THE PROCESSES OF SPONTANEOUS RECOMBINATION IN VEGETATIVE NUCLEI OF ASPERGILLUS NIDULANS[1,2]

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IN the normally haploid strains of the fungus *Aspergillus nidulans* stable diploid strains are produced spontaneously as a rare event by fusion of nuclei (ROPER 1952). Such spontaneous diploids can easily be isolated from heterokaryons between differently marked haploid strains. They are visually distinguishable from the haploid since they show less regularly arranged and less uniformly shaped conidial heads. The size of the individual conidia increases proportionally with ploidy and provides an easily measurable criterion of the number of chromosome sets. That the diploid state is abnormal in some respects is indicated by the fact that diploid strains are almost completely sterile, meiosis of the diploid proceeding only up to metaphase I (ELLIOTT 1960). Also, in competition, the haploid shows the higher viability. Diploids are fairly stable, but produce rare mitotic recombinants, some of which are again haploid (ROPER 1952; PONTECORVO and ROPER 1953). So far, little is known about the occurrence of diploidy in nature and therefore about the actual evolutionary contribution of this potentially efficient type of parasexual recombination (PONTECORVO 1956). Also, while some mitotic segregation has been found in a variety of organisms, the question of its general occurrence in fungi and in tissues of higher organisms is still open.

Ever since the discovery of mitotic recombination in diploid strains of *Aspergillus nidulans*, attempts have been made to analyse the underlying processes. While one process, mitotic crossing over, was recognized early (PONTECORVO, TARR-GLOOR and FORBES 1954), and has been analysed in detail (PONTECORVO and KÄFER 1958), it was more difficult to elucidate the mechanism(s) producing the other types of segregants (haploids, nondisjunctional diploids and aneuploids). It became clear that for the analysis of these mitotic recombinants, which are all products of some kind of chromosomal segregation, it was necessary to use diploids with markers on all chromosomes, preferably on both chromosome arms. Mapping procedures making use of mitotic recombination have therefore been worked out, the available useful markers mapped and crossed together in suitable arrangements and the chromosome number established genetically (KÄFER 1958). Various chromosomal aberrations, especially translocations, were encountered and it became possible to recognize several types of characteristic distortions of the patterns of mitotic recombination caused by their presence in heterozygous condition.

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The present work was undertaken to elucidate the process or processes of mitotic *chromosomal* segregation, making use of well-marked diploids and non-selective methods for the isolation of segregants. The aim was to gain a detailed knowledge not only of the basic process(es) but of the many other factors which obviously influence the frequency of the recovered recombinants (e.g. presence of aberrations, conditions of growth, methods of isolation).

It was hoped that such information could provide decisive criteria for the interpretation of results obtained in the analysis of recombination processes in many asexual species. In some cases a remarkable similarity in the results justifies the assumption that the underlying processes in other fungi are the same as in *Aspergillus nidulans* (e.g. in *Aspergillus niger*, HUTCHINSON 1958 and LHOAS 1961; in *Penicillium chrysogenum*, SERMONTI 1957; in yeast, ROMAN and JACOB 1958; and possibly in *Fusarium oxysporum*, BUXTON 1956). On the other hand, results not following the same pattern are obtained in many cases, either with different strains of the same species (e.g. in Penicillium, especially after treatment with mutagens, MUCHNIK 1961; and in Fusarium, TUVESON and GARBER 1959) or with other species (in Streptomyces, e.g. BRAENDLE and SZYBALSKI 1959).

A better understanding of the processes of mitotic recombination in fungi might also permit a more fruitful comparison with the phenomena encountered in tissues of higher organisms, where it is not possible to breed from recombinant cells. Here analysis must be indirect and evidence is usually based on patterns produced by the phenotypically expressed recombination of genetic markers or by cytologically recognizable variations of the karyotype (e.g. in endosperm of maize, JONES 1937; MCCLINTOCK 1956; or in root tips of Allium, HUSKINS 1948; in body surface cells of Drosophila, STERN 1936; or of Ephestia, KÜHN 1960; or in human cells grown in tissue culture, PUCK 1958).

MATERIALS AND METHODS

Strains: All mutant markers used in this analysis have been induced or selected in the same wild-type strain of *Aspergillus nidulans* which normally possesses a haploid set of eight chromosomes. Details are given elsewhere (KÄFER 1958), except for a new spontaneous mutant, "chartreuse" (*cha*), which determines light green conidial color and is located distal to *ribo2* in the right arm of linkage group VIII (Figure 1). Four suitably marked haploid strains were obtained by intercrossing the original mutant strains isolated after X-ray or ultraviolet irradiation.

