INFLUENCE OF CHROMOSOMAL ABERRATIONS ON MEIOTIC AND MITOTIC NONDISJUNCTION IN *ASPERGILLUS NIDULANS*[1]

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ANALYSIS of all types of mitotic segregants from diploids of *Aspergillus nidulans* with non-selective methods has shown that among conidia aneuploids resulting from *mitotic* nondisjunction occur with a relatively high frequency of 1–2% (KÄFER 1961). It seemed interesting to find out whether *meiotic* nondisjunction is also frequent in this species. In haploid organisms the only products of meiotic nondisjunction viable enough to be isolated are aneuploids of the n + 1 type. In *Neurospora crassa*, in which no diploids have yet been isolated, rare n + 1 aneuploids have been obtained from many crosses as pseudo-wild types, heterozygous for complementing alleles (PITTENGER 1954). In Aspergillus aneuploid colonies of this type are visually distinguishable from haploid ones since they grow poorly and produce large haploid sectors by loss of one of the disomic homologues. In standard crosses, and under standard conditions, colony morphology is very uniform and "abnormal" colonies have been found only rarely. This has made it possible to detect a small, but higher than normal, frequency of aneuploid looking colonies in a few crosses between related strains and to identify some of these as hyperhaploids of the n + 1 type. It seemed likely that *meiotic* nondisjunction was increased in these crosses as a result of heterozygosis for one or more chromosomal aberrations since aberrations are known to increase meiotic nondisjunction in higher organisms (STURTEVANT 1926; ANDERSON 1929; DOBZHANSKY 1930, 1933; DOBZHANSKY and STURTEVANT 1931; BEADLE 1932; GLASS 1933, 1935) and translocations have been found in the pedigrees of several Aspergillus strains (KÄFER 1965). The results presented here are consistent with this hypothesis. On the other hand, no similar effects on the frequency of the various types of n + 1 aneuploids resulting from *mitotic* segregation were detectable in diploids heterozygous for translocations.

MATERIALS AND METHODS

Strains: All mutant strains used in these experiments are descendants of the same wild type strain of *Aspergillus nidulans* (PONTECORVO, ROPER, HEMMONS, MACDONALD and BUFTON 1953) which normally possesses a haploid set of 8 chromosomes (KÄFER 1958). Details of all genetic markers used here have been summarized recently (DORN 1967).

The strains employed in the investigation of meiotic nondisjunction are shown in Figure 1. An eighth generation backcross strain, strain C (FGSC No. 85 in BARRATT, JOHNSON and OGATA 1965) was used as the best available reference strain. Strain B was employed as a common

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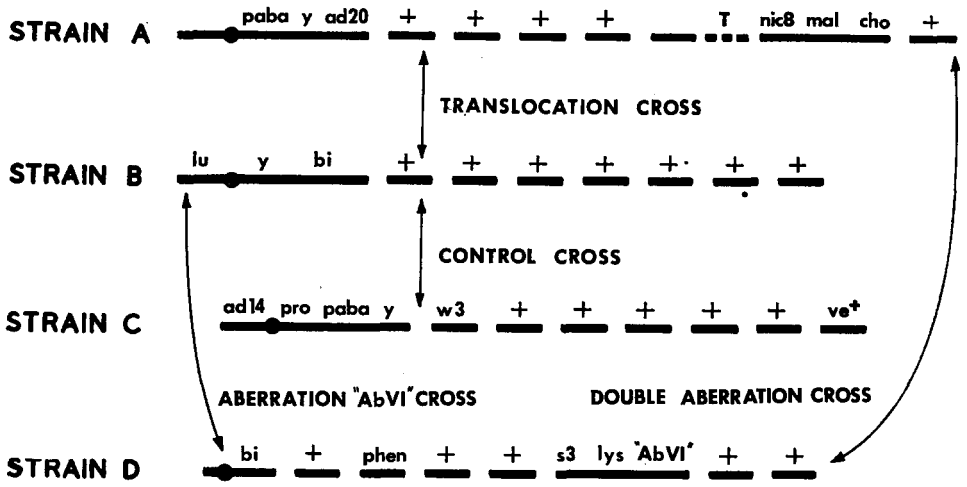


FIGURE 1.—Genotype of the four strains used for meiotic analysis, with arrows indicating the four crosses analysed in detail. + = standard chromosomes without markers. T = reciprocal VI; VII translocation. "Ab VI" = aberration linked to *lys1* in linkage group VI. (For explanation of mutant symbols, see Figure 2.)

