Genet. Res., Camb. (1970), **16**, *pp.* 29–35
Printed in Great Britain

A genetic analysis of non-disjunction and mitotic recombination in *Neurospora crassa*

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(*Received* 22 *December* 1969)

SUMMARY

The frequency of the more common patterns of non-disjunction in Neurospora was studied. Detection of these asci was accomplished by the use of pantothenate requiring strains grown on reduced amounts of pantothenate, so that combinations of dark, pale, and aborted spores provided evidence of non-disjunction. The isolates from five non-disjunction asci were examined in detail and an apparently high frequency of mitotic recombination was detected for presumed disomic isolates. An hypothesis concerning a mechanism of non-disjunction is presented.

1. INTRODUCTION

Pseudowild types (PWTs) in *Neurospora crassa* are thought to arise from non-disjunction during meiosis, followed by the production of aneuploid nuclei disomic for chromosomes with complementing mutant loci. The disomic nuclei are unstable and break down to form heterokaryons (Mitchell, Pittenger & Mitchell, 1952). Later it was shown that mitotic recombination may occur between the homologous chromosomes of the disomic nucleus (Pittenger & Coyle, 1963; Threlkeld & Stephens, 1966). Much of the earlier work was based on PWTs isolated from collections of random spores. Results from a limited investigation of asci segregating for pairs of aborted spores, presumed to be nullisomic, agreed with the concept that non-disjunction forms the basis of PWT production (Pittenger, 1953; Coyle & Pittenger, 1964).

The work to be described in this paper makes use of the presence of aborted spores in asci, together with spore colour alternatives obtained through the use of complementing *pan-2* mutants grown on medium containing reduced amounts of pantothenate (Threlkeld, 1965). Crosses with parents heteroallelic for markers at the *pan-2* locus, grown on medium with reduced pantothenate, give rise to pale coloured ascospores with *pan-2* genotypes, and to black spores which are either wild type or PWT. Asci containing pairs of pale spores, black spores, and aborted spores, may be explained on the basis of non-disjunction, with the black spores disomic or heterokaryotic for the *pan-2* linkage group, the aborted spores nullisomic for that linkage group, and the pale spores normal haploids. This paper describes such asci both with regards to frequencies of the more common types of non-disjunction and to subsequent mitotic crossing-over.

2. MATERIALS AND METHODS

Methods not described below have been described elsewhere (Threlkeld, 1962; Threlkeld, 1965; Threlkeld & Stephens, 1966).

Asci with segregation for aborted spore pairs (presumed nullisomic) together with segregation for black spore pairs (presumed disomic or heterokaryotic) were selected for dissection from crosses where parents were heteroallelic for the *pan-2* locus, and in addition carried markers on each side of that locus. The spores were germinated and the resulting cultures were tested for biochemical requirements in the usual way. A number of asci were analysed in more detail. Each of the cultures derived from these asci was examined for heterokaryosis, by seeking the presence of genetically different nuclei through serial conidial isolates. Isolates were considered homokaryotic when forty conidial isolates taken from the culture of a single isolate were found to be genetically identical for the markers studied. Where heterokaryosis existed it was usually detected within ten conidial isolates.

Pan-2 alleles were usually identified by back-crossing to two strains with each one of the alternative alleles. Prototrophs obtained from one of the crosses, but not from the other, confirmed the allele designation. When complementation tests were used as a basis for allele identification, the tests were carried out on liquid medium containing Vogel's (Vogel, 1956) salt solution.

The following parental strains were used in these studies:

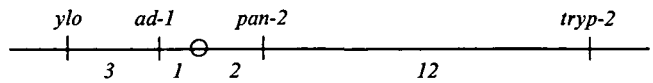
(i)	*ad-1* (3254), *pan-2* (B5) *a*	designated 467,
(ii)	*ylo* (Y 30539 Y), *pan-2* (B 3), *tryp-2* (75001), *A*	designated 469,
(iii)	*al-2* (15300), *ad-1* (3254), *pan-2* (B 5), *a*	designated 14-5,
(iv)	as for (iii)	designated 14-6,
(v)	*al-2* (15300), *pan-2* (B3), *tryp-2* (75001), *A*	designated 46-5.

