mm tubes as described by TAYLOR (1965). Cultures were scored as *Ab* or inversion

only if the frequency of white (defective) spores appeared significantly greater than the 5 to 10% background level found in crosses of OR wild types to each other or to our standard *fl* testers. Strains were scored N (normal sequence) when white spores did not exceed 10%. In critical cases the frequencies of tetrads with specific patterns of black (viable) and white (defective) spores were determined in unordered tetrads obtained by the method of STRICKLAND (1960). With *fr* isolates, it was necessary to wait 3 weeks after fertilizing to get reliable fertility tests.

Spores to be scored for DA were ordinarily isolated onto glycerol-complete medium, a modification of medium 2 of TARUM *et al.* (1950); arginine (0.3 mg/ml) was added when an arginine requirement was involved. After 3 to 6 days growth at 25°C, DAs were scored on the basis of pigmentation, morphology, and restricted growth.

In scoring coverage of biochemical mutants in DAs, the DAs were allowed to escape completely before testing for the requirement. Mis-scoring occurs if slow-growing nonescaped material is used as a source of test inoculum.

Somatic segregation was analyzed by plating escaped-DA conidia on sorbose medium. Segregants of *fr* and *morph(D317)* were also obtained by picking mutant sectors before they were overgrown by the wild type, and were purified by repeating the process as often as necessary.

All platings and biochemical, fertility, and progeny tests on DAs were performed on escaped-DA cultures, unless specifically noted; the word "escaped" is often omitted for brevity.

RESULTS

When H4250 Ab is crossed to wild type, about $\frac{3}{4}$ of the viable ascospores yield phenotypically normal progeny. These show good allele ratios and the expected linkage of *arg-1* and mating type, both of which loci are in the left arm of group I. The remaining $\frac{1}{4}$ of the viable ascospores (actually 15–35%) form the characteristic "inhibited" colonies called Dark Agars (symbol DA).

Description of Dark Agars: The initial colonies have long, fuzzy irregular outgrowths which make them resemble spiders; the outgrowths penetrate the agar but there is little aerial growth and no conidia (Figure 1). After about four days on glycerol-complete medium at 25°C, they form intense dark brownish-black pigment which is concentrated in the trunk hyphae but also diffuses into the agar. On minimal medium the morphology is the same, but no pigment is formed. After 5 to 8 or more days at 25°C, when the colonies are 5 to 10 mm in diameter, the DAs "escape" from the inhibition and yield cultures which look essentially like wild type, except that the initial dark spot is still visible. When the escaped DAs are transferred the initial phenotype does not recur, and the linear growth rate is essentially wild type. Slow-growing morphologicals such as hemizygous *dot* or homozygous *fr* modify the DA morphology, and produce tiny dark colonies without the spidery outgrowths; homozygous *fr* DAs eventually escape to yield normal *fr* morphology.

The original DA cultures, after escape, are still abnormal in two ways: (1) About $\frac{1}{3}$ of them react with both *A* and *a* mating-type (*mt*) testers. It was shown by plating that such cultures actually consist of a mixture of pure *A* and pure *a* conidia; they will be called *Mixed*. *Mixed* cultures are very unstable, giving rise to pure *A* or pure *a* cultures after one or two transfers. When the initial DA cultures are tested for mating type before they are fully conidiated, and the inoculum is taken from several points in the tube, the proportion of DAs scored as *Mixed* goes up to about $\frac{2}{3}$. From these and other observations it appeared that