"standard" strain in the various crosses; generally, "standard" strains are descendants from fully viable crosses between an eight generation backcross strain and the original *bi1* strain (FGSC 26) containing a UV-induced mutant. Strains A and D are two aberration strains of different types. They were chosen for detailed analysis when it was observed that the cross between them produced an unusually high frequency of abnormal, possibly aneuploid, descendants. Strain A was tested for the presence of a translocation in combination with a suitably marked translocation free tester strain (a segregant from cross 474, Figure 2, BARRATT *et al.* 1965). From the heterozygous diploid containing these two strains mitotic haploids were isolated to test for abnormal mitotic segregation (KÄFER 1962). Complete linkage for the markers of linkage groups VI and VII was found (44 haploids analysed). It appears, therefore, that strain A contains the same chromosomal aberration, presumably a reciprocal translocation T (VI; VII), which had been observed in one of its parent strains (a recombinant from cross 352, Figure 4, KÄFER 1965). Strain D was similarly tested, but was shown to be translocation free since no case of complete linkage between groups was observed (44 haploids tested; lowest recombination frequency: 13/44 for markers in groups I and VII; no other case of significantly less than 50% recombination). It was concluded that in strain D an intrachromosomal aberration or a genic mutation must be causing the meiotic abnormalities. The mutant effect was retained by *lys1* recombinants, after standard chromosomes were substituted for linkage groups I to IV via the parasexual cycle, and after ten generations of "backcrossing" to standard or T (VI; VII) strains. This mutation appears, therefore, to be either associated with, or closely linked to the X-ray induced mutant *lys1* in linkage group VI. At this time, no further tests are available to map it precisely since the few available markers of linkage group VI are practically unlinked in meiosis. From the effects observed here it seems likely that it is a chromosomal aberration, possibly a transposition or an inversion, rather than a point mutation; it is, therefore, called "aberration VI" (Ab VI in Figure 1 and Table 1).

The diploids used for the isolation of mitotic aneuploids of the n + 1 type are shown in Figure 2. Diploids T and A contain identical markers on all homologues and differ only by the presence of T (VI; VII) in the former. The haploid strains combined to form these diploids are very closely related, being either identical (b) or sister strains (a and c). Diploid B, on the other hand, is quite unrelated and contains many different markers. A summary of the data

from this diploid has been published earlier (KÄFER 1960); additional details which are relevant for judging marker effects on phenotype and frequency of the various aneuploids are presented here for comparison.

Methods: Standard media and methods were used for testing, plating, setting up of crosses etc. (PONTECORVO *et al.* 1953) and for the synthesis of heterozygous diploids (ROPER 1952). Diploid, aneuploid, and haploid types were distinguished by the presence or absence of sectors and segregating heterozygous markers as well as visually, by differences in the arrangement and shape of conidial heads.

To isolate aneuploids or any other unusual products of *meiosis*, ascospores were plated at a