To make sure that a standard set of chromosomes would be present for analysis, several strains were checked for translocations (by mitotic analysis) and mutants which could not be separated from aberrations were excluded. In spite of this, it turned out that one of the final strains (diploid R, homologue a, Figure 1), which was expected to have standard chromosomes, carried a translocation between linkage groups VI and VII. It was decided to use this strain in any case, since valuable information on the influence of translocations on mitotic segregation

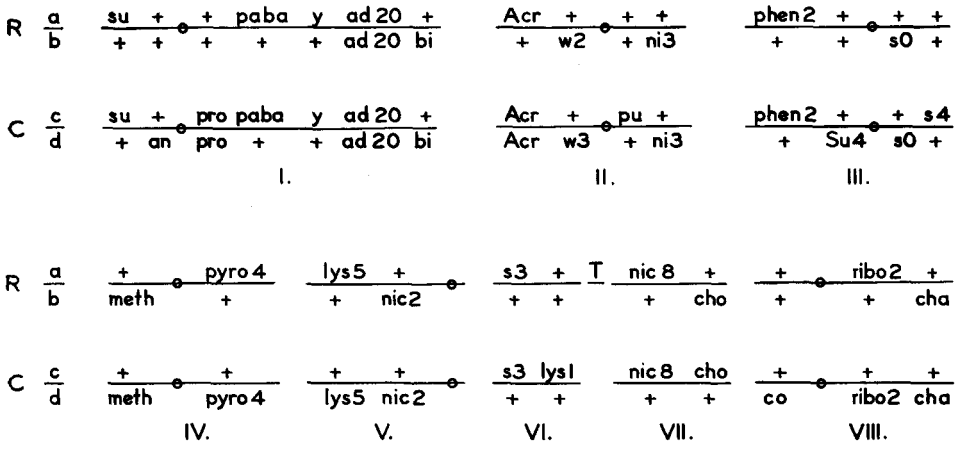


FIGURE 1.—Genotypes of repulsion diploid R (synthesized from the two haploids a and b) and coupling diploid C (c/d) with markers on linkage groups I–VIII. Conidial color mutants: *y* = yellow, *w2*, *w3* = white (allelic), *cha* = chartreuse. Colonial mutant: *co* = compact. Mutants determining requirements: *ad20* (allele of *ad8*) = adenine, *an* = aneurin (= thiamin), *bi* = biotin, *cho* = choline, *lys1* and *lys5* = lysine, *meth* = methionine, *ni3* = nitrite, *nic2* = nicotinic acid, *nic8* = nicotinic acid or tryptophan, *paba* = p-aminobenzoic acid, *pro* = proline, *pu* = putrescine, *pyro4* = pyridoxine, *ribo2* = riboflavin, *s0*(*s12*), *s3* and *s4* (allele of *s1*) = sulphite. Suppressors: *su1ad20* (*su*): suppressing *ad20*, *Su4pro* (*Su4*) suppressing *pro*. Resistance mutant: *Acr* = acriflavine. T = translocation (VI–VII). (All unnumbered alleles have isolation number 1).

could be obtained in this way. At the moment it is assumed, but not certain, that the so-called "standard" arrangement of chromosomes present in the other three strains used in this analysis is the same as that of the original wild type.

The two test diploids were selected from heterokaryons between two each of the four well-marked haploid strains, using ROPER's technique (1952). One of these diploids showed an extremely early mitotic crossing over in linkage group VIII before analysis was started. The new diploid (diploid C, Figure 1) carried the two reciprocal crossover strands, with *cha* and *ribo2* heterozygous but now in coupling, while each haploid strain had carried one of the markers. The two diploids used for analysis carried markers on all eight linkage groups. Their complete genotype is given in Figure 1. In the repulsion diploid R it was possible to follow segregation of all chromosomes (because of the presence of the translocation VI–VII the only unmarked chromosome of linkage group VI is not found to segregate independently). The coupling diploid C has 23 heterozygous markers, wherever possible arranged in coupling on both chromosome arms to permit distinction of nondisjunctional types from mitotic crossovers showing segregation in single chromosome arms; but it has the disadvantage that in five linkage groups (IV–VIII) one homologue does not carry any markers. The most likely positions of six of the centromeres are indicated in Figure 1 (full evidence only available for linkage groups I and II, PONTECORVO and KÄFER 1958).

Techniques: Standard media (KÄFER 1958) and testing methods (PONTECORVO, ROPER, HEMMONS, MACDONALD and BUFTON 1953) were used. To obtain

and analyse mitotic segregants, platings of samples of conidia on complete medium (CM) were made. A new plating method, "needle plating" was used extensively to plate samples from very small or badly conidiating colonies (or parts of colonies): conidia were collected from one or several conidial heads by touching with a fine needle and rinsing off in 0.2–1.5 ml of saline. Platings onto CM plates were made from these suspensions without diluting or counting and usually showed the desired low density of a few colonies per plate.