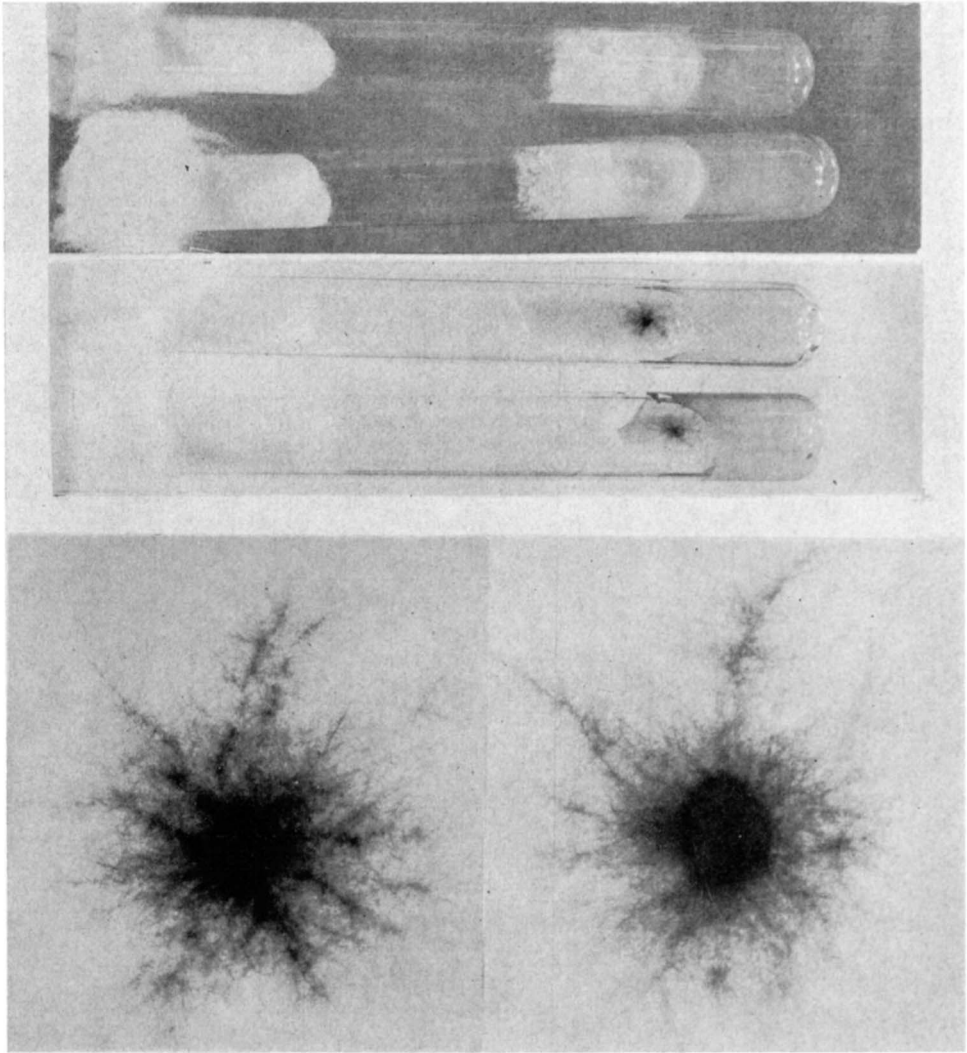


FIGURE 1.—Upper—Dark Agar and non-Dark Agar isolates from the same cross, both grown for six days at 25°C on glycerol complete medium ($\times 1.2$). Lower—Dark Agar isolates like those above ($\times 7.4$).

one mating type often overgrows the other already in the original culture, suggesting that escaped DAs that seem pure *A* or *a* are really overgrown *Mixed* cultures. (When no selective marker is present, “pure” *A* and “pure” *a* cultures are found among the escaped DAs from the same cross.) Similar overgrowth where there is no obvious selective advantage has been found by COYLE and PITTEGER (1965) in pseudowild-type cultures (heterocaryons arising from single ascospores which appear to be heterozygous disomics). (2) Although escaped DAs form abundant perithecia, most of them are barren; i.e., the perithe-

cia usually stop development just before forming necks, and very few spores are produced. But about 20% of the DAs are much more fertile (often with completely normal spore production) in crosses with one or the other mating type (rarely with both).

Distribution of defective spores in individual asci: Crosses of H4250 Ab to wild type yield about 25% white spores. Pooled data on 220 unordered tetrads from many Ab \times N crosses gave the following frequencies of tetrads with different patterns of viable (black) and defective (white) ascospores: 8 black:0 white—17.3%; 6 black:2 white—64.5%; 4 black:4 white—12.7%; 2 black:6 white—4.5%. Asci with 0 black:8 white were rare.

In 25 asci from which the ascospores were dissected in order (see later), each ascospore pair that gave rise to DAs was balanced by a white spore pair.

Possible interpretations: Most of these facts could be accounted for by an aberration which, in crosses to normal sequence, yields reciprocal duplication and deficiency products, one of which is viable (and presumably DA) and the other inviable. This might be the case with an insertional translocation, or with a reciprocal translocation that had one break in a dispensable chromosome tip. However, crosses to two linkage testers (*alcoy*, marked in six linkage groups, and LT2a, marked in all seven groups) showed no linkage of either *arg-1* or DA to anything except the group I marker (*al-2*).

An alternative explanation was that the DAs were really pseudowild types (PWTs). PWTs are single-ascospore cultures that appear to originate as heterozygous disomics, which quickly break down into euploid heterocaryons (PITTENGER 1954). This would explain the somatic segregation, and also the fact that in the initial crosses (*arg-1* Ab \times *arg-1*⁺ N) almost all escaped DAs were *arg*⁺. But when the aberration was obtained free of *arg*, and the cross was made in the opposite coupling phase (*arg-1*⁺ Ab \times *arg-1* N), the escaped DAs were now predominantly *arg*. Also, crosses to normal stocks carrying other left-arm markers produced DAs which were predominantly mutant for markers proximal to *mt* (*phen*, *ad-5*, and *arg-3*, here called *cit*), and predominantly wild-type or sectoried for markers distal to *mt* (*un(55701t)* and *fr*). (The gene order is *fr un mt phen ad-5 arg-1 cit* centromere.) Therefore, if DAs were really pseudowild types, they could only be heterozygous for the part of the left arm that is distal to *phen*.

These data suggested a break between *phen* and *mt*. A break somewhere distal to *arg-1* was also suggested by the experiment from which the *arg-1*⁺ Ab stock was obtained. Crossovers were selected immediately distal and immediately proximal to *arg-1*, in the hope that one or the other might recombine *arg-1*⁺ with the aberration. Only one *ad-5*⁺ *arg-1*⁺ (distal) crossover was obtained, and it carried the original aberration. The *arg-1*⁺ *cit*⁺ (proximal) crossovers were either DA or normal.

When these data are considered in connection with the inversion hypothesis below, it is clear that *ad-5*⁺ *arg-1*⁺ crossovers should have survived only when there was a second crossover within the inversion loop, to yield the Ab sequence; this is consistent with their extreme rarity (<.03%). The *arg-1*⁺ *cit*⁺ crossovers should be DA (if singles) or normal (if there was a second crossover further out in the loop).

Inversion hypothesis: The correct explanation was suggested by the discovery that recombinants between *al-2* and *arg-1* were more frequent among the DAs than among non-DAs, suggesting that DAs are produced by crossing over in group I. It was therefore supposed that the aberration is a pericentric inversion with one break between *phen* and *mt* and the other beyond the rightmost markers *os* and *so* (Figure 2a, b). When there are single crossovers in the inverted region, pericentric inversions produce reciprocal duplication-deficiency classes (rather than the anaphase bridges characteristic of paracentrics). Thus DAs would represent the crossover class that is duplicated (and usually heterozygous) for most of the left arm, and deficient for the tip of the right arm (Figure 2c). The white spores would represent the reciprocal crossover class, which is deficient for most of the left arm and duplicated for the tip of the right arm.

It was further supposed that the initial inhibition of the DAs is due to heterozygosity for mating type (*A/a*), and that the escape from inhibition is due to production of homozygous *A/A* and *a/a* nuclei by mitotic crossing over (Figure 2d). By this hypothesis, all DAs must be *Mixed* when they first escape—every exchange that makes one product homozygous should automatically produce the complementary homozygote.