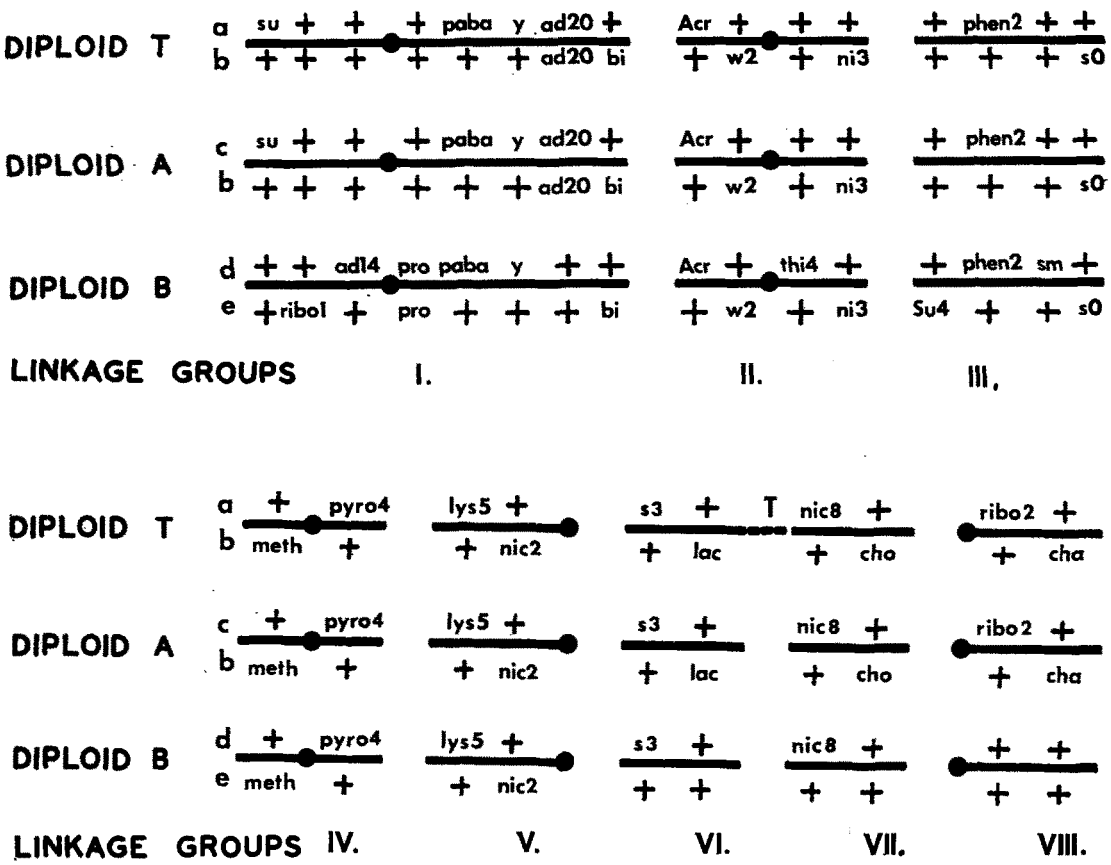


FIGURE 2.—Genotypes of diploids T, A, and B synthesized from haploids a–e with markers on linkage groups I–VIII. Conidial colour mutants: *y* = yellow: *w2* and *w3* = white, *cha* = chartreuse. Morphological mutants: *sm* = small; ve$^+$ = fluffy conidiation like the original wild type (practically all *A. nidulans* strains carry *ve* = velvet-like conidiation). Mutants determining requirements: *ad14* and *ad20* (allele of *ad8*) = adenine; *bi* = biotin; *cho* = choline; *lu* = leucine; *lys1* and *lys5* = lysine; *meth* = methionine; *ni3* = nitrate; *nic2* = nicotinic acid; *nic8* = nicotinic acid or tryptophan; *paba* = para-benzoic acid; *phen2* = phenylalanine; *pro* = proline; *pyro4* = pyridoxine; *ribo1* and *ribo2* = riboflavine; *s0(s12)* and *s3* = sulphite; *thi4* = thiazole. Suppressors: *su* (=*su1ad20*) = recessive suppressor of *ad*20; *Su4pro* = dominant suppressor of *pro*. Resistance marker: *Acr* = acriflavine. Mutants unable to use a carbon source: *lac* = lactose; *mal* = maltose. (All unnumbered alleles have isolation number 1.)

low density (<10 per plate) and all "abnormal" looking, potentially aneuploid, colonies were analysed.

For the isolation of *mitotic* aneuploids of a comparable (n + 1) type two selective methods were used, both based on the visual recognition of the extremely irregular conidial heads formed by aneuploid nuclei in conidiophores. The first is essentially the one used earlier for Diploid B (KÄFER 1960): irregularly shaped, potentially *aneuploid*, heads of yellow or white colour are identified among the wild-type green conidial heads of heterozygous diploid colonies grown on complete medium (with a yield of up to 75% aneuploids among the selected colour segregants). For the second method of selection conidia from diploids A and T were plated on minimal medium supplemented with all requirements except adenine which selects for hemi- or homozygosis of *su1ad20*. Aneuploid looking patches of any colour can then be visually selected among the "suppressed" sectors. Both methods yield aneuploids of two general types, either hyperhaploid and hemizygous for the selected mutant, or hyperdiploid and homozygous for the mutant. Both types of segregants are the result of more than one step of segregation; hyperhaploids probably result from chromosome loss from 2n-1 types produced by nondisjunction, and hyperdiploids from mitotic crossing-over in the chromosome arm carrying the selected mutant in addition to nondisjunction in one of the other linkage groups (no hyperdiploids resulting from more than one event of nondisjunction were observed). From the analysis of the absolute frequency of all types of mitotic segregants (KÄFER 1961) it is expected that the latter are the rarer types. This was found to be the case when the first selection method was used which selects for a single mutant in addition to aneuploid phenotype (e.g. among the aneuploids isolated in this way from diploid A only 20 out of 125 were hyperdiploid). When more than one mutant is used for selection, as was the case in the second method where "suppressed" segregants of different colours were isolated, the relative frequency depends on the relative positions of the various markers used (e.g. yellow suppressed aneuploids were mainly hyperhaploids, and wild-type green suppressed aneuploids were mainly hyperdiploids, because *y* was present in the opposite arm of the same homologue as *su1ad20*).

For a preliminary purification of *mitotic* segregants isolated by either method, the conidia from the aneuploid heads were streaked on complete medium (rather than plated directly as done previously for diploid B, KÄFER 1960). This procedure was found to be more economical and the recovery of aneuploids is quite satisfactory, even though a few aneuploids are lost since they become overgrown by the parental type. Also it is not always possible to recover the original very unstable types with more than one extra chromosome.

For the detailed analysis of all *meiotic* and *mitotic* aneuploids or other "abnormal" looking types, conidia from the abnormal looking areas were replated (using the method of "needle plating," KÄFER 1961). Pure platings of low density (5–15 per plate) were obtained for all isolates, which were used for phenotypic comparisons and photographed. From all sectoring types a sample of 13—26 or more stable sectors and some centers were then tested for segregation of genetic markers. In hyperhaploids, segregation of alleles identifies disomic linkage groups if these contain heterozygous markers (as shown in Table 2 for the *mitotic* n + 1 types from diploid A). When in *meiotic* aneuploids, no segregation of alleles was found in sectors or centers, these were classified by phenotype, making use of identified mitotic types for comparison. Similarly, the two most frequent *mitotic* types, n + 1 and 2n + 1 for linkage group III from diploid A, were in the latter part of the work identified by phenotype, after a large number of tests had shown the reliability of such visual identification. "Apparently non-sectoring" abnormals from the aberration crosses were transferred by up to 50 mass inocula. Certain types produced a few sectors under these circumstances and are called "rarely sectoring" types, while others never produced sectors ("non-sectoring" types, Table 1).