Allelism tests were made for the various sulphite-requiring mutants which can easily be characterized by their ability to crossfeed alleles of different genes, but not of the same gene, on medium without sulphite. Complementarity tests needed to determine the presence of other epistatic markers in haploids have not been carried out, since no case occurred where crucial evidence could have been obtained in this way.

EXPERIMENTS AND RESULTS

The experiments consisted simply of conidial platings from two well marked diploids under optimal conditions and detailed analysis of all segregants obtained in this way. The detailed procedure was as follows: A small number of colonies ("primary colonies") was grown from single uninucleate conidia from each of the two diploids and the genotype of these nuclei was verified by isolating mitotic haploids from the conidia in the center of each of these colonies. To analyse the mitotic segregation in these primary colonies batches of conidia (about 10^6) were harvested from each of them, diluted, and a small sample of conidia (usually several hundred, for details see Table 1) plated at low density on complete medium (CM). Most of these conidia are of the original type, while the rare segregant nuclei, produced by mitotic recombination during the growth of the primary colonies, are present only in very few of the conidia. The recombinant conidia form colonies of "aberrant" types which, on objective criteria, fall into two classes (1) colonies with mutant phenotype and normal growth (stable haploid and diploid segregants) and (2) colonies with abnormal growth patterns (e.g. unstable aneuploids are expected in this class).

1. Of the *stable* segregants which grow normally, but show a mutant phenotype, one category can be detected visually by inspecting all colonies grown from the plated conidia, namely (a) those showing mutants which affect the conidial *color* (*y*, *w*, *cha*); the others, (b) showing nutritional *requirements*, can only be found by transfer to minimal medium (MM). Only part of the colonies from each plating were tested on MM for requirement (Table 1). To characterize the "color" segregants and the "requiring" segregants, their phenotypes and ploidy are determined by further tests.

2. The *unstable* segregants producing abnormal growth patterns are also detected visually. All colonies deviating in size or conidiation are designated as "*abnormal*" segregants. They are further analysed by replating conidia from the center of each one and testing the obtained colonies as well as the stable sectors many of them produce.

TABLE 1

Size of analyzed samples and frequency of segregants

	Numbers		Frequency	
	Diploid R	Diploid C	R	C
Inspected colonies				
Number of platings	19	13	..	..
Colonies per plating (range)	169–1189	61–687	..	..
Total colonies	9877	3595	..	..
Average density: colonies/plate	20	10	..	..
Colonies tested for requirements				
From original platings	2054	1893	..	..
Secondary isolates	3520	1446	..	..
Total	5574	3339	..	..
1. Stable segregants				
(a) "color" segregants (3 markers)				
Whole colonies	19	16	0.2%	0.4%
Mixed colonies	6	1	..	..
(b) "requiring" segregants (14 chromosome arms marked)				
From original platings	12	7	0.7%	0.5%
From secondary isolates	27	10		
2. Unstable segregants				
"Abnormals", mostly aneuploids sectoring,				
With segregant sectors	41	36	0.6%	1.3%
With sectors like centers	14	12		
"Lost", probably sectoring,				
Some sectors recovered	14	11	0.3%	0.4%
No segregant recovered	16	3		
Translocation segregants				
With segregant sectors	22	..	0.2%	..
With sectors like centers	1?	..	..	..
"Misjudged", later normal				
Analysed	17	6	..	..
Not analysed	11	2	..	..

This method of isolation of segregants is completely nonselective and suitable for recovery of types with reduced viability which have been missed in earlier experiments. It has the disadvantage that each batch of conidia, harvested from the surface of a "primary" colony, contains segregant clones of various sizes, depending on the size of segregant patch formed from a single event in the primary colony. The experiments were therefore originally designed to permit calculation of the absolute frequency of each type of segregant with statistical correction for clonal distribution (LURIA and DELBRÜCK 1943). However, the size of the different sample platings varied considerably because the density (average given in Table 1) was decreased during the course of the experiments (since it turned out that only low densities of less than ten colonies per plate permit recovery of practically all the very slow growing types). Also the size of each sample was found to be small enough and the segregation events rare enough that

even relatively frequent types occurred in not more than about one quarter of the independent platings. It seemed therefore to be a reasonable assumption that the few cases of two or three segregants of exactly the same type within one sample all represent clones, each from a single event. They are treated as such throughout the analysis and a minimum estimate of frequencies is calculated: number of independent events per total number of tested colonies (Tables 1, 2 and 6).

Isolation of stable segregants: (a) Most *"color" segregants*, detected visually in the original platings, were isolated as colonies with pure mutant color and normal growth pattern. In a few cases, however, mixtures of mutant and wild type color were analysed (Table 1). It was hoped that in some of these cases, where segregation occurred in one of the first mitotic divisions, a "twin spot" consisting of the two complementary, phenotypically recognizable, products of mitotic crossing over might be recovered. One of the five cases analysed from diploid R was such a "twin spot" (the two diploid recombinant types were *paba y bi*$^+$ and *paba*$^+$ *y*$^+$ *bi*, resulting from a mitotic crossing over in linkage group I between *paba* and the centromere). Only one further such twin spot was encountered: two reciprocal diploid sectors from diploid R (homozygous *cha* and *ribo2* respectively, resulting from a single event of mitotic crossing over in linkage group VIII) were present among the sectors of a single unstable colony. Two other cases, showing high frequency of apparent twin spotting, will be discussed later.