The hypothesis that the aberration is a pericentric inversion has been confirmed by numerous lines of evidence, described below. Therefore, the aberration will henceforth be called *In(ILR)H4250*. The mechanism of escape it still under study.

Marked crosses heterozygous for the aberration: Random isolates from crosses marked with *arg-1 aur os* or *arg-1 aur nic-1 so* strongly support the inversion hypothesis (Table 1, crosses 1 and 2). All markers are within the proposed inversion loop, with *arg-1* near the left break point, *os* and *so* near the right break point, and *aur* roughly in the middle; *so* is a few units distal to *os*.

Note that most single crossovers between *arg* and *os* (or *arg* and *so*) occur in the DAs; these are all *arg⁺ os⁺* (or *arg⁺ so⁺*). This type comes from a single crossover in the loop. The reciprocal single crossover (*arg os* or *arg so*) should be grossly deficient and lethal; no DAs of this type were found. Conversely, most parental and double crossover progeny are non-DA, as they should be.

Two kinds of exception are found: (1) *Crossovers between the extreme markers and the break points are not detectable.* Thus most non-DA “singles” are in all likelihood double crossovers having one exchange beyond the genetic markers (*c*, *d*, *e*, *g*, *h*, Table 1). A parentally marked DA can be attributed to a single crossover between an extreme marker and the adjacent break point (*‡*, *‡*, Table 1). (2) *Duplications that are not DA may be produced.* A 3-strand double crossover with one exchange in the loop, and the other between mating type and the left break point, would produce a duplication structurally identical to that in the DAs, but homozygous for mating type (*mt*). If the inhibited DA phenotype is indeed due to heterozygosity for *mt*, such a duplication should not *look* like a DA, but should usually resemble most DAs in having an *arg-os* crossover (in this case *arg⁺ os⁺*) and in having barren perithecia. Two such isolates were found (*b*, *f*, Table 1).

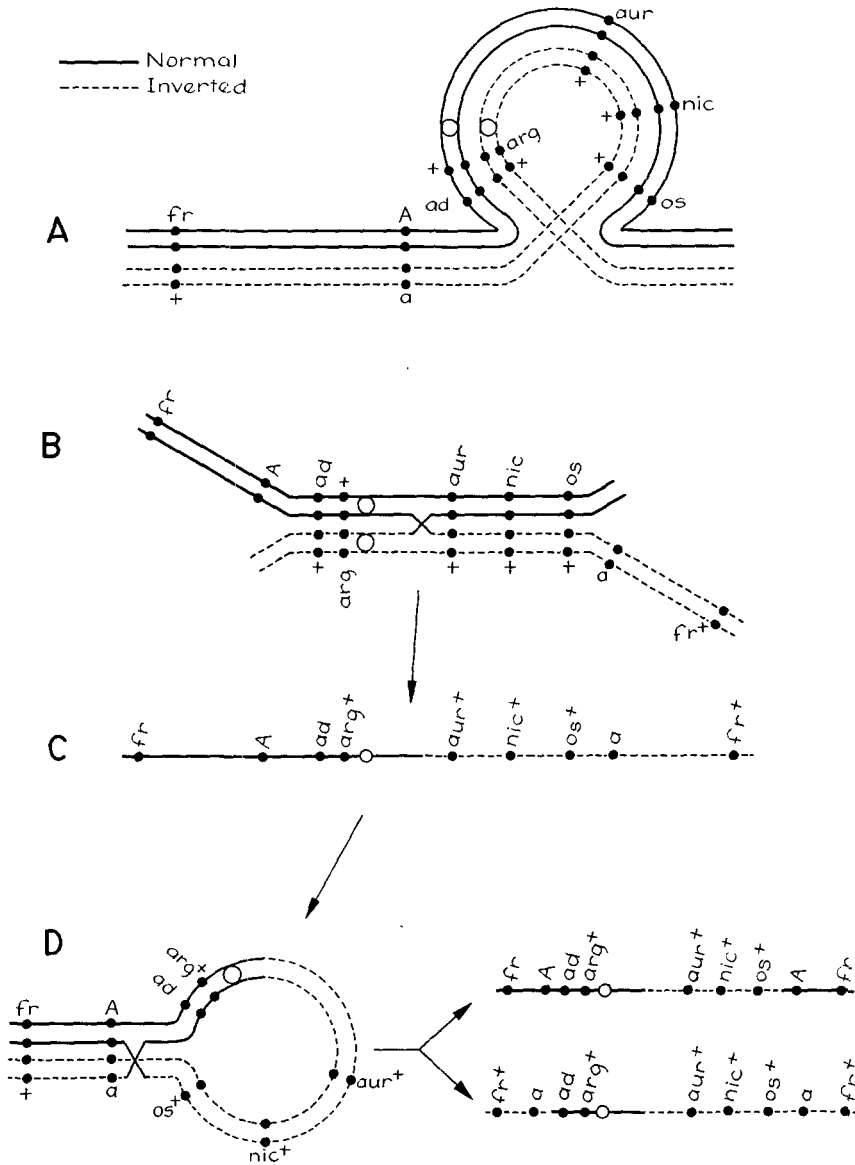


FIGURE 2.—Diagrams of a cross heterozygous for the proposed inversion. Not to scale, *ad* = *ad-5*, *arg* = *arg-1*, *nic* = *nic-1*. **A** and **B** show synapsis during meiosis. **A** shows complete synapsis to yield an inversion loop. **B** shows only the inverted region synapsed, in order to illustrate better how a crossover in the inverted region forms complementary duplication-deficiency products. **C** shows the viable product deficient for the right tip and duplicated for most of the left arm, heterozygous for mating type (*A/a*) and *fr*. **D** shows how mitotic crossing over in the duplicated region might yield products homozygous for mating type and *fr*.