EXPERIMENTS AND RESULTS

MEIOTIC ANEUPLOIDS: Nondisjunction of single chromosomes in meiosis of ascomycetes is expected to produce mainly aneuploid ascospores of the hyper-

haploid, n + 1, type—the n − 1 product being completely inviable. It was, therefore, attempted to obtain information on the frequency of meiotic nondisjunction by plating ascospores at low density which permits detection of such aneuploids as slow-growing, abnormal colonies. Since the viability of aneuploids is lower than that of haploids, especially when they are in competition with each other, the aneuploid frequency can at best be a minimum estimate of the nondisjunction frequency. However, some information on the influence of different genotypes, e.g. chromosomal aberrations, on this frequency can be obtained by comparing the frequency of aneuploids from crosses between different strains.

The genotypes of the strains used in the four analysed crosses are indicated in Figure 1. To reduce variation in genetic background a common "standard" strain B was crossed to three other strains: i) to the eight generation backcross strain C to produce a standard or control cross; ii) to the translocation strain A; and iii) to strain D containing "aberration VI". In addition, in the fourth cross, the "double aberration cross", strains A and D were intercrossed.

Frequency of abnormal colonies: To obtain a reliable estimate of the frequency of aneuploids in the various crosses three platings of ascospores were made from each cross (with the total number of inspected colonies in each plating varying from about 500 to 2000). The average frequencies of abnormal colonies of all types were calculated from these three platings using transformed percentages (see Table 1).

No abnormal colonies were found among the 5000 colonies inspected from the control cross, indicating a frequency of nondisjunction of less than 0.02% in crosses between standard strains. On the other hand, both crosses heterozygous for a single aberration showed a measurable frequency of abnormal colonies (ca. 0.4% and 2.5%, Table 1) and significantly more than the sum of these frequencies (4.2%) is observed in the double aberration cross. This relatively high frequency of abnormal, mainly aneuploid, colonies has been observed in several further crosses heterozygous for *lys1*, i.e. "Ab VI", and T (VI; VII) in repulsion. For example, among about 200 descendants from each of two recent crosses involving two sister *lys1* strains, fifth generation descendants from strain D, both crossed to the same *paba y; co;* T (VI; VII) strain, the frequencies of "abnormal" types were 5.8% and 6.7%, respectively.

Classification of abnormal colonies from aberration crosses: All abnormal colonies from the heterozygous aberration crosses were analyzed for genetic segregation. Identification of n + 1 aneuploids is easy if heterozygous markers of the disomic linkage group segregate in the haploid sectors, as is always the case for *mitotic* aneuploids from well-marked diploids. Because of the occurrence of crossing over, the possibility of first and second division nondisjunction and, more pertinent in this study, the presence of aberrations, segregation of markers is not expected to occur regularly in the *meiotic* n + 1 types obtained here: indeed very few cases were found with segregation of heterozygous markers from disomic types. However, as has been demonstrated in detail for hyperdiploids (KÄFER 1961) and since confirmed for induced hyperhaploids (UPSHALL and CROFT 1967), all aneuploids form poorly conidiating centers and

TABLE 1

Classification and frequency of abnormal colonies from control and aberration crosses

Classification		Frequency in crosses							
		Control		Ab VI/+		T(VI;VII)/+		T(VI;VII)/Ab VI	
According to frequency and type of sectors	Visual; compared to standard n+1 disomics	Number	Percent	Number	Percent	Number	Percent	Number	Percent
1. Regularly sectoring types:									
A. with haploid sectors:									
A-1	n+VI-like	.	.	9	0.2	20	1.3	71	1.9
A-2	n+VII-like	.	.	.	.	15	0.9	24	0.7
A-3	n+III	.	.	6	0.1	1	0.06	19	0.5
A-4	n+IV	.	.	.	.	1	0.06	.	.
A-5	n+II(?)	.	.	2	0.04	.	.	.	.
B. with diploid sectors:	2n+1	.	.	.	.	.	.	1	0.03
C. with "aneuploid" sectors:		.	.	.	.	3	0.2	1	0.03
2. Rarely sectoring types:									
D. with haploid sectors from aneuploid sectors:	n+VI-like center	.	.	.	.	.	.	22	0.6
E. with haploid sectors:	n+VI-like center	.	.	.	.	.	.	3	0.08
3. Non-sectoring types (class F):	n+VII-like center	.	.	.	.	1	0.06	9	0.3
Total number of abnormal colonies		0		17		41		150	
Total number of colonies analysed		5806		4888		1590		3641	
Average frequency (from 3 platings) in percent		<0.02		0.35±0.17		2.45±0.43		4.2±0.09	

frequent euploid sectors, in patterns characteristic for each linkage group. It was, therefore, possible to classify the meiotic n + 1 aneuploids phenotypically, using for comparison the simultaneously isolated mitotic hyperhaploids, disomic for the various linkage groups.