(b) Under "requiring" segregants are included all those which differed from the original diploids not only with respect to requirement but also (from diploid R) with respect to resistance to acriflavine. They are detected by testing on MM, on which the original diploids (*ad20/ad20 su1ad20/*+) show adaptive growth and form sectors (*su1ad20/su1ad20*) by mitotic crossing over, so that an additional requirement of homozygosis for *su1ad20* or *su1ad20*+ can be detected (similarly on CM supplemented with acriflavine homozygosis for *Acr* or *Acr*$^+$ can be detected; PONTECORVO and KÄFER 1958). Two sets of such segregants were obtained, one consisting of the requirers found among the normal looking colonies of the sample platings which were transferred to MM for this purpose, and a second one resulting from incidental events of segregation in replatings of "abnormal" colonies (secondary isolates), which produced rare requiring sectors (Table 1).

Testing and classification of stable segregants: The phenotypes of the stable segregants were determined by replication on the various differently supplemented test media. The ploidy of these segregants was judged by several criteria; haploids can be distinguished from diploids visually (they show very dense and regular conidiation), by the absence of heterozygosity (testable markers: *su1ad20*, *Acr*, *y*, *w* and *cha*) and by the presence of all the mutants carried on one homologue of each linkage group. Only two were found (among the yellow segregants, Table 2). All other segregants, classified as diploids, were found heterozygous for at least two markers, and generally showed only segregation for mutants of a single chromosome. Three color segregants were exceptions (two of

them homozygous *su1 ad20*, and one, from diploid R, *pyro4*) and probably were produced by two consecutive events of segregation. In these and in a few other cases the diameter of the conidia was measured which provides a reliable criterion for distinction of the various levels of ploidy in stable (euploid) segregants (ROPER 1952).

Further classification of the diploid segregants into mitotic "crossovers" or "nondisjunctionals" was possible in all those cases, where segregation involved a chromosome with markers located on both chromosome arms: They are likely to be products of mitotic crossing over when only markers of one arm are found homogygous, and "nondisjunctionals" when all markers on both arms have become homozygous simultaneously (coincidence of crossing over in both arms being very rare, PONTECORVO and KÄFER 1958). Most color segregants (except the *cha/cha* types from diploid R) are classifiable in this way and their frequencies are given in Table 2. The number of "requiring" segregants classifiable for the two types is so small (24) that no comparable frequencies are calculated. Of the 11 from diploid R and 13 from diploid C (listed among the "requiring" segregants of Table 1), 18 were mitotic crossovers, six nondisjunctionals.

Isolation and testing of "abnormal" segregants: "Abnormal" segregants were detected in the original platings as colonies which showed abnormal growth rate and/or conidiation after two days at 35°C. Since all earlier identified aneuploids showed abnormal growth, this class was expected to include aneuploid types which could provide information about the process of chromosomal segregation. To avoid any bias, it was decided to classify as "abnormal" any colony deviating

TABLE 2

Frequency of the different types of stable "color" segregants

Color and phenotype			Number: Diploid R	Diploid C	Total		Frequency
Yellow	*y*	Mitotic CO	0	2	2		
(I R)	*paba y*		5	2	7	9	0.07%
	su paba y	Nondisj.	1	3	.	4	0.03%
		Haploids	2	0	.	2	0.015%
White							
(II L)	*w*	Mitotic CO	5	2	.	7	0.05%
	w ni3	Nondisj.	0	1	.	1	0.007%
		Haploids	0	0	.	0	<0.01%
Chartreuse	*cha*	Mitotic CO	(6*)	2	2		
(VIII R)	*cha ribo2*	(in C)	.	3	3	5	0.14%
	cha ribo2 co	Nondisj.	.	1	.	1	0.03%
		Haploids	0	0	.	0	<0.01%
Average mitotic crossing over/chromosome arm							2 × 0.07%
Average nondisjunction/chromosome							2 × 0.02%
Total haploids						2	0.015%

* Mitotic crossovers not distinguishable from nondisjunctionals.