All other crosses heterozygous for the aberration give results consistent with those just described. In cross 5, where the *arg/In* coupling phase is reversed, the DAs become predominantly *arg*, instead of *arg*⁺. In crosses 4 and 5, many DAs appear to be noncrossovers because there is no marker near the right tip. Cross 5 (footnotes) shows that DAs are often mosaic for *leu-3* as well as for *fr* and *mt*, as predicted. Most of the DAs homozygous for *fr* or *leu-3*, in crosses 4 and 5, are presumably due to additional meiotic crossovers distal to *mt*.

There are, however, wide variations in the relative frequencies of crossing over in different regions. For example, in cross 3 much more of the crossing over occurs near the break points. Cross 4 shows high crossing over in all marked regions, including the left arm; cross 5 has very little crossing over distal to *mt*, but resembles cross 4 elsewhere. A cross to be described later (Table 3) had many crossovers in the loop but none between *mt* and *arg-1*, in contrast to the abnormally high frequency between *mt* and *arg-1* found in crosses 4 and 5. (The highest percent recombination ever found between *mt* and *arg-1* (B369) in the absence of an aberration is about 15%, as opposed to 21% and 26% in crosses 4 and 5.) The unusual left-arm crossover values in cross 5 are not due to an abnormality in the normal parent, because this same parent, when crossed to wild type, gave rather typical values (*fr/leu* = 31.1%; *leu/mt* = 9.7%; *mt/arg* = 6.5%). Because of the small number of isolates used, several of the variations encountered are not statistically significant.

Location of the undetected crossovers between extreme markers and break points can be deduced from the data on *mt* and white-spore frequency given in the footnotes to Table 1. Analysis of these indicates that several crossovers occurred between *arg-1* and left break point, and several between *os* and right break point, but none between *so* and right break point.

Mapping by duplication coverage: The exact location of the left break point was determined by testing escaped DAs to see which markers were covered by the duplication. In these tests all markers came in with the normal parent, as shown for *ad-5* and *fr* in Figure 2. The figure shows that for recessive markers in the loop and close to break point (like *ad-5*), the escaped DAs will usually have the mutant phenotype. For markers distal to break point (like *mt* and *fr*), the DAs will usually be heterozygous, and the mixed cultures formed after escape will have the dominant phenotype or be sectored. By this method the break point was located between *suc* and *phen*, which are less than one unit apart (Table 2). Most DAs did have the mutant phenotype in the case of all markers right of break point. Most DAs were sectored or wild type for all markers left of break point except *acr*, which is resistant and therefore dominant over its wild-type allele in mixed culture. It has not been tested for dominance in the heterozygote. That *acr* is really covered by the duplication was shown by plating conidia from an escaped DA. These segregated for resistance versus sensitivity.

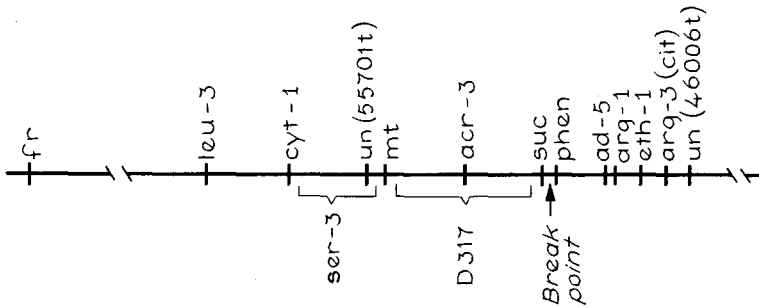
Thirty-eight escaped DAs have been analyzed by plating conidia or picking sectors. All seven markers from the duplicated region which were tested (*fr*, *leu-3*, *ser*, *mt*, *acr*, *morph(D317)* and *suc*) did segregate somatically in at least some DAs. In contrast, the three markers tested from the nonduplicated region (*arg-1* (both B369 and H4250), *aur*, and *os*) did not. In 24 of these DAs there was somatic segregation for a marker in the duplicated region (usually *leu-3*),

TABLE 2

Location of break point by duplication coverage

Markers covered by H4250 duplication (Most DA's look wild-type or sectored)		Markers not covered by H 4250 duplication (Or covered but dominant) (Most DA's have mutant phenotype)	
<i>fr</i>	<i>un(55701t)</i>	<i>phen</i>	<i>eth-1*</i>
<i>leu-3</i>	mating type	<i>ad-5</i>	<i>arg-3</i>
<i>cyt-1</i>	<i>morph(D317)</i>	<i>arg-1</i>	<i>un(46006t)</i>
<i>ser-3</i>	<i>suc</i>		<i>acr-3†</i>

Map order in IL by conventional methods:



Location of break point by duplication coverage. In all cases gene order is based on three-point crosses not involving the inversion (see METHODS). Genes listed below the line lie within the regions indicated by brackets. Centromere is near *un(46006t)*.

* *eth-1* was scored by heat-sensitivity, not by resistance to ethionine.

† *acr-3* is covered but dominant in mixed culture.

but no somatic segregation for *arg-1*, although both markers were present in the crosses from which these 24 DAs were derived.

The possibility that *phen* might actually be covered but dominant has been eliminated by showing that *phen* is recessive in the duplications produced by insertional translocation *T(1;II)39311* (PERKINS 1966). It is therefore concluded that the break point is really between *suc* and *phen*.