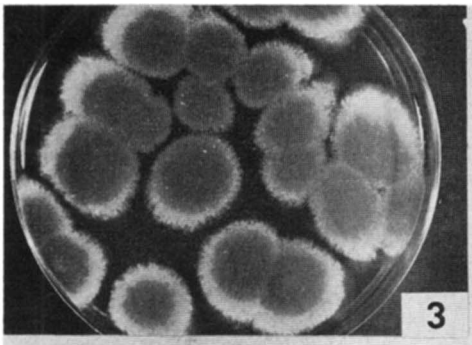
Phenotypic classification of all abnormals from the heterozygous crosses was preceded by grouping, using objective criteria: first into three groups (1. = regularly sectoring, 2. = rarely sectoring, 3. = stable, non-sectoring) with respect to the frequency of sectors, and then within each group according to the ploidy of the sectors produced (Classes A-F, see Table 1). Within each class obtained in this way, most abnormals appeared to be of a very few, phenotypically distinct, types. They were compared with standard aneuploids and with each other and tentatively identified as indicated in the various subclasses of Table 1. Figure 3 shows the plating of conidia from a normal colony to compare with platings of some of the meiotic abnormals and the corresponding mitotic n + 1 types shown in Figures 4, 5 and 6.

The majority of the abnormals from each of the three aberration crosses belong to sub-class A-1 (Figure 6b) and/or A-2, which closely resemble the *mitotic* aneuploids disomic (n+1) for linkage groups VI (Figure 6a) and VII, respectively—the two linkage groups involved in the aberrations. They cannot be designated standard aneuploids for these linkage groups because all haploid sectors contained the same homologue of the disomic linkage group as is expected if one of the disomic homologues is structurally abnormal. However, the striking similarity of the phenotypes of these colonies and those of the standard VI or VII disomics makes the classification unequivocal. The frequency of these main types varies for the three aberration crosses. In the translocation cross, where both linkage groups VI and VII are involved in the aberration, types A-1 and A-2 occur with about the same frequency. In the double aberration cross, where linkage group VI is involved in both heterozygous aberrations, abnormals of type A-1, resembling n + VI aneuploids, are found in excess of type A-2, resembling aneuploids disomic for VII; and in the aberration VI cross, where linkage group VII is structurally normal, no n + VII-like aneuploids are found.

An appreciable number of abnormal colonies, especially from the double aberration cross, but also from the aberration VI cross, are found in sub-classes A-3, A-4, and A-5, which represent aneuploids disomic for linkage groups III, IV, and II, respectively (see Figures 4 and 5).

The remaining classes of abnormal colonies have not been identified with certainty. They look like the centers of aneuploid types but do not show the regular sectoring typical of standard aneuploids. Since practically all of them were found only among the products of the double aberration cross, they appear to be the result of the presence of both aberrations. On the original ascospore platings or on replating, these colonies never showed sectoring (Figure 6c). However, when large numbers of transfers were made some sectors were found in two phenotypically different types ("rarely sectoring" classes D and E), while a few others remained "non-sectoring" (class F).