Strains 467 and 469 were obtained from the Fungal Genetics Stock Center; the other strains were synthesized in our laboratory. Although strains 14-5 and 14-6 have identical genotypes for the markers studied, and although they were recovered from the same cross, it does not follow that the whole of their genomes are identical. They are therefore considered separately.

Phenotypic characteristics used in the detection of the alleles described above are as follows:

ad-1	A requirement for adenine.
pan-2 (B 3)	A requirement for pantothenic acid. Complements with *pan-2* (B 5).
pan-2 (B 5)	A requirement for pantothenic acid. Complements with *pan-2* (B 3).
ylo	Yellow conidia.
tryp-2	A requirement for anthranilic acid.
al-2	White (albino) conidia.
A and *a*	Mating type.

The loci *ylo*, *ad-1*, *pan-2* and *tryp-2* are on linkage group VI; the mating type and the *al-2* (albino) locus are on linkage group I. A genetic map of linkage group VI with approximate values for recombination frequencies is as follows:



The crosses examined were

467 × 46-5 (*ad pan* (B 5) *a* × *al pan* (B 3) *tryp A*);

469 × 14-5 (*ylo pan* (B 3) *tryp A* × *al ad pan* (B 5) *a*);

and 469 × 14-6 (*ylo pan* (B 3) *tryp A* × *al ad pan* (B 5) *a*).

The crosses were abundantly fertile, producing few hyaline or aborted spores and when grown on medium fully supplemented with pantothenic acid the germination of ascospores was in excess of 95 %. When grown on medium with reduced amounts of pantothenic acid, the pale spores germinate at a frequency of approximately 80 %. It is possible that pale spores are more susceptible to mechanical damage than dark spores during isolation.

3. RESULTS

Only the four most frequent classes of non-disjunction type asci for linkage group VI were studied in this investigation. They were:

Class I: two pairs of black spores and two pairs of aborted spores, segregating at the first meiotic division, e.g. 1st pair, black; 2nd pair, black; 3rd pair, aborted; 4th pair, aborted.

Class II: two pairs of black spores and two pairs of aborted spores in a second division type of segregation, e.g. 1st pair, black; 2nd pair, aborted; 3rd pair, black; 4th pair, aborted.

Class III: one pair of black spores, two pairs of pale spores, and one pair of aborted spores, with the pair of black spores adjacent to one of the pairs of pale spores at one end of the ascus, e.g. 1st pair, black; 2nd pair, pale, 3rd pair, aborted; 4th pair, pale.

Class IV: similar to class III, but with the pair of black spores adjacent to the pair of aborted spores, at one end of the ascus, e.g. 1st pair, black; 2nd pair, aborted; 3rd pair, pale; 4th pair, pale.

These classes of asci together occurred with a frequency of approximately 1 in 300; in a total count of 11379 random asci 39 fell in the above classes. The frequencies of each of the classes of asci with respect to the three crosses are shown in Table 1. In all cases, as expected, the aborted spores were not viable, and the dark spores were PWTs.

Five of the asci from the cross 467 × 46-5 (*ad pan* (B 5) *a* × *al pan* (B 3) *tryp A*) were examined in detail, they are described in Table 2. The loci on linkage group I segregate in all the asci without evidence of disomy or heterokaryosis. The nuclear components of the cultures derived from the PWT spores were determined through

serial conidial isolates. Initially 100 conidial isolates were taken from each PWT, and it was observed that these conidial isolates contained isolates wild type for all the linkage group VI loci (presumed PWT) at a freqency of $0{\cdot}54 \pm 0{\cdot}20$, the remainder of the isolates within the set showed mutant phenotypes (or genotypes) for one or more of the loci. None of the original wild-type spores (presumed PWT) initially gave rise to homogenous conidial isolates.