at all from the majority of normally growing ones. In several cases colonies showing slight variations in appearance were classified as segregants, but later turned out to be normal by all criteria; these cases are presumed to be caused by slight environmental differences and are listed in Table 1 as "misjudged." To analyse all other, genetically "abnormal" segregants, conidia from their centers were harvested and plated on CM. In most cases colonies grown from these conidia showed a deviant phenotype like the original segregant, but generally grew larger and regularly produced better growing sectors under the favorable conditions of a low density plating with almost no competition. It was found that simple transfer of conidia from the unstable centers of tiny "abnormal" colonies only led to the recovery of one or a few stable second order segregants, since the unstable center type was not able to compete. In a few cases, the center type was therefore not recovered, while several sector types were obtained; other tiny colonies were too close to normal colonies and could not be analysed at all (both these groups are listed as "lost" in Table 1). The method of needle plating used in the later part of the analysis permits recovery of most very abnormal types, provided they form a few conidial heads. When the sectoring or otherwise abnormal types were very rare in the first replating of a segregant a secondary replating was made using the conidia from the center of one of them. However, only segregants analysable in the first replating are sure to have been present in the analysed sample; later ones may possibly represent new events of segregation. This slightly reduces the accuracy of the frequencies calculated for the various "abnormal" segregant types. All colonies originally designated as "abnormal" are listed in Table 1, classified by objective criteria, except for segregants interpreted as caused by the presence of the translocation in diploid R. All "abnormals" which could be replated were analysed by testing and classifying at least 26 isolates, mostly stable sectors, from each plating (using the same procedure as for stable segregants). The total numbers of all these tested sectors are listed as "secondary isolates" in Table 1.

Classification of "abnormal" unstable segregants

The "abnormal" segregants turned out to be of a variety of types. Their appearance on the original plating as well as their recovery in secondary replatings depends to a large extent on the plating conditions (especially crowding) and on the number of mitoses preceding the occurrence of secondary segregation events. Meaningful classification of the original appearance (attempted with a similar group of abnormals from an earlier diploid, KÄFER 1960) was therefore not possible. But it has been found that abnormal segregants replated under standard conditions at very low density show a growth pattern characteristic for their genetic constitution. Most of them show a slowly growing central part of characteristic size and reduced conidiation of a specific type (except for tufts of conidiating aerial mycelium in the very center), and form at the edge better growing sectors with normal, diploid or haploid, growth characteristics (see Figures 2–5). They were objectively classified into 11 different types according to the mutants expressed by the unstable type of the center and the pattern of segrega-

tion shown by the sectors. The 11 types are interpreted to represent six major groups (a–f) as follows:

(a) *Hyperdiploids:*

(1) centers are heterozygous (like the original diploid), producing two types of sectors, some like the center and some of a mutant type, nondisjunctional for a single chromosome;

(2) centers are mutant, nondisjunctional for one chromosome, giving two types of sectors, some like the centers and others nondisjunctional for a second chromosome as well;

(3) centers are heterozygous (like the original), producing four types of sectors which show nondisjunctional segregation for the markers of two chromosomes;

(4) centers are of heterozygous (original) or mutant (nondisjunctional) types, the sectors showing nondisjunction for three or four chromosomes in various combinations.

(b) *Unstable types, sectoring without segregation of markers (unmarked chromosomes, diploid C):*

(5) heterozygous centers giving sectors of the same type (all like the original diploid);

(6) centers and sectors are of the same mutant phenotype (nondisjunctional for a single chromosome).

(c) *Hyperhaploids:*

(7) centers are mutant producing haploid sectors only.

(d) *Breakdown types:*

(8) centers produce a variety of nondisjunctional or haploid sectors.

(e) *Lost unstable types:*

(9) centers lost, but stable segregants recovered;

(10) no segregant recovered.

(f) *Translocation segregants:*

(11) (from diploid R only) centers show sectoring; any diploid sectors only segregate for markers of linkage groups VI or VII, while haploid sectors only segregate for all other markers.

(a) *Hyperdiploids:* The majority of segregants were of type (1), that is their centers were heterozygous for all mutant markers, like the original, and produced diploid sectors of two types, one like the original diploid, the other nondisjunctional for a single chromosome. Segregants of type (2) showed the same type of sectoring with segregation for a single chromosome but were in addition homozygous for markers of another chromosome. In 24 cases the segregating chromosome carried markers on both arms and all sectors obtained were recognizably of a nondisjunctional type, segregating simultaneously for the markers on both arms. The ratio of heterozygous:homozygous sectors was roughly 2:1, somewhat lower if the homozygous sectors showed mutant color and were preferentially isolated for testing, somewhat higher if the homozygous mutant sectors had slightly reduced viability (details for all these segregants are shown in Table 3).

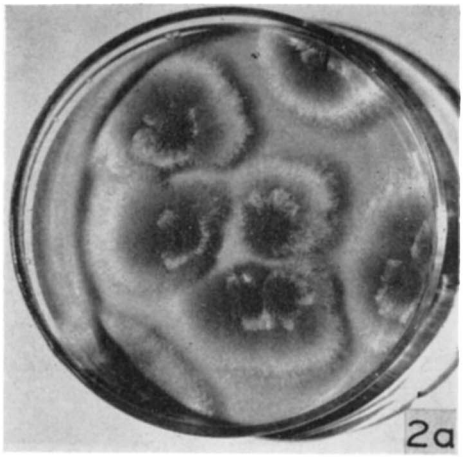
TABLE 3

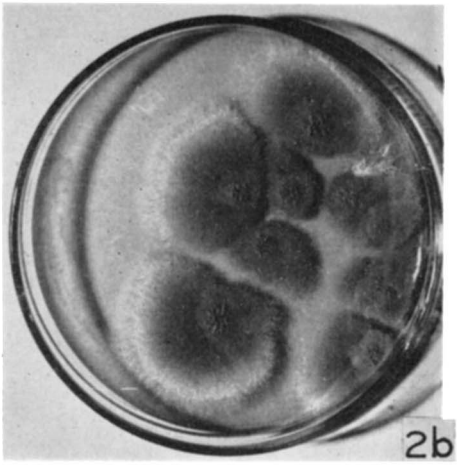
Hyperdiploid segregants showing high frequency sectoring for one linkage group

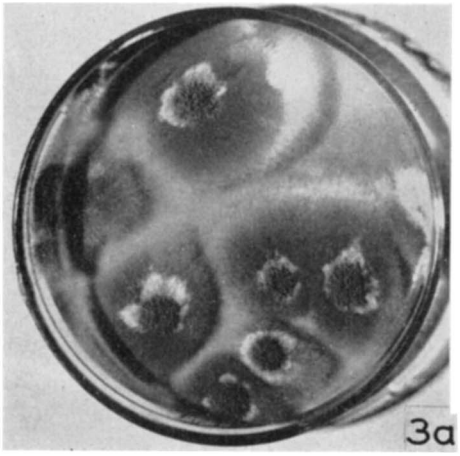
Inferred type of original aneuploid	Linkage group	Diploid R				Diploid C			
		Homologue				Homologue			
		Segregating	Homozygous	No.	Sector ratio +:nondisj.	Segregating	Homozygous	No.	Sector ratio +:nondisj.
2n + 1	I	Ia	. .	1	11:10	Ic	. .	1	9:10
		Ib	. .	4	97:23				
2n + 2						Ic	IVd	1	10: 5
						Ic	Vd	1	15: 5
2n + 1	II	IIa	. .	1	26:13				
		IIa-b*	. .	1	3 types				
		IIb	. .	2	22:26	IId	. .	1	17: 8
2n + 2		IIa	VIIIa	1	5: 6	IIc	IVd	1	30: 6
2n + 1	III	IIIa	. .	5	87:38	IIIc	. .	2	34:11
		IIIa-b*	. .	1	3 types				
		IIIb	. .	5	147:66	IIId	. .	2	24: 8
2n + 2		IIIa	Vb	1	15: 2	IIIc	Vc	1	15:11
		IIIb	Ia	1	22: 4				
		IIIb	IVa	1	26: 5				
2n + 1	IV	IVb	. .	1	3: 2				
2n + 2						IVd & IVc-d	VIIc	1	45:10
2n + 1	V	Vb	. .	2	30:13				
2n + 1	VI	. .				VIc	. .	3	47:21
2n + 2						VIc	Vc	1	15: 6
2n + 2	VII	VIIb	IVa	1	10:10				
2n + 1	VIII	VIIIa	. .	3	46:25	VIIId	. .	3	49:22
2n + 1	Total			26	469:216			12	180:80
2n + 2	Total			5	78:27			6	130:43
Total				31				18	

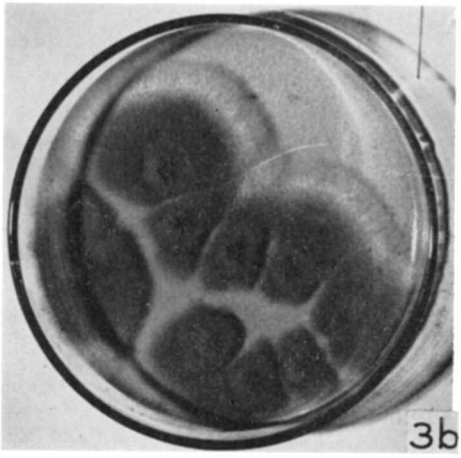
* Aneuploidy preceded by mitotic crossing over.

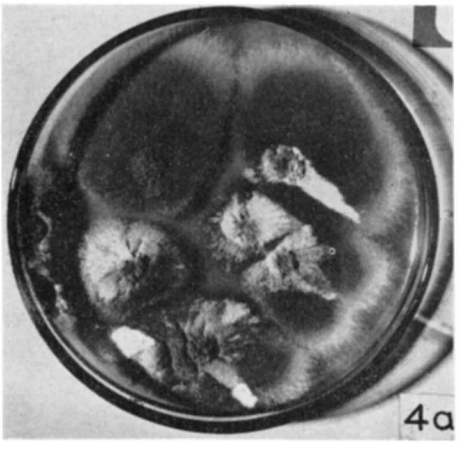
It is concluded that the centers of these segregants are aneuploids of the hyperdiploid type, trisomic for a single chromosome (2n + 1). The reduced growth rate of the center is due to general unbalance produced by the additional chromosome, rather than gene dosage effect of the mutant present in any one case. This became clear when it was found that aneuploids trisomic for the same linkage groups look exactly the same, if originating from the same diploid regardless of which homologue is present twice (Figures 2b and 3b), and even if originating from differently marked diploids. Aneuploids trisomic for different linkage groups on the other hand show different appearances. Two of the eight 2n + 1 types, trisomic for one of the eight linkage groups are shown in Figures 2 and 3. The replated segregants shown in Figure 2 each originated as an independent event, while the corresponding types in Figure 3 are intermediate segregation products replated from a 2n + 3 segregant (R 133). Figures 2a and 3a show hyperdiploids with an extra homologue of linkage group I (Ib), Figure 2b a trisomic with an extra homologue IIIa, Figure 3b with an extra IIIb. This remarkable phenotypic identification of specific trisomics closely parallels the different recognizable tri-











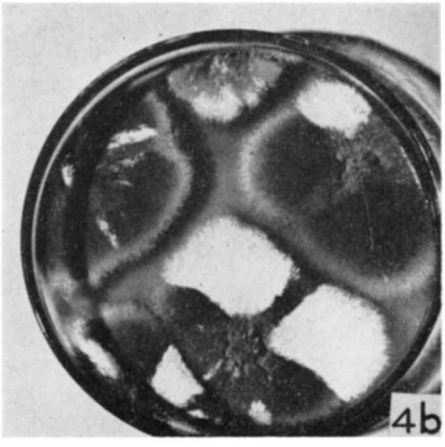


FIGURE 2.—Trisomics of independent origin (a) 2n + Ib; (b) 2n + IIIa. FIGURE 3.—Trisomics isolated as intermediate segregation products from a hyperdiploid (R 133) (a) 2n + Ib; (b) 2n + IIIb. FIGURE 4.—(a) Hyperdiploid R 133, 2n + Ib + IIb + IIIb; (b) Segregant from R 133, 2n + IIb + IIIb.

somic types in various species of higher plants (e.g. in Datura, for which a wide variety of aneuploids have been identified, BLAKESLEE and BELLING 1924).

Abnormal segregants of the types (3) and (4), showing frequent and regular nondisjunctional segregation for markers on two or three chromosomes, are then logically classified as 2n + 2 or 2n + 3. This seems all the more justified since secondary 2n + 1 or 2n + 2 intermediate types were usually recovered from these (all identified ones are listed in Table 4). From a particularly well analysed 2n + 3 segregant (R 133) ten different intermediate types, either 2n + 1 (Figure 3) or 2n + 2 (one shown in Figure 4) were recovered and identified (over 1000 sectors from 11 secondary platings were tested). Four of these intermediate aneuploid types were found to be homozygous for one of the three extra chromosomes, as can be expected to occur in a third of the cases. If care is taken in the collection of conidia from the center of aneuploids, such platings are amazingly uniform, giving mostly centers of the original type and only occasional ones of a type closer to diploid.

It seemed logical, therefore, to conclude that sectoring segregants which show homozygosity for markers of one or two chromosomes (the types 2 and 4) are such intermediate segregation products and that the original aneuploid segregant producing them was trisomic for the corresponding linkage groups. The ploidy of

TABLE 4

Hyperdiploid segregants showing high frequency sectoring for more than one linkage group

Inferred type of original aneuploid	Diploid of origin	Genotype of original aneuploid	Recovered intermediate aneuploid stages	No. of sectors tested
2n + 2	C	2n+Ic+IId	2n+Ic, 2n+IId, 2n(Ic/Ic)+IId	78
	R	2n+Ia+IIIa	2n+Ia	52
	R	2n+IIb+IIIb	2n+IIIb	20
	C	2n+IId+Vd	2n+Vd	23
	R	2n+IIb+VIIa	2n+IIb, 2n(IIb/IIb)+VIIa, 2n(VIIa/VIIa)+IIb	41
	C	2n+IIIc+IV(c-d)*	2n+IIIc	105
	R	2n+IIIa+VIIIb	2n+IIIa, 2n+VIIIb	26
2n + 3	R	2n+Ib+IIb+IIIb	2n+Ib+IIb, 2n+Ib+IIIb, 2n+IIb+IIIb, 2n(IIIb/IIIb)+Ib+IIb, 2n(IIb/IIb)+Ib+IIIb, 2n+Ib, 2n+IIb, 2n+IIIb, 2n(IIb/IIb)+Ib, 2n(IIIb/IIIb)+Ib	1250
2n + 3	Inferred from intermediate recovered stages			
	C	2n+Ic+IId+VIc	2n+VIc, 2n(VIc/VIc)+Ic+IId	21
	C	2n+Ic+IId+VIIc	2n(VIIc/VIIc)+Ic, 2n(VIIc/VIIc)+IId	71
	C	2n+IId+IVd+Vd	2n(IVd/IVd)+IId+Vd	10
	C	2n+IId+VId+VIIId	2n(VId/VId)+IId)+VIIId	18
2n + 4	Inferred from intermediate recovered stages			
	C	2n+IId+IIId+Vd+VIIId	2n+IId+Vd+VIIId, 2n+IIId+Vd	69
	C	2n+IVd+Vd+VIc+VIIc	2n(Vd/Vd, VIc/VIc)+IVd+VIIc	52

* Mitotic crossover chromosome.

the original segregant is therefore inferred correspondingly and, for example, all segregants homozygous for one chromosome, which segregate in the sectors for a second one (type 2), are listed as originally 2n + 2 (Table 3). Similarly, trisomy for three or four chromosomes is inferred in most of the cases listed in Table 4. That their centers were not recovered, can be accounted for by the sparsity of conidia in these higher aneuploid types so that conidia collected for replating are likely to be of a segregant, less abnormal, type.

In three cases many diploid recombinant sectors were found which did not show the expected "nondisjunctional" phenotype. Haploids isolated from these segregants showed that in all cases a mitotic crossing over had preceded aneuploidy and one or two of the trisomic chromosomes were products of a single mitotic exchange. Of the two cases from diploid R the segregant R 26 contained three different homologues of linkage group III, the two original *phen2 s0*$^+$ and *phen2*$^+$ *s0* and a third, a crossover type, *phen2 s0*. It produced frequent diploid sectors, wild type or homozygous for either *phen2* or *s0*, showing apparently high frequency of twin spotting. The segregant R 21 carried three homologues of linkage group II (*Acr*$^+$ *w2 ni3*, *Acr*$^+$ *w2 ni3*$^+$, *Acr w2*$^+$ *ni3*), two of them the reciprocal products of a mitotic exchange, and produced frequent segregant sectors, either homozygous *w2* or *ni3*. The third case, from diploid C, gave *pyro* and + sectors, and contained the two original (*meth pyro*, and *meth*$^+$ *pyro*$^+$) and a crossover (*meth*$^+$ *pyro*) homologue of linkage group IV.

(b) *Unstable types, sectoring without segregation of markers (unmarked chromosomes, diploid C)*: The most difficult types to interpret are unstable segregants which form sectors but do not show segregation for any marker, and are classified as types (5) and (6). Segregants of this type from diploid C may contain an extra chromosome which carries no marker and many show an appearance consistent with this assumption. Their frequency (compared to the corresponding identifiable types) and the fact that three of the total of 12 such cases from diploid C are found to be of type (6), with centers and sectors nondisjunctional for the markers of one chromosome, support this hypothesis.

Similar segregants from diploid R cannot be explained in this way. Most of them also show a very different appearance, namely extremely aconidial centers and relatively small conidating sectors. One of them shows extremely small conidial sectors and resembles very closely a rare type of translocation segregant. It is therefore classified as such. The nine others looked very much alike, all of them with aconidial vigorous centers; one of them was found as a large sector. Detailed analysis in one case did not reveal any change in the genotype. The absence of chromosomal segregation and the origin of these types as sectors calls to mind similar, cytoplasmically determined, sectors found in *Aspergillus glaucus* (SUBAK-SHARPE 1956). Also, acriflavine which has been shown to induce cytoplasmic aconidial types (ROPER 1958) appeared to increase the frequency of such aconidial sectors. But further analysis will be needed, to decide, whether any of these aconidial, sectoring types are in fact cytoplasmic segregants.

Furthermore, among these "abnormals" from diploid R which produce sectors but not segregation for any of the markers there are five cases which did not show

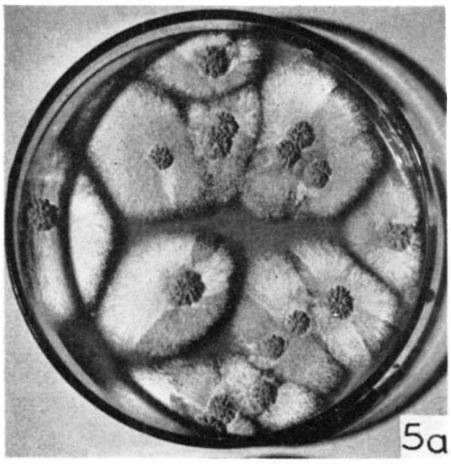