A two-step segregation appears to occur in the abnormals of type D: abnorm-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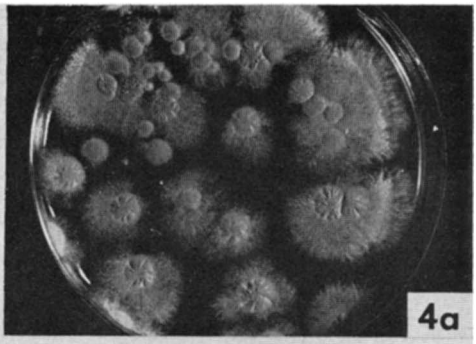
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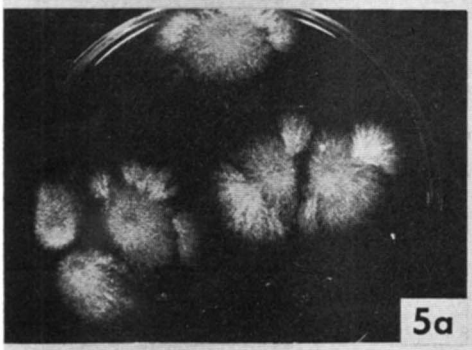
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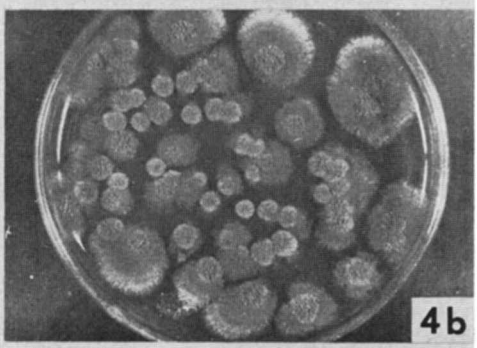
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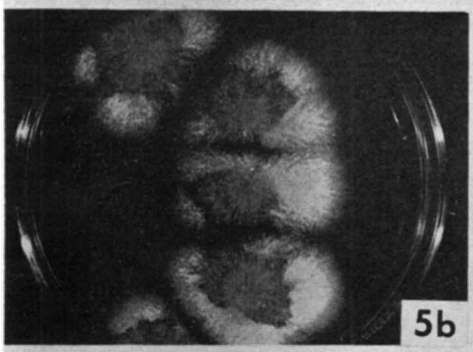
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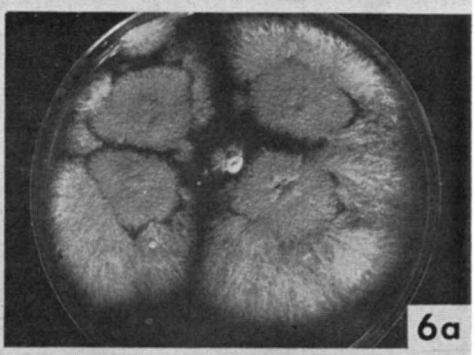
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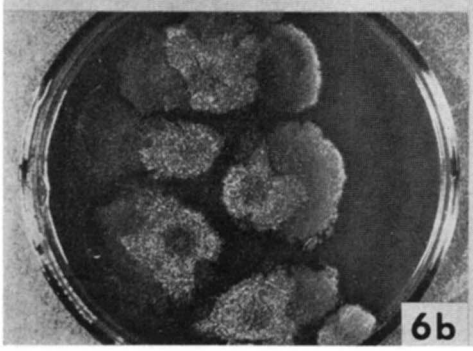
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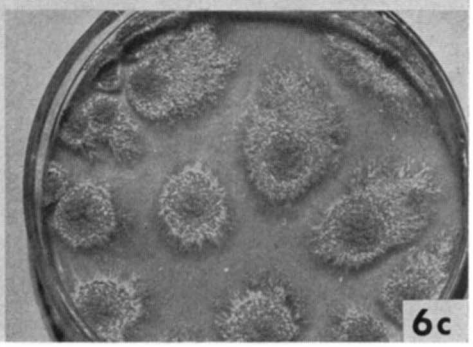
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ally conidiating, aneuploid-like sectors are found as well as rare secondary sectors of a normal haploid type. The few cases of type E may represent isolations of the intermediate step; they produce rare haploid sectors only. The phenotypic appearance of these colonies resembles that of aneuploids of subclass A-1 (n + 1 for VI) except that sectors are much rarer. It is thought that these colonies are aneuploids disomic for linkage group VI plus some other chromosome segment (VII?), the arrangement being such that an event or events other than chromosomal loss, possibly mitotic crossing over, are necessary to obtain a haploid product from the original aneuploid center. Mitotic crossing over of a reciprocal type has been found in the disomic pseudo-wild types of Neurospora by PITTENGER and COYLE 1963, and COYLE and PITTENGER 1965.

The completely stable types (class F) which look more like the centers of type A-2 might be haploids with duplications (of VI and/or VII) rather than n + 1 types.

MITOTIC HYPERHAPLOIDS FROM STANDARD DIPLOIDS AND TRANSLOCATION HETEROZYGOTES: As described above, mitotic segregants of the aneuploid n+ 1 type were isolated by two methods of "double selection", combining selection for aneuploid-looking, irregular, conidial heads with selection for the mutant allele of either the colour markers *w* or *y* on complete medium or of *su1 ad20* on supplemented minimal medium. Since selective techniques are used, no estimate of the absolute frequencies of these aneuploids can be made. Even the relative frequency of aneuploid compared to euploid isolates cannot be determined meaningfully, since it obviously depends on the efficiency of the visual selection (which varies with minor variations in technique and skill of the investigator). Among the aneuploids, in addition, the relative frequency of hyperdiploids depends very much on the relative map position of the mutants used for selection (for details see MATERIALS AND METHODS). Diploid, haploid and hyperdiploid isolates have, therefore, been omitted from all tables. On the other hand, no significant difference was found in the relative frequency of the different hyperhaploid types between sets with different selection and combined figures are given throughout.

Details of the analysis leading to the identification of all hyperhaploid segregants from the standard diploid A (Figure 2) are given in Tables 2 and 3. In Table 2 the observed number of n + 1 segregants disomic for each of the eight linkage groups is given, as well as the number of tested haploid sectors and their allele ratios for the disomic markers. As expected, these ratios are close to 1:1 in most cases. They deviate significantly only in one case, presumably as a consequence of viability effects of the marker gene *phen2* (on homologue c,

FIGURE 3.—Normal diploid colonies.

FIGURE 4.—Aneuploids disomic for linkage group III: (a) n+III from standard diploid A; (b) n+III from double aberration cross.

FIGURE 5.—Aneuploids disomic for linkage group IV: (a) n+IV from standard diploid A; (b) n+IV from double aberration cross.

FIGURE 6.—Aneuploids of the n+VI type: (a) normal n+VI from diploid A; (b) unstable sectoring type A-1 (n+VI-like) from double aberration cross; (c) apparently stable type D from double aberration cross: plated, no sectors formed.