Ordered asci: Additional evidence was obtained from ordered asci (Table 3). The DAs always occur as spore pairs, as expected on the inversion hypothesis. If it is true that apparently pure *A* and pure *a* escaped DAs are derived from *Mixed* DAs in which one mating type has overgrown the culture, then the two members of a DA spore pair should, after escape, differ frequently as to *mt*, and might be any combination of *A*, *a*, and *Mixed*; this is found. One would also expect most 6 black:2 white asci to have a single crossover between centromere and right break point; such an ascus should have a normal spore pair in the same half-ascus as the DA pair, and a spore pair carrying the original inversion in the same half-ascus as the white spore pair. This is found in 17 of the 21 6:2 asci. The other four (Types VI, VIII, IX, and X) are multiple-crossover types, three of which have different arrangements of the N, DA, In, and white spore pairs; these are explainable if one crossover is between *arg-1* and centromere, as shown in Table 3.

Every DA pair was balanced by a white spore pair. However, in one ascus, a white pair was not balanced by a DA pair (Type IV). This case is not readily explained as due to a duplication homozygous for *mt*, like those described earlier, because the only non-DA pair in this ascus is fully fertile, normal, and *suc*. Since in our hands about 10% (16/156) of the asci in crosses of our standard wild types to each other, or to our standard *mt* testers, contain one or more white spore-pairs, it seems likely that the extra white spore-pair in the one ascus of Type IV is due to this background level of white spores, and is unrelated to the H4250 aberration.

Crosses homozygous for the inversion: Marked crosses homozygous for the H4250 aberration provided definitive proof that it is an inversion (Table 4). In this case no DAs are produced, and the percent of white spores is no greater than in crosses between standard wild types. Markers were used that cover essentially the whole known length of linkage group I. Gene order in the inversion, determined by conventional mapping methods, is *fr leu-3 mt * os nic-1 aur me-6 nic-2 arg-1**, where the segment between the asterisks is inverted relative to the normal sequence. The data also clearly indicate the order *acr nic-1 aur*, confirming that *acr* is not included in the inverted region. Map distances and coincidence values vary from cross to cross, but in all cases the data favor the order given. H4250 is thus unquestionably an inversion.

It was thought possible that there might be a third break, distal to *fr*, with the tips of II and IR exchanged. The result would be an inverted insertion with the same sequence as above, except that the right tip would be next to *fr* and the left tip next to *arg-1*, instead of vice versa. This "inverted insertion" hypothesis was attractive because it might explain the occurrence of fertility in the DAs. On this hypothesis most DAs would initially carry one left tip and one right tip (instead of two left tips as in the simple inversion), but a single mitotic crossover would eliminate one tip or the other; if one or both tips were needed for fertility, only certain mitotic crossover types would be fertile. However, this explanation of fertility is largely excluded by cross 5 of Table 1. If it were true, one or both classes of non-DA single crossovers between *fr* and the *suc/phen* break point should yield barren perithecia. In fact (with only one possible exception), all such crossovers were fully fertile. Therefore, the explanation could hold only if the distance between *fr* and the third break point were so great that all these crossovers were really doubles. This is unlikely, since *fr* is the leftmost known marker. The "inverted insertion" hypothesis is therefore untenable unless fertility does not require either the right tip or the left tip.

Cytological evidence: BARRY (1967) has made a cytological examination of crosses heterozygous for *In(ILR)H4250*, and has found partially synapsed inversion loops at pachytene. In his figures the left arm, outside the loop, appears to be completely synapsed; the "inverted insertion" hypothesis therefore appears unlikely.

Basis of the DA phenotype: The idea that the initial inhibited DA phenotype is due to heterozygosity for mating type (*mt*), and that escape is due to an event which makes *mt* homozygous, was originally suggested by three facts: (1) *A* and *a* strains of *Neurospora crassa* ordinarily do not form healthy heterocaryons. Instead, hyphal fusion is followed by the protoplasmic incompatibility reaction typical of strains differing in heterocaryon compatibility factors (GARNJOBST and WILSON 1956). (2) Conidia from escaped *Mixed* DAs are almost always pure

TABLE 3

Ordered asci from the cross *suc aur os Na* × *arg-1 In A*

Type	Number of asci*	Phenotypes of the four products						Interpretation
		†	<i>mt</i> ‡	<i>suc</i> §	<i>arg</i>	<i>aur</i>	<i>os</i>	
I	11	N	<i>a</i>	—	+	—	—	
		DA	‡	+	+	—	+	
		W	—	—	—	—	—	
		In	A	+	—	+	+	
II	5	N	<i>a</i>	—	+	—	—	
		DA	**	+	+	+	+	
		W	—	—	—	—	—	
		In	A	+	—	+	+	
III	1	<i>ng</i>						
		DA	<i>a,M</i>	+	+	—	—	
		In	A	+	—	+	+	
		W	—	—	—	—		
IV	1†† (see text)	N	<i>a</i>	—	+	—	—	
		DA	—	—	—	—	+	
		W	—	—	—	—	—	
		W	—	—	—	—		
V	2	DA	‡‡	+	+	+	+	
		W	—	—	—	—	—	
		DA	‡‡	+	+	—	+	
		W	—	—	—	—		
VI	1	N	<i>a</i>	—	+	—	—	
		In	A	+	—	—	+	
		W	—	—	—	—	—	
		DA	<i>A,M</i>	+	+	+	+	
VII	1	N	<i>a</i>	—	+	—	—	
		N	<i>a</i>	—	+	+	—	
		In	A	+	—	+	+	
		In	A	+	—	—	+	
VIII	1	N	<i>a</i>	—	+	—	—	
		DA	<i>a,ng</i>	+	+	+	+	
		W	—	—	—	—	—	
		In	A	+	—	—	+	
IX	1	In	A	+	—	—	+	
		DA	<i>M,ng</i>	+	+	—	+	
		W	—	—	—	—	—	
		N	<i>a</i>	—	+	+	—	
X	1	DA	<i>A,a</i>	+	+	+	+	
		W	—	—	—	—	—	
		N	<i>a</i>	—	+	+	+	
		In	A	+	—	—	—	

A and pure *a*. (3) Neither the isolated pure *A* and pure *a* cultures nor the original escaped *Mixed* culture produces a DA phenotype when transferred. (It was also shown, by germinating *A* and *a* normal ascospores together, that a simple mixture of *A* and *a* does not produce a DA phenotype, even under the conditions existing just after ascospore germination.)

Several additional lines of evidence now support the hypothesis that heterozygosity for mating type causes the inhibited DA phenotype.

1. As described earlier, duplications analogous to DAs should occasionally be homozygous for *mt* from the start, because of an additional meiotic crossover between *mt* and break point. These should not show the initial inhibition but should resemble escaped DAs in usually having a demonstrable crossover in the loop and in producing barren perithecia. Several isolates of this type were found (Table 1, b, f, j, l, q).

2. If *mt* heterozygosity causes the DA phenotype, then all DAs must be *Mixed* at the time of escape, providing that escape occurs either by mitotic crossing over or by any other event which yields reciprocal *A* and *a* products. Escaped DAs that are apparently pure *A* or pure *a* would then be due to overgrowth of the culture by one of the products. The following experiment employed selective markers to demonstrate that all escaped DAs are indeed initially *Mixed*.

An inversion strain carrying *un(55701t)*, which can only grow at temperatures below 34°C, was crossed to *ser-3* normal sequence. (Both markers are very close to *mt*.) Ascospores were isolated to complete medium at 19°C. Part of the initial colony of each of the 23 DAs was removed before escape and transferred to solid minimal medium at 19°C to select for the *un* component; the rest of the initial culture was moved to 30°C to select for *ser*, which grows poorly at low temperatures. The selection was not drastic enough to yield pure cultures of *A* or *a* in most cases; most of the resulting cultures were *Mixed*. However, the method did succeed in retaining both mating types in one or both cultures from each of the 23 DAs.

Strong evidence for overgrowth by one component of the mixture, in the absence of any obvious selection, was obtained by conidial platings of escaped DAs from a cross segregating for *leu-3*. Ratios as different as 47 *leu*⁺*A* : 1 *leu* *a* and 1 *leu*⁺*A* : 335 *leu* *a* were obtained from different escaped DAs from the same cross.

3. According to the hypothesis, any kind of duplication that is heterozygous

A broken horizontal line separates products found in opposite halves of the ascus. Ascii are pooled when they are identical except for the arrangement of products within the same half-ascus.

* All ascii had at least one member of each black spore-pair germinated, except for three ascii of Type I, 1 of Type II, and 1 of Type III, where one black pair failed to germinate. The nongerminated pair was always presumptive normal or presumptive inversion.

† N=normal; In=inversion; W=white spores; DA=Dark Agar. Scoring for normal and inversion was based on the frequency of white spores in crosses to standard mating type testers.

‡ Mating types of the two members of a DA pair are separated by a comma. M=*Mixed* mating type ng=not germinated. Scoring is based on original tests and does not include additional components detected only on plating.

§ Phenotype. DA's may really be *suc*⁺/*suc*.

¶ In each diagram, the upper chromosome is the normal parent; the lower chromosome is the inversion parent.

¶ Five ascii with one member of the DA pair *A*, the other member *Mixed* (*A*, *M*). One ascus *a*, *M*. One *a*, *a*. One *M*, one *M*, *M*. Two *A*, *a*.

** One ascus *A*, *M*. One *a*, *a*. One *A*, *A*. One *a*, *M*. One *A*, *a*.

†† DA's lost before finished testing.

††† One ascus *aur*⁺ *a*, *M* and *aur*⁻ *A*, ng. One ascus *A*, *A* in both DA pairs.

for mating type should be phenotypically DA. This is true of the only other known duplication which covers this region—the duplication produced in crosses of normal \times T(I;II)39311. Translocation $T(I;II)39311$ has a long segment of II, including *mt* but not the distal third of the arm, inserted into IIR (PERKINS 1966).

4. Disomics heterozygous for mating type should resemble DAs phenotypically. PITTENGER (1957) isolated pseudowild types carrying both *A* and *a*, but noted no unusual morphology. However, his methods (personal communication) were such that a DA phenotype might not have been detected. More recently, a morphology similar to that of DAs was observed by DR. S. R. GROSS (personal communication) in pseudowild types heterozygous for *leu-4*, which is very close to *mt*. He very kindly provided us with *leu-4* strains for comparison. The *leu-4* pseudowild types (PWTs) were selected by plating on sorbose minimal, and compared with H4250 DAs isolated on other plates of the same medium. (The initial morphology of the DAs was not affected by sorbose.) Presumptive PWTs were chosen as colonies which looked considerably like the DAs, although they were decidedly smaller. Both PWTs and DAs were isolated to GCP. The DAs remained inhibited quite a bit longer than the presumptive PWTs, and developed typical pigment. Some presumptive PWTs grew like wild type within 24 hours of isolation; these were discarded to eliminate any possibility of contamination with adjacent euploid *leu* spores. But other PWTs remained inhibited for one or two days and developed a small amount of pigment very similar to that found in DAs. Representatives of this latter class were shown by plating to be really PWT; also, they were *Mixed* and usually fully fertile with both *A* and *a* testers. It therefore appears that PWTs which are heterozygous for *mt* have an initial phenotype similar to that of DAs, but they escape much faster, and possibly for this reason develop much less pigment. The more rapid escape of the PWTs is expected, because PITTENGER (1959) has shown that the disomic condition in *Neurospora* is very transitory.

5. If escape is due to mitotic crossing over between *mt* and break point, markers in this region could be either homo- or heterozygous after escape, depending on the position of the mitotic crossover. The data so far suggest that this is so. Platings of three different escaped DAs from crosses heterozygous for *suc* have given phenotypically *suc*⁺*A* and *suc*⁺*a* colonies, but no *suc*. (*suc*⁺/*suc* heterozygotes would presumably look *suc*⁺.) *suc*⁺ and *suc* were shown to segregate from the same DA only in two circumstances. They segregated (1) when we specifically chose for analysis the only two escaped DAs (out of 69) which had an intermediate *suc* phenotype, and (2) when the escaped DAs were fertile, which might suggest a mechanism of escape other than somatic crossing over.

Mechanism of somatic segregation: Analysis of the mechanism of somatic segregation, including the construction of multiply marked stocks to test the mitotic crossing over hypothesis, is still in progress. Numerical data will therefore be presented elsewhere. Preliminary data have been obtained from escaped DAs by plating conidia (or by picking *fr*⁺ and *fr* sectors), using crosses with one or two markers other than *mt* in the duplicated region. These support three generalizations:

1. Where the original escaped DA was scored as *Mixed* (either barren or not scored for fertility) and both mating types were recovered in pure form, the most common result was that both the mutant and wild-type form of the covered marker were recovered, in the parental combinations with respect to mating type, and both purified components remained barren.

Exceptions were obtained, of four kinds: (a) The two components were alike for a marker distal to *mt*, e.g., *leu*⁺*A* and *leu*⁺*a*, probably because a meiotic crossover had made the initial duplication homozygous for the distal marker. (b) The two components were alike for a marker proximal to *mt* (*suc*⁺*A* and *suc*⁺*a*); this could occur if the presumed mitotic crossover occurred between *suc* and *mt*, so that the escaped DA remained *suc*⁺/*suc*. (c) In addition to the two barren parental types, a third component occurred which was fertile. (d) Three combinations of *mt* and a covered marker were obtained from the same DA; in three such DAs, one of the combinations was not easy to explain except by a double somatic crossover, or two successive crossovers.

2. Where the original escaped DA was fertile in crosses with one *mt* tester, both fertile and barren isolates of the same mating type were found on plating, and several combinations of mating type, covered marker, and fertile *vs.* barren were often obtained from the same DA.

3. Several escaped DAs (both barren and fertile) also segregated for various morphological traits not detected in the original escaped DA culture. (These could conceivably be due to a double dose of cryptic IL morphological mutants present in the parents.)

These three generalizations are entirely consistent with the somatic crossover hypothesis, except that it is difficult to see how somatic crossing over could determine the presence or absence of fertility.

The simplest explanation is that fertility results from the removal of part or all of the duplication by breaking off a terminal piece of chromosome. This is being tested. If so, it appears that the breakage usually regenerates the normal sequence, since fully fertile escaped DAs usually (1) produce very few white spores, (2) carry the markers of the normal parent, and (3) segregate normally when crossed to wild type.

A second possible explanation for the fertility is that fertile escaped DAs, unlike those that are barren, originate either as ordinary disomics (pseudowild types), or as disomics consisting of one normal or inversion chromosome plus one duplication chromosome. This alternative is ruled out, at least for the nine fertile DAs obtained from the ordered asci of Table 3. (Five of these came from Type I asci, two from a Type II ascus, one from Type III and one from Type VI. Of the six spore pairs involved, three had both members fertile; the other three had one member fertile and one barren). The reasoning is as follows.

The fertile DAs of Table 3 come from asci with 6 black:2 white spores and therefore cannot be due to nondisjunction at Meiosis I. The results are also incompatible with nondisjunction at Meiosis II because: (a) If there were no crossing over, a disomic formed by nondisjunction at Meiosis II would be homozygous and therefore could not produce a *Mixed* culture, and it would have no crossover between *arg* and *os*. Actually, all fertile DAs came from spore pairs in which

both mating types were represented and/or in which there was a crossover in *arg-os*. (b) If there were a crossover in the inversion loop, followed at Meiosis II by nondisjunction of the centromere carrying the duplication strand, the resulting ascus would be 4 black:4 white rather than 6 black:2 white. If the nondisjunction involved the other centromere, the resulting fertile product would not be *Mixed* and would be *arg*; actually, most were *Mixed* and all were *arg*⁺. (c) If there were no crossover (or a double) in the loop, plus a crossover between *mt* and the left break point, nondisjunction at Meiosis II would give a *Mixed* DA in a 6 black:2 white ascus, but this DA should (1) produce either fertile Normal products of both mating types or fertile Inversion products of both mating types; (2) it should also usually be noncrossover for *arg* and *os*. None of the fertile DAs conformed to (1), and only one conformed to (2). (d) A crossover in the loop plus a crossover between *mt* and left break point would result in most of the difficulties listed under (b). (e) If the centromeres split prematurely so that an inversion strand and a normal strand could end up in the same spore pair, the resulting DA should segregate for markers in the loop. This was not found; it should have been detectable in three fertile DAs which were plated (from Ascus Types I and II) and in two additional fertile DAs which were progeny-tested with both *mt* testers (from Ascus Types I and III).

DISCUSSION

The right tip: The data show clearly that most of linkage group I is inverted with respect to the *fr-suc* segment, such that the latter is now attached at or near the right tip, distal to *os*. However, there is no proof that this attachment is sub-terminal, rather than terminal; the break point close to the right tip is postulated only because of the evidence from higher organisms that broken ends do not attach to unbroken ends. We therefore have no proof that DAs carry a tiny deficiency in addition to their proven duplication. Assuming that a break point close to the tip does exist, we also cannot prove that it is right of the rightmost marker *so*, since no recombination was detected between them. Theoretically the *so* locus could even be distal to the break point and therefore deficient in the DAs, but this could be so only if *so* is an antimorph, since the DAs do not have a *so* phenotype.

A dominant gene, Round-spore (*R*) (MITCHELL 1966) has recently been mapped by CAMERON (1967) 23 units distal to *os*. CAMERON also crossed *R* to *In(ILR)H4250* and found that, like *so*, *R* failed to recombine with the right break point, and all DAs were *R*⁺. It is questionable whether there is room for the 23-unit *os-R* distance inside the inversion loop, because of the low map distances found for *os-mt*, *nic-1-mt*, and *nic-1-acr* in crosses homozygous for the inversion. Conversely, if most of the *os-R* region is distal to break point, one wonders that such a long region can be dispensed with in the DAs. The explanation may lie in the great variability of recombination frequency in *Neurospora*; however, it may be that in the wild type the right tip is redundant.

Somatic segregation and fertility: The evidence from the preliminary somatic segregation analysis makes it clear that the DAs must initially carry a duplication for most of the left arm. However, it is not yet proved that the duplication is still present after escape.

Somatic crossing over is known to occur in *Neurospora* pseudowild types (COYLE and PITTINGER 1965) and somatic crossing over is certainly a tenable

explanation for the production of the barren escaped segregants. If this is the mechanism, the escaped material should still carry the duplication.

However, the preliminary somatic segregation data could equally well be explained if escaped DAs were a mixture of hemizygous rather than homozygous products, and progeny tests so far suggest that at least some of the fertile DAs have indeed lost the duplication. The simplest hypothesis is that the barren DAs still carry the duplication and that fertility results from the loss of some or all of the duplicated material. This would be consistent with the fact that reduced fertility, in varying degrees, appears to be characteristic of duplications so far described in *Neurospora* (BARRY 1961; ST. LAWRENCE, personal communication).

BAINBRIDGE and ROPER (1966) have reported an unstable duplication type in the progeny of a translocation in *Aspergillus*. In their case the duplication is fertile, but grows slowly and has a distinct morphology. Their duplication strains produce frequent fast-growing sectors, which segregate for the only known covered marker. There is good evidence that fast-growing sectors arise by loss of some of the duplicated material; how the loss occurs is unknown.

In the unstable duplications produced by the insertional translocation $T(I;II)39311$ in *Neurospora* (PERKINS 1966), the mechanism of somatic segregation is not yet deciphered. In this case loss of the duplication seems less likely, because it is nonterminal. However, a brief report by NGA and ROPER (1966) indicates that, in a second unstable duplication in *Aspergillus*, nonterminal loss may occur.

The existence of these four cases of unstable duplication in *Neurospora* and *Aspergillus*, plus a probable additional case in *Aspergillus* (BALL 1966) and two more possible cases in *Neurospora* (PERKINS, personal communication) indicates that unstable duplications may be a common phenomenon in the fungi.

THRELKELD (1962) described *Neurospora* isolates segregating for *arg-3*, which he tentatively interpreted as due to a centric fragment. However, his data are also consistent with an ordinary duplication undergoing somatic segregation, as described here. It is conceivable that unstable duplications might also explain the unconventional genetic data described by MITCHELL (1963).

In diploid organisms the frequency of somatic crossing over is very low, and might not be greatly increased by the presence of three copies of a region instead of two. This might explain why duplications in higher organisms do not appear to cause variegation except in cases explainable by the single-inactive-X hypothesis (LYON 1963). But if there is indeed a common breakage process which tends to get rid of the duplication, it should have been detectable in such organisms. OHNO *et al.* (1966) have reported evidence, based on karyotypes, for some type of somatic segregation occurring in deer mice heterozygous for pericentric inversions; in this case, however, there is no evidence for the formation of viable duplications.

The DA phenotype: While the data strongly suggest that the initial DA phenotype is due to heterozygosity of the mating-type region, all we know for certain is that it is due to heterozygosity (or merely duplication) for something between the left break point of *In(ILR)H4250* and the left break point of $T(I;II)39311$, that is, between *phen* and *un(b39)*. It should be possible to pinpoint the respon-

sible region by means of another aberration now being studied. This aberration (NM176) is probably broken just distal to mating type, and produces IL duplications that are not DAs.

Effect of In(ILR)H4250 on recombination frequency: It is difficult to judge the effect of the inversion on recombination frequency because there is much variation between crosses of different parentage (as is generally true in *Neurospora*). It is clear, however, that for each region there are at least some crosses in which the inversion in heterozygous condition does not reduce the recombination frequency compared to normal \times normal. The occurrence of double (or presumptive triple) crossovers having one exchange in the left arm and at least one exchange in the inverted region (Table 1) implies that inversion loops must be formed in at least some meioses, in agreement with the cytological evidence.

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SUMMARY

The arginine-1 strain H4250 contains a pericentric inversion, *In(ILR)H4250*, which is separable from the *arg-1* locus by crossing over. One break point is between two closely linked proximal markers in linkage group II; the other is at or near the tip of IR. Break points were located approximately using marked crosses heterozygous and homozygous for the inversion; exact location of the left break point is based on the recessive genes covered by the viable duplication progeny.—Single crossovers within the inverted region give two reciprocal products. One is duplicated for the terminal 50 units of the left arm and is fully viable. The other is deficient for the same segment and is lethal. In ordered asci, each spore-pair carrying the duplication is balanced by a white (defective) spore-pair, and the segregation of markers and of duplication, deficiency, inversion, and normal products is as predicted.—The viable duplication progeny initially form characteristic dark, inhibited colonies called Dark Agars (DAs). These later “escape” from the inhibition and grow like wild type.—Conidial platings show that DAs segregate somatically for markers in the duplicated region; this has been demonstrated for seven markers, including mating type. It was shown by means of selective markers that all DAs initially carry both mating-type alleles; without selective markers, one mating type often overgrows the other. There are several reasons for believing that the initial inhibition is due to heterozygosity for the mating-type locus, and that escape is due to formation of homozygous or hemizygous *A* and *a* nuclei. Preliminary analysis suggests that somatic segregation occurs sometimes by mitotic crossing over and sometimes by loss of duplicated material.—Most escaped DAs form barren perithecia, but about 20% of

the DAs are fertile. The fertile DAs from the ordered asci arose from the same type of crossover as barren DAs; they cannot be explained as disomics.

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