SOME ASCI WITH NONIDENTICAL SISTER SPORES FROM A CROSS IN *NEUROSPORA CRASSA*

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MEIOSIS in *Neurospora crassa* results in the formation of an ordered tetrad; each nucleus of the nuclei undergoes a mitotic division to give rise to four pairs of sister spores which make up the eight spores of an ascus; a further mitosis results in each spore possessing two nuclei. This sequence of events has been described by CATCHESIDE (1951).

The existence of unusual asci in which a pair of alleles occurs in the ratio of 3:1 is now well established in ascomycetes, and particularly in *Neurospora crassa* (MITCHELL 1957; CASE and GILES 1958). However, unusual asci with a pair of alleles segregating in a 5:3 ratio (i.e. with nonidentical sister spores) have rarely been found. The only group of such asci to be well substantiated were those found by OLIVE (1959) in *Sordaria fimicola*. The present paper describes and attempts to interpret some asci with 5:3 ratios, obtained from a *Neurospora crassa* cross.

MATERIALS AND METHODS

The parents of the cross from which the initial asci with nonidentical sister spores were derived, were of the following genotypes: *A*, *arg-3* (30300), *cr*, *tryp-2* (75001), *ylo* (Y30539y), and *a*, *hist-2* (C94), *pan-2* (Y153). *A/a*, *arg-3*, *hist-2* and *cr* are on chromosome I; *ylo*, *pan-2*, and *tryp-2* are on chromosome VI, (throughout this paper the terms chromosome I and chromosome VI designate the chromosomes carrying linkage groups I and VI, respectively). Except for *pan-2*, these markers have been described by BARRATT, NEWMAYER, PERKINS and GARNJOBST (1954); *pan-2* has been described by CASE and GILES (1958). They may be detected as follows: *A/a*, mating type; *arg-3* (30300), responds to arginine; *cr*, morphological character (crisp); *hist-2* (C94), responds to histidine; *pan-2* (Y153), responds to pantothenic acid; *tryp-2* (75001), responds to anthranilic acid; *ylo* (Y30539y), yellow conidia.

All growth media used were uniform with respect to inorganic salts and the essential *Neurospora* growth factor biotin. These ingredients were made up as described by WESTERGAARD and MITCHELL (1947), and are referred to below, collectively as "4x salt solution". The initial crosses were made on a malt-peptone medium (4x salt solution 250 ml; sucrose 5 g; glucose 10 g; malt extract 10 ml; peptone (BDH) 2 g; Difco bacto-agar 20 g; distilled water up to 1000 ml).

Asci were dissected approximately 25 days after the crosses were made. Each spore was placed in a separate small tube of glucose medium (4x salt solution 250 ml; glucose 20 g; Difco bacto-agar 20 g; distilled water up to 1000 ml), with biochemical supplements added at the rate of 80 mg/l. Spores were germinated in the usual way by heat activation, and biochemical markers were scored by the standard procedure of inoculating series of petri dishes of appropriately supplemented sorbose medium (4x salt solution 250 ml; sorbose 4 g; sucrose 2 g; Difco bacto-agar 20 g; distilled water up to 1000 ml).

When necessary, genotypes were determined by crossing the isolates concerned with one or more of the following strains, on appropriately supplemented reproductive medium (WESTERGAARD and MITCHELL 1947): wild types; Lindgren 25a and 1A; *a ad-3* (38701) *arg-3* (30300); *a ad-1* (3254); *a pan-2* (Y153) *hist-2* (C94); and *a hist-2* (C94) *ylo* (Y30539y). The markers *ad-3* (38701) (on chromosome I) and *ad-1* (3254) (on chromosome VI) have been described by BARRATT, NEWMAYER, PERKINS and GARNJOBST (1954); they both may be detected by their response to adenine.