TABLE 2

Disomics from diploid A : allele ratio of heterozygous markers observed in tested haploid sectors

Type	Number of disomic segregants	Number of tested sectors	Allele ratio homologues c : b	χ^2-test for 1 : 1 ratio P value
n + I	1	9	5 : 4	> .90
n + II	7	91	43 : 48	0.4
n + III	38 (+15*)	528	204 : 324	<0.001
n + IV	16	224	111 : 113	>0.90
n + V	10	132	75 : 57	0.10
n + VI	2	23	12 : 11	> .90
n + VII	4	89	44 : 45	> .90

* Classified visually.

linkage group III). For the relatively few hyperhaploids with more than one disomic linkage group the complete genotype and all isolated intermediates, mainly of the n + 1 type, are given in Table 3.

For all mitotic n + 1 aneuploids the classification on the basis of segregating alleles was consistent with the grouping by phenotypes. As predicted, it was found that for each of the eight linkage groups an n + 1 type with a characteristic sectoring phenotype is produced which corresponds to and resembles the previously isolated 2n + 1 types (KÄFER 1961). In Table 4 the frequencies of disomy for the various linkage groups in all hyperhaploids from diploids A and B are summarised and compared to the corresponding figures from the translocation heterozygote T. The results from the three diploids are very similar and no difference between the translocation heterozygote and the structurally normal diploids is found. On the other hand, it is evident that in all diploids the eight disomic types are isolated with very different and possibly characteristic frequencies, e.g. the n + III types are by far the most frequent, followed by the relatively frequent n + IV types, while at the other extreme, not a single n + VIII type was found. It appears likely that these frequencies reflect the relative viability of the different disomic types, possibly related to the size of the chromosomes involved (also among the 2n + 1 trisomics the 2n + III types are by far the most frequent; e.g. 30 out of 48 isolated from diploid A).

DISCUSSION

From the results presented here it can be concluded that in crosses between structurally homozygous strains viable aneuploid types are produced with extremely low frequency, indicating that in standard crosses the incidence of meiotic nondisjunction must be very rare. In crosses heterozygous for chromosomal aberrations, on the other hand, a considerable frequency of aneuploids or aneuploid-like types is produced. The hypothesis is put forward that, as

TABLE 3

Genotypes of hyperhaploids from diploid A disomic for more than one linkage group

Inferred type of original aneuploid	Recovered homologue for 8 linkage groups I	II	III	IV	V	VI	VII	VIII	Recovered intermediate aneuploid stages	Number of haploid sectors tested
n + 2 (18)										
n + II + IV	c*	c/b	b	c/b	c	c	b	c	n + IV	26
	c*	c/b	c	c/b	c	b	b	c	n + IV	11
	c*	c/b	b	c/b	c	c	b	c	n + II	15
	c	c/b	?	c/b	c	c	c	c	n + IV	12
n + III + IV	b	b*	c/b	c/b	b	c	c	c	n + III; n + IV	35
	c*	c	c/b	c/b	b	c	b	c	n + III; n + IV	51
	?	b*	c/b	c/b	b	c	?	?	n + III; n + IV	26
	b	b*	c/b	c/b	b	c	c	c		15
	b	c	c/b	c/b	b	c	b	b*	n + III; n + IV	11
	c*	c	c/b	c/b	b	c	b	c	n + III; n + IV	16
n + III + V	c*	c	c/b	b	c/b	b	b	c	n + III; n + V	35
	c	b*	c/b	c	c/b	c	b	b	n + III; n + V	19
	c*	c	c/b	b	c/b	c	b	b	n + III	18
	c	b*	c/b	c	c/b	b	b	b	n + III; n + V	50
	b	c	c/b	c	c/b	c	b	b*	n + III; n + V	25
n + III + VI	c	b*	c/b	b	b	c/b	c	b	n + III; n + VI	27
	b	b*	c/b	c	c	c/b	c	b	n + III; n + VI	26
n + IV + V	c	b*	c	c/b	c/b	b	c	c	n + IV; n + V	57
n + 3 (4)										
n + II + III + IV	c*	c/b	c/b	c/b	?	?	?	c	n + III	13
n + II + IV + V	c*	c/b	b	c/b	c/b	b	b	b	n + IV + V; n + IV; n + V	57
n + III + IV + V	b	b*	c/b	c/b	c/b	c	b	b	n + III; n + V	25
n + III + V + VI	c	b*	c/b	c	c/b	c/b	c	c	n + III; n + V; n + VI	13
n + 4 (1)										
n + I + III + IV + V	c/b	b*	c/b	c/b	c/b	c	b	b	n + III + V; n + III	72
Total	1	6	17	15	10	3	0	0		

* Selected marker; ? not tested.

TABLE 4

Hyperhaploids from different diploids and their relative frequencies of disomic linkage groups

Inferred ploidy	Number isolated	Disomic linkage groups							
		I	II	III	IV	V	VI	VII	VIII
Diploid A									
n + 1	93	1*	7*	53	16	10	2	4	0
n + 2, n + 3, n + 4	23	1*	6*	17	15	10	3	0	0
Total	116	2*	13*	75	31	20	5	4	0
Diploid B									
n + 1	40	0	1*	21	12	3	2	4	.†
n + 2, n + 3	19	1*	8*	11	12	9	1	1	.
Total	59	1*	9*	32	24	12	3	2	.
Approximate average ratio		1* :	4* :	20 :	11 :	7 :	1 :	1.5 :	0
Diploid T									
n + 1	34	0*	0*	18	11	4	1	0	0

* Reduced probability for detection because of selection for hemizygosis of mutant alleles.
† Linkage group VIII unmarked.

observed in higher organisms, structurally non-identical chromosome pairs show increased frequency of meiotic nondisjunction.