Table 1. *Numbers of each class of non-disjunction ascus*

Spore type	Class I	Class II	Class III	Class IV	Total per cross
	Dark	Dark	Dark	Dark	—
	Dark	Dark	Dark	Dark	—
	Dark	Aborted	Pale	Aborted	—
	Dark	Aborted	Pale	Aborted	—
	Aborted	Dark	Aborted	Pale	—
	Aborted	Dark	Aborted	Pale	—
	Aborted	Aborted	Pale	Pale	—
	Aborted	Aborted	Pale	Pale	—
Cross					
467 × 46-5	21	5	27	1	54
469 × 14-5	0	1	4	1	6
469 × 14-6	7	0	8	1	16
Total	28	6	39	3	76

Average frequency of combined classes in the crosses studied was 1 in 300.

Table 2. *Asci isolated for detailed analysis from cross 467* (ad pan (*B5*) a) × *46-5* (al pan (*B3*) tryp A)

Ascus	1st spore pair	2nd spore pair	3rd spore pair	4th spore pair
97	Aborted	Pale spores: *al pan (3) tryp a*	Dark spores: PWT *al a*	Pale spores: *ad pan (5) tryp A*
98	Dark spores: PWT *al a*	Pale spores *ad pan (5) A*	Aborted	Pale spores: *pan (3) tryp a*
100	Dark spores: PWT *al A*	Aborted	Aborted	Dark spores: PWT *a*
101	Aborted	Dark spores: PWT *al A*	Aborted	Dark spores: PWT *al a*
135	Dark spores: PWT *A*	Dark spores: PWT *al a*	Aborted	Aborted

Table 3 provides the information gathered from the analysis of serial conidial isolates taken from the PWTs. In some cases in order to establish a homogeneous set of conidial isolates, and so prove the existence of a single nuclear type of a particular genotype, serial conidial isolates were continued through six serial isolations. In all but three (101-3, 135-2, and 135-3) of the PWTs more than two types of nuclei were detected. The number of classes of nuclei actually detected must necessarily be the minimum number present in each PWT culture.

Table 3. *Types of nuclei identified in the PWT isolates, with respect to linkage group VI*

PWT	Nuclei	PWT	Nuclei
97-5	*ad pan* (5) *pan* (3) *tryp* *ad pan* (*) *tryp*	97-6	*ad pan* (5) *pan* (3) *tryp* *ad pan* (*) *tryp* *pan* (5)
98-1	*ad pan* (5) *pan* (3) *tryp* *ad pan* (3) *tryp* *pan* (5)	98-2	*ad pan* (3) *pan* (5) *tryp* *ad pan* (5) *tryp* *pan* (3)
100-1	*ad pan* (5) *pan* (3) *tryp* *ad pan* (3) *tryp* *pan* (3)	100-2	*ad pan* (5) *pan* (3) *tryp* *pan* (5)
100-7	*ad pan* (3) *pan* (3) *tryp* *ad pan* (5) *tryp* *ad pan* (3) *tryp* *pan* (5)	100-8	*ad pan* (5) *pan* (3) *tryp* *pan* (3)
101-3	*ad pan* (5) *pan* (3) *tryp*	101-4	*ad pan* (5) *pan* (3) *tryp* *pan* (3)
101-7	*ad pan* (5) *pan* (3) *tryp* *pan* (5)	101-8	*ad pan* (5) *pan* (3) *tryp* *ad pan* (3) *tryp*
135-1	*ad pan* (5) *pan* (3) *tryp* *ad pan* (3) *tryp* *pan* (3)	135-2	*ad pan* (5) *pan* (3) *tryp*
135-3	*ad pan* (5) *pan* (3) *tryp*	135-4	*ad pan* (5) *pan* (3) *tryp* *ad pan* (3) *tryp* *pan* (3)

* Allele not identified.

4. DISCUSSION

The data appear to support the following hypothesis: in the system studied, the precocious division of the centromere at meiosis I accounts for more than half of the non-disjunction events. The disomic nucleus formed as a result of non-disjunction is unstable and PWT cultures became heterokaryotic rather than remaining disomic. A likely mechanism for the breakdown of the disomic is through mitotic chromosome pairing, with crossing-over and chromosome segregation, with the resultant loss of one member of the disomic pair, and the establishment of a haploid nucleus. Somatic pairing and crossing-over probably occur with a relatively high frequency in the disomic nuclei. A consideration of the evidence for the hypothesis follows.