THE 5:3 ASCUS RATIOS AND THEIR INTERPRETATION

A total of 332 asci were dissected from the cross *A arg-3 cr ylo tryp-2* × *a hist-2 pan-2*. Germination was such that, assuming no undetected nonidentical sister spores, 306 tetrads could be fully scored. Tetrad analysis gave the following uncorrected linkage data:

chromosome I	<i>A/a</i>	8.8	<i>arg-3</i>	2.1	0	<i>hist-2</i>	6.5	<i>cr</i>
	— ————— ————— ————— ————— ————— ————— —————							
chromosome VI	<i>ylo</i>	2.9		2.4	<i>pan-2</i>	11.7	<i>tryp-2</i>	
	— ————— ————— ————— ————— ————— ————— —————							

(0 represents a centromere)

Thirteen asci were found to be abnormal in that each included one or more nonidentical sister spores. Eleven of the asci involved sister spores which were nonidentical for the *arg-3* locus (or possibly nonidentical where one member of the pair failed to germinate); a situation which usually resulted in a 5:3 ratio of *arg-3*⁺:*arg-3*, and which always resulted in an excess of *arg-3*⁺ over *arg-3* (the other markers segregated normally). Of the other two asci, one included a 5:3 ratio of *pan-2*⁺:*pan-2*, and the other a 5:3 ratio of *tryp-2*⁺ *ylo*⁺:*tryp-2 ylo*. Details of these 13 abnormal asci are shown in Table 1.

The isolate X221-1 (Table 1) was crossed with the Lindgren wild-type strain 25a. A total of ten asci were isolated from four perithecia. All six asci from three of the perithecia showed normal segregation for all markers including *arg-3*; in each ascus, at least one member of each spore pair germinated. The phenotypes of three of the four asci from the final perithecium showed unusual

TABLE 1

Asci with nonidentical sister spores

Ascus	Phenotypes	Members of aberrant pair(s)
X221	Spore 1: A cr pan-2 tryp-2 Spore 2: A arg-3 cr pan-2 tryp-2 Spores 3 and 4: a pan-2 Spores 5 and 6: a hist-2 cr ylo tryp-2 Spores 7 and 8: A arg-3 hist-2 ylo	Spores 1 and 2
DK624	Spore 1: cr ylo Spore 2: arg-3 cr ylo Spore 3: cr ylo tryp-2 Spore 4: arg-3 cr ylo tryp-2 Spores 5 and 6: hist-2 pan-2 tryp-2 Spores 7 and 8: hist-2 pan-2	Spores 1 and 2 and spores 3 and 4
DL631	Spores 1 and 2: arg-3 cr ylo tryp-2 Spore 3: cr tryp-2 Spore 4: arg-3 cr tryp-2 Spores 5 and 6: hist-2 pan-2 Spore 7: hist-2 ylo pan-2 Spore 8: Failed to germinate	Spores 3 and 4 (assuming spore 8 to be <i>hist-2</i> <i>ylo pan-2</i>)
DN651*	Spore 1: Failed to germinate Spore 2: cr tryp-2 Spores 3 and 4: arg-3 cr tryp-2 ylo Spores 5 and 6: hist-2 pan-2 Spores 7 and 8: hist-2 pan-2 ylo	Spores 1 and 2 (assuming spore 1 to be <i>arg-3</i> <i>cr tryp-2</i>)
AT385†	Spore 1: Failed to germinate Spore 2: a hist-2 pan-2 Spores 3 and 4: a hist-2 cr pan-2 Spore 5: A arg-3 cr Spore 6: A arg-3 cr ylo tryp-2 Spores 7 and 8: A arg-3 ylo tryp-2	Spores 5 and 6 (assuming spore 1 to be a <i>hist-2</i> <i>pan-2</i>)
AV399	Spores 1 and 2: A arg-3 cr ylo tryp-2 Spore 3: A cr ylo tryp-2 Spore 4: A arg-3 cr ylo tryp-2 Spores 5, 6, 7 and 8: a hist-2 pan-2	Spores 3 and 4
AV403	Spores 1, 2, 3 and 4: a hist-2 ylo tryp-2 Spores 5, 6 and 7: A arg-3 cr pan-2 Spore 8: A cr pan-2	Spores 7 and 8
AV404	Spores 1 and 2: a hist-2 ylo pan-2 Spores 3 and 4: a hist-2 ylo tryp-2 Spore 5: A cr pan-2 Spore 6: A arg-3 cr pan-2 Spores 7 and 8: A arg-3 cr tryp-2	Spores 5 and 6
AV413	Spore 1: A arg-3 cr pan-2 Spore 2: A cr pan-2 Spore 3: A arg-3 pan-2 Spore 4: A pan-2 Spores 5 and 6: a hist-2 cr ylo tryp-2 Spores 7 and 8: a hist-2 ylo tryp-2	Spores 1 and 2 and spores 3 and 4

TABLE 1—Continued
Asci with nonidentical sister spores

Ascus	Phenotypes	Members of aberrant pair(s)
AW420	Spores 1 and 2: <i>hist-2 ylo</i> Spores 3 and 4: <i>hist-2 ylo tryp-2</i> Spore 5: <i>cr pan-2 tryp-2</i> Spore 6: <i>arg-3 cr pan-2 tryp-2</i> Spore 7: <i>arg-3 cr pan-2</i> Spore 8: Failed to germinate	Spores 5 and 6 (assuming spore 8 to be <i>arg-3</i> <i>cr pan-2</i>)
AX422	Spore 1: <i>arg-3 cr ylo tryp-2</i> Spore 2: Failed to germinate Spore 3: <i>arg-3 cr ylo</i> Spore 4: <i>arg-3 cr ylo pan-2</i> Spores 5 and 6: <i>hist-2 tryp-2</i> Spores 7 and 8: <i>hist-2 pan-2</i>	Spores 3 and 4 (assuming spore 2 to be <i>arg-3</i> <i>cr ylo tryp-2</i>)
AY438	Spore 1: Failed to germinate Spore 2: <i>hist-2 cr pan-2</i> Spores 3 and 4: <i>hist-2 pan-2 tryp-2</i> Spores 5 and 6: <i>arg-3 ylo tryp-2</i> Spore 7: <i>cr ylo</i> Spore 8: <i>arg-3 cr ylo</i>	Spores 7 and 8 (assuming spore 1 to be <i>hist-2</i> <i>cr pan-2</i>)
BT530	Spore 1: A <i>cr pan-2 tryp-2</i> Spore 2: A <i>arg-3 cr pan-2 tryp-2</i> Spores 3 and 4: A <i>arg-3 cr pan-2</i> Spores 5 and 6: a <i>hist-2 ylo tryp-2</i> Spores 7 and 8: a <i>hist-2 ylo</i>	Spores 1 and 2

* Possibly nonidentical.

† Possibly due to technical error.

segregations for the *arg-3* locus, and they are given in detail below, together with the apparently normal ascus from the same perithecium:

Ascus 307

Spores 1,2,3 and 4: a *pan-2 tryp-2*

Spores 5 and 6: A *cr*

Spores 7 and 8: A *arg-3 cr*

Ascus 308

Spores 1 and 2: A *cr*

Spores 3 and 4: a *cr*

Spores 5 and 6: a *pan-2 tryp-2*

Spores 7 and 8: A *arg-3 pan-2 tryp-2*

Ascus 309

Spores 1,2,3 and 4: a *pan-2 tryp-2*

Spores 5,6,7 and 8: A *cr*

Ascus 310

Spore 1: Failed to germinate

Spores 2,3 and 4: A *arg-3 cr*

Spores 5,6,7 and 8: a *pan-2 tryp-2*.

The tetrad analysis indicates that the *arg-3* locus may possibly be heterozygous in the isolate X221-1. It is apparent that an additional chromosome I, or part of the chromosome, with the wild-type allele (*arg-3*⁺) may be present. This wild-type allele is detected in one perithecium, but not in the other three, suggesting that two types of nuclei may be present in the mycelium of X221-1; one type aneuploid and the other haploid.

The cross X221-1 × *arg-3 ad-3* (the *ad-3* locus is about one and a half cross-over units on the right of the centromere of chromosome I, i.e. on the opposite arm to the *arg-3* locus (BARRATT, NEWMAYER, PERKINS and GARNJOBST 1954)) was made on arginine deficient medium, and of 29 spores germinated out of 32, one had the phenotype *cr*⁺ *arg-3*⁺ *ad-3 pan-2 tryp-2*. The genotype of this isolate was checked by crossing it to the Lindegren 1A wild-type strain; of 53 spores isolated, 42 germinated, 15 of which were *arg-3 ad-3* (the markers on chromosome VI were not scored). It thus appears that the allele *arg-3*⁺, present in the isolate X221-1, is carried on a fragment of chromosome I which is exclusive of, and does not extend to the right of, the *ad-3* locus. Clearly the fragment must include the centromere or it would not be transmitted in cell division. The fragment is possibly a telocentric chromosome for the left arm of chromosome I.

The isolate AV404-5 appears to behave in crosses similarly to X221-1. The cross AV404-5 × Lindegren 25a was made, and five asci were isolated from three perithecia; they included one abnormal ascus from each perithecium. Their details are given below:

CP584 Spores 1,2,3 and 4: a wild type for all loci

Spores 5 and 6: A *arg-3 cr pan-2*

Spore 7: A *cr pan-2*

Spore 8: A *arg-3 cr pan-2*

CQ586 Spore 1: A *cr pan-2*

Spore 2: Failed to germinate

Spores 3 and 4: A *arg-3 cr pan-2*

Spores 5,6,7 and 8: a wild type for all loci

CR587 Spore 1: A *cr pan-2*

Spore 2: A *arg-3 cr pan-2*

Spores 3 and 4: A *arg-3 cr pan-2*

Spores 5,6,7 and 8: a wild type for all loci

AV404-5 was also crossed with *a hist-2 ylo* on arginine deficient medium, and five asci were isolated from four perithecia. Four of the asci, from three perithecia, showed normal segregations for all loci. The one ascus isolated from the remaining perithecium had spores of the following phenotypes:

EW794 Spores 1 and 2: *hist-2 ylo*

Spores 3 and 4: *hist-2*

Spore 5: Failed to germinate

Spore 6: *cr pan-2*

Spore 7: *cr ylo pan-2*

Spore 8: Failed to germinate

The isolate AV404-5 appears to be heterozygous for the *arg-3* locus. Again there is evidence of a fragment, for not only is the *cr* allele expressed in the mutant form in the isolate, but also in crosses a complete extra chromosome would be unlikely to result in the appearance of nonidentical sister spores such as those in asci CP584 and CR587.

In similar crosses, the isolate AV413-2 gave somewhat different results. Thus from the cross AV413-2 $\times$ Lindegren 25a, 36 out of 40 spores germinated and, with respect to chromosome I, they were of the following phenotypes: 21 wild type; eight *cr*; seven *agr-3 cr*. (For chromosome VI the ratio of *pan-2⁺*:*pan-2* was 19:17.) When the *cr* isolates were backcrossed to the wild-type parent they were found to be heterozygous for the *arg-3* locus.

From the cross AV413-2 $\times$ *a arg-3 ad-3*, made on arginine deficient medium, 56 spores were germinated out of 60; their phenotypes were as follows: 14 *arg-3 cr*; 16 *arg-3 ad-3*; ten *cr*; nine *ad-3*; two *arg-3*; three *ad-3 cr*; and two wild type. (For chromosome VI the ratio of *pan-2⁺*:*pan-2* was 25:31.) One of the *ad-3* isolates, and one of the *ad-3 cr* isolates was crossed to the Lindegren wild-type strains; both isolates appeared to be A mating type. Progeny from both crosses included those possessing the *arg-3* marker.

The results from the two crosses with AV413-2, again provide evidence for the possible existence of a telocentric chromosome for the left arm of chromosome I. Unlike the crosses with the isolates X221-1 and AV404-5, it would appear that the telocentric, or fragment, is probably present at every meiosis and has the alternatives of segregating with *arg-3 cr* or with *arg-3 ad-3* (or with a recombinant of these).

There is a third type of isolate that also appears heterozygous for the *arg-3* locus. AV403-8, AV413-4, and AY438-7 in crosses with normal strains, all produced progeny with a low percent germination; and, at least for crosses with AV403-8, the reduced germination is of a particular type.

Thus from the cross AV403-8 $\times$ Lindegren 25a, six asci were isolated from four perithecia. Asci from two of the perithecia showed normal segregation for all markers, including *arg-3*, and good germination. The three asci from the other two perithecia appeared to segregate for nongermination, with segregation for the markers, including *arg-3*, appearing normal; the asci are described in detail below:

- CD565 Spores 1,2,3 and 4: *pan-2*
 Spores 5,6,7 and 8: Failed to germinate
- CD566 Spores 1,2,3 and 4: Failed to germinate
 Spores 5,6,7 and 8: *arg-3 cr*
- CH572 Spores 1 and 2: *pan-2*
 Spores 3 and 4: Failed to germinate
 Spores 5 and 6: *arg-3 cr*
 Spores 7 and 8: Failed to germinate

A further cross, AV403-8 $\times$ *hist-2 ylo*, made on arginine deficient medium, produced similar results; ten asci from two perithecia segregated for "nongermination." Three showed second division segregation for "nongermination."

So far as could be detected, both the markers *cr* and *arg-3* each showed only one second division segregation, which were independent of each other, and in neither instance was there a concomitant second division segregation for "nongermination." Second division segregations were not detected for either marker on chromosome VI.

Isolates AV413-4 and AY438-7 are thought to be similar to AV403-8, for when they were crossed to normal strains, on arginine deficient medium, germination of the progeny was < 50 percent; and when the crosses were made on supplemented medium germination was > 50 percent. Thus from a cross of AV413-4 $\times$ Lindegren 25a made on supplemented medium, 26 spores germinated out of 40, and 11 were genotypically mutant for the *arg-3* locus; from a cross of AV413-4 $\times$ *arg-3 ad-3* on arginine deficient medium, 20 spores germinated out of 40 and all were mutant for the *arg-3* locus. Similarly from a cross of AY438-7 $\times$ Lindegren 25a on supplemented medium, 25 spores were germinated out of 40, and 11 were mutant for the *arg-3* locus; from a cross of AY438-7 $\times$ *arg-3 ad-3* on arginine deficient medium, 15 spores were germinated out of 32, and all were mutant for the *arg-3* locus. No abnormal isolate with the *arg-3*⁺ phenotype was detected in the crosses with AV403-8, AV413-4, and AY438-7, and although germination was low the progeny showed normal segregation for all the markers concerned.

From all the crosses studied above, it would appear that the abnormal isolates involving the *arg-3*⁺ allele may be divided into three groups:

1. Those which appear to possess two types of nuclei, one aneuploid and one haploid; and which, when crossed to normal strains, produce spores which appear to germinate normally, and to give rise occasionally to isolates which are similar to the abnormal parent, e.g. X221-1 and AV404-5.
2. Those which when crossed to normal strains give rise to a relatively large number of isolates similar to the abnormal parent, e.g. AV413-2.
3. Those which when crossed to normal strains give rise to isolates which segregate for "nongermination"; and it appears that "nongermination" may be selected for by growing the cross on arginine deficient medium, i.e. there are two types of nuclei present. An example of these abnormal isolates is AV403-8 and possibly also AV413-4 and AY438-7.

Crosses have not yet been made with all the abnormal *arg-3*⁺ isolates in Table 1; limited crosses concerning AV399-3 and BT530-1 provided evidence which suggests that in these isolates chromosome I is again heterozygous for the *arg-3* locus.

A unifying hypothesis may be formed to account for the various results discussed above, based on the assumption that heterozygosity at the *arg-3* locus is due to the presence of a telocentric chromosome, or an isochromosome. The assumption is reasonable but lacks cytological evidence.

The hypothesis should account for the presence of three phenotypically similar, but genotypically different sets of aberrant isolates for the *arg-3* locus. It should also show how one initial unusual event may account for the different results.

By postulating the presence of aneuploid nuclei, possessing the normal complement of chromosomes plus an isochromosome for the left arm of chromosome I, in the mycelium of the original parent, *a hist-2 pan-2*, the events described above may be accounted for. The origin of such an isochromosome is likely to have occurred during a previous meiosis (SEARS 1952); there is no report of isochromosomes or telocentric chromosomes arising from normal chromosomes during mitosis. It is known that isochromosomes may misdivide during meiosis to form telocentric chromosomes (SEARS 1952). Thus during meiosis in the initial cross (*A arg-3 cr ylo tryp-3* $\times$ *a hist-2 pan-2*) the aneuploid nucleus may occasionally be involved; the isochromosome may misdivide to form telocentric chromosomes, so that some of the progeny may receive one or more telocentric chromosomes or isochromosomes. It follows that various combinations of telocentric chromosomes and isochromosomes may well result in isolates which are phenotypically similar, but genetically different for the *arg-3* locus.

Thus a heterocaryon with normal nuclei and aneuploid nuclei which have an additional telocentric chromosome for the left arm of chromosome I, may account for the isolates of Group I above. Group II isolates may be homocaryotic for the same type of aneuploid nucleus. Group III isolates require a rather more involved explanation; "nongermination" should be associated with the type of aneuploidy under discussion. Thus it may be supposed that nuclei with three doses of the left arm of chromosome I (i.e. the normal nucleus plus an isochromosome) may be unable to initiate germination, in which case spores giving rise to Group III isolates must be heterocaryotic (normally all mature *Neurospora* ascospores are binucleate (CATCHESIDE 1951)), for the presence of a normal nucleus (or one with less than three doses of the left arm of chromosome I) would be required for germination. The appearance of "nongermination" is thus accorded to spores homocaryotic for the aneuploid nucleus with the isochromosome. Second division segregation for nongermination may be explained on the basis of misdivision of the isochromosome at first metaphase of meiosis. It is of interest that five asci from the original cross appeared to segregate for nongermination.

There are two other abnormal isolates from nonidentical spore pairs, one with respect to both loci *tryp-2 ylo*, and the other with respect to the locus *pan-2*.

AT385-5 was crossed to a wild-type strain. Two types of perithecia were isolated from the cross: those in which the marker *pan-2* was absent, and the markers *tryp-2* and *ylo* were present and segregating normally; and those in which the *tryp-2 ylo* markers were absent, and the *pan-2* marker was present and segregating normally. In both types of perithecia the remaining markers segregated normally. Isolate AT385-5 may have originated as a pseudowild (PITTENGER 1954), disomic for chromosome VI, which later broke down to form a heterocaryon. However, although the tetrad analysis demonstrates the likely presence of a heterocaryon, there is no evidence of disomy; thus isolate AT385-5 may reflect a technical error in ascus dissection resulting in the isolation of two spores instead of one (*A arg-3 cr tryp ylo* and *A arg-3 cr pan*).

Results of crosses with the isolate AX422-3 are summarized in Table 2. It

TABLE 2
Crosses with AX422-3

Cross [‡]	Other parent	Medium	Fraction germinated	Phenotypes with respect to chromosome VI
A	Lindegren 25a	Complete	47/72	{ pan-2 ylo : 22 pan-2 ⁺ ylo ⁺ : 24 ylo : 1
B	Lindegren 25a	Pantothenic acid deficient	30/37	{ ylo* : 14 ylo ⁺ : 16
C	(a pan-2 hist-2)	Pantothenic acid deficient	75/80	{ pan-2 : 28 ylo* : 47
D a	ad-1 [†]	Pantothenic acid deficient	72/80	{ ad-1 : 28 ylo : 44
E	Lindegren 25a	Pantothenic acid deficient	26/40	{ ylo : 13 ylo ⁺ : 13
F a	ad-1	Pantothenic acid deficient	30/40	{ ylo : 14 ad-1 : 16
G	Lindegren 25a	Complete	37/40	{ ylo : 22 ylo ⁺ : 15
H a	ad-1	Complete	32/40	{ ylo : 14 ad-1 : 18

* Subsequent backcrosses showed this also to be the genotype.

[†] Located about one crossover unit to the right of the centromere on chromosome VI (BARRATT, NEWMAYER, PERRINS and GARNJOBST 1954).

[‡] In all crosses the ratio of *cr:cr*⁺ (chromosome I) was a good 1:1 fit.

would appear that with respect to chromosome VI three types of nuclei are present in the mycelium of AX422-3. Thus Cross A involves a nucleus monosomic for chromosome VI with the markers *pan-2* and *ylo* present. Crosses B, E, F, G and H involve a nucleus monosomic for chromosome VI but with only the marker *ylo* present. Crosses C and D seem to involve a nucleus disomic for chromosome VI, with the marker *ylo* present in both chromosomes. It is suggested that originally the isolate contained two types of nuclei with respect to chromosome VI: one monosomic for that chromosome with the markers *pan-2* and *ylo* present, and the other disomic with only the marker *ylo* present, but present in both chromosomes. Thus in Cross A, which was made about ten weeks before the other crosses, the original normal nucleus was selected for. In two of the later crosses, made on pantothenic acid deficient medium (Crosses C and D), the nucleus disomic for chromosome VI was selected for. Finally, it is supposed that the nucleus disomic for chromosome VI gave rise to nuclei monosomic for the chromosome, with the marker *ylo* present; this type of nucleus appears to be involved in Crosses B, E, F, G and H, and is apparently selected for in preference to the other normal nucleus even in crosses grown on a complete medium.

The possible origin of the three nuclei is of considerable interest. The first

point to make is that there are five products of meiosis so far as chromosome VI is concerned, and they are: *ylo tryp-2* (Spore 1), *ylo* (included in Spore 3), *ylo pan-2* (Spore 4 and included in Spore 3), *tryp-2* (Spores 5 and 6) and *pan-2* (Spores 7 and 8). Secondly, if it is assumed that spore 2, which failed to germinate, is genotypically identical with spore 1, then each of the five products of meiosis occurs as a pair; spore 3 possessing a pair of chromosome VIs each with the marker *ylo*, as well as a chromosome VI with the markers *pan-2 ylo* which makes up a pair with that present in spore 4. Thirdly chromosome VI with the marker *ylo*, is a recombinant chromosome and supposedly originated through some form of recombination event prior to anaphase I. The reciprocal recombinant, i.e. *pan-2 tryp-2* is not present, therefore the recombination event is likely to have been nonreciprocal. In view of the later discussion it is important to note that apparently this nonreciprocal recombination gave rise to two extra identical chromosome VIs in the ascus.

DISCUSSION

In addition to 5:3 ratios reported here, similar ratios have been reported in *Sordaria fimicola* by OLIVE (1959). Although aneuploidy was not detected by OLIVE, it may nevertheless have been present. The 5⁺:3 type ratio occurred more frequently than the 3⁺:5 type, and in fact in only one ascus of the latter type did both nonidentical sister spores germinate. OLIVE makes direct mention of only one testcross; it concerned the wild-type member of a nonidentical spore pair, but no details are given as to the number of progeny inspected. In OLIVE's data 5:3 ratios occurred less frequently than 6:2 ratios; in one group of 2,700 hybrid asci OLIVE reported 11 asci with 6:2 ratios and none with 5:3 ratios.

Several workers (e.g. CASE and GILES 1958; STADLER 1959) have sought an explanation for unusual ascus ratios on the basis of genetic recombination, chromatid replication, and DNA replication. PRITCHARD (1960), in a general review of recombination, points out that if it is accepted that DNA replication is semiconservative, and that recombination is through a copy-choice process, then nonidentical sister spores should be expected. It is clear from existing data that nonidentical sister spores are of rare occurrence, and from the present paper that those detected are probably due to forms of aneuploidy.

If there is a contradiction between genetic data from tetrads and the evidence concerning the molecular structure of the chromosome and its mode of replication (MESELSON and STAHL 1958; TAYLOR 1958), as PRITCHARD believes there to be, then as he suggests, it is possible that some form of conservative replication or conservative distribution of DNA may take place during meiosis or recombination, while at mitosis DNA replicates semiconservatively.

Alternatively it may be that the DNA double helix replicates conservatively at mitosis as well as at meiosis. Thus although the subunits involved in replication may replicate semiconservatively, the DNA double helix may remain intact, and not become unzipped, so that the double helix in fact replicates conservatively, and is itself a single subunit rather than two subunits. Evidence for

this view has been presented in a series of papers by CAVALIERI and ROSENBERG (1961a,b,c).

It is of interest to note that the evidence presented in this paper regarding the existence of nuclei disomic for the nonreciprocal recombinant chromosome VI in isolate AX422-3, suggests a misreplication involving a double unit (i.e. conservative).

SUMMARY

Thirteen abnormal asci, with apparently nonidentical sister spores were isolated from a cross in *Neurospora crassa*. Genetic analysis suggested that non-identical sister spores may have resulted from various forms of aneuploidy. The bearing of ascus ratios on the concept of DNA replication is discussed.

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