Consistent with this hypothesis is the observation that in the crosses heterozygous for the translocation T(VI; VII) aneuploids resembling the n + VI and the n + VII standard types are found (types A-1 and A-2, Table 1) but only one of these, the n + VI type, is observed in the cross which is heterozygous only for the aberration on linkage group VI. The more than additive effect and the production of many rarely sectoring n + VI-like aneuploid types (classes D and E, Table 1) in the cross heterozygous for both aberrations is presumably due to the fact that these involve the common linkage group VI. Similar, rarely sectoring, mitotic aneuploids have previously been identified from a well marked diploid heterozygous for two translocations involving a common linkage group and were found to be of a pseudo-wild type heterozygous for markers on both homologues of this group (KÄFER unpublished).

However, a fair number of other aneuploid types (sub-classes A-3 to A-5, Table 1), observed especially in the two crosses involving strain D, are not explained by this simple hypothesis. These types are indistinguishable from standard aneuploids for other linkage groups and by far the largest number of them is of the n + III (A-3) type. One possible explanation is to assume that chromosomal aberrations also increase nondisjunction for the normal chromosome pairs as proposed by NOVITSKI (1964) and GRELL (1962) to account for similar observations in Drosophila. The excess of n + III types would then be due to its relatively high viability as evident from its preponderance among the mitotic n + 1 segregants from all diploids and the corresponding relatively high frequency of 2n + III types. Under the much more competitive conditions of the meiotic system such viability effects are expected to be even more pronounced. The alter-

nate possibility would be the presence of a recent chromosomal mutation in strain D involving only linkage group III which could cause the increased nondisjunction of the corresponding chromosome pair. While it is felt that the latter explanation is the less likely one, it cannot be ruled out at this time. Further crosses, heterozygous for the aberration in group VI and isogenic for a standard homologue of linkage group III might provide an answer to this question. Unfortunately, the substitution of the linkage group III chromosome in strain D, with the corresponding one of strains A or B, while theoretically possible via the parasexual cycle, has not been successful so far because of difficulties in the formation of balanced heterokaryons with strain D and its descendants.

Practically all *meiotic* aneuploids had to be classified by phenotype only, using identified *mitotic* aneuploids for comparison. This is feasible because in *A. nidulans* the morphology of colonies grown from ascospores of conidia of identical genotype is very uniform. Various other types of chromosomal imbalance have been found to produce a characteristic deviant morphology which permits phenotypic classification: for example duplication progeny can be identified visually in crosses heterozygous either for a duplication (PRITCHARD 1956) or for a nonreciprocal translocation (BAINBRIDGE and ROPER 1966).

Comparing the frequencies of the various *mitotic* n + 1 aneuploid segregants from the standard diploid A with those from the identically marked translocation heterozygote T, it can be seen that the mitotic situation is not analogous to the meiotic one: mitotic nondisjunction does not appear to be influenced by chromosomal aberrations, since no difference is observed in the relative frequencies of the different types of mitotic hyperhaploids isolated from these two diploids. Similarly, the results from the differently marked diploid B agree closely with those from A. This indicates that the relative frequencies of the various n + 1 types are not influenced much by the different methods of selection, the presence of heterozygous markers or differences in genetic background. However, they vary considerably for the different linkage groups, possibly as the result of differences in viability related to differences in the size of the extra chromosomes. It is concluded that either the process of chromosomal segregation giving rise to meiotic aneuploids is not the same as that giving rise to mitotic aneuploids, or, in meiosis, aberrations have specific effects on the process, possibly by interacting with recombination, which are not in force in mitosis.

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SUMMARY

The frequency of aneuploids of the n + 1 type, which is presumed to reflect the frequency of *meiotic* nondisjunction, has been studied in standard crosses and in crosses heterozygous for chromosomal aberrations. No aneuploids were observed in large samples from standard crosses indicating that normally meiotic nondisjunction is very rare. Both aberrations, a translocation involving linkage groups VI and VII and an aberration affecting VI only, increased the rate of non-

disjunction, primarily for the linkage groups in which these aberrations reside. The effect of the translocation was more pronounced, and there was a synergistic effect when the two aberrations were crossed together, possibly due to the fact that both segregating abnormalities involved a common linkage group.—The relative frequency of the different n + 1 types was found to be the same among *mitotic* segregants isolated from three different heterozygous diploids. It appears to be independent of the presence of translocations and not much influenced by isolation methods and heterozygous markers. It presumably reflects the relative viability of the various aneuploids and may be some indication of the relative lengths of the corresponding chromosomes. As expected from results obtained with hyperdiploids, it is found that n + 1 hyperhaploids disomic for each of the different linkage groups show characteristic phenotypes. Even hyperhaploids with more than one extra chromosome have specific phenotypes and always produce n + 1 segregants of the expected types by regular loss of single chromosomes.

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