Whereas the simplest explanation for class I asci is non-disjunction at meiosis I,

and for class IV asci non-disjunction in one of the two nuclei undergoing meiosis II divisions, an additional factor appears to be involved in the class II and class III asci. Thus the class III asci are best explained by hypothesizing the precocious division of one of the centromeres associated with the linkage group VI bivalent, at meiosis I, followed by a daughter chromosome from one member of the bivalent accompanying the other member of the bivalent at anaphase I. Class II asci may be derived from the precocious division of both centromeres of the bivalent at meiosis I, so that essentially the mitotic-like division of meiosis II takes place in meiosis I for the linkage group VI chromosomes. In meiosis II a further mitotic-like division for linkage group VI may not be expected, with the result that the two linkage group VI chromosomes are together included in only one of the two meiotic products associated with each diad nucleus. Thus class II asci are thought to reflect precocious division of both centromeres at meiosis I, and class III asci the precocious division of one centromere. The frequency of class III asci is approximately 0·5 in the sample studied, the frequency of the double event (class II asci) is approximately 0·12. Allowing for the fact that the method by which the asci are selected (namely, the dark spores are an expression of the presence of both *pan* alleles) does not permit detection of disomics with like *pan* alleles, the frequency 0·12 is not surprising. If the frequency of precocious division of one centromere of the bivalent is 0·5, then assuming the double event to occur at the combined frequency of two independent single events, the frequency of precocious division of both centromeres would be 0·25. In order for dark spores to be manifest, both the (B 3 B 5$^+$) and the (B 3$^+$ B 5) allele of the *pan-2* locus must be present, one on each of the two linkage group VI chromosomes. The dark spores result from complementation between the (B 3 B 5$^+$) and (B 3$^+$ B 5) allele which segregate together to form a disomic or heterokaryon. Half the segregation is expected to be (B 3 B 5$^+$) + (B 3$^+$ B 5) and half should be (B 3 B 5$^+$) + (B 3 B 5$^+$) or (B 3$^+$ B 5) + (B 3$^+$ B 5). Thus only half the double events will actually be detected.

Certainly it seems unlikely that class II asci reflect a double event of the class IV type. No reasonable explanation can be offered for non-independence of a double non-disjunction event at meiosis II to account for the high relative frequency of class II asci compared to class IV.

Of the sixteen PWT isolates studied in detail, three (101-3, 135-2, and 135-3) appeared to be heterokaryotic for two types of nuclei; the remainder are heterokaryotic for three or more types of nuclei. It is postulated that the additional nuclei arise from a mitotic recombination event. It is possible that the disomic nucleus breaks down through mitotic pairing and concomitant crossing-over of the disomic chromosomes with segregation of a complete haploid set of chromosomes from the remaining single chromosome of the disomic pair, so that each nuclear type results from mitotic pairing, and different genotypes in excess of two are evidence of the mitotic crossing over. If this is so, detection of reciprocal types from a crossover event would not be expected. The fact that stable PWTs were never detected in the asci studied suggests that the disomic nucleus is readily involved in mitotic pairing and recombination of the disomic chromosomes, to

result in the formation of a haploid nucleus and the establishment of heterokaryons rather than disomics.

The apparent relatively high frequency of mitotic recombination in the disomic nuclei of Neurospora suggests the lack of restrictions on mitotic recombination in this organism. The fact that diploid nuclei have never been detected in Neurospora suggests that such restrictions may only be present in organisms where diploid nuclei are usual. These suggestions are in accordance with the view that mitotic pairing and exchange are primitive traits in evolution, and that with the evolution of meiosis there also evolved genetic systems to repress somatic pairing and crossing-over (Westergaard, 1964; Stack and Brown, 1969). In Neurospora such repression appears to be weak, as might be expected where diploid nuclei, with the exception of the fusion nucleus, are absent.

This study was made possible through a grant from the National Research Council of Canada and through the technical assistance of Mr Leslie Gyorkos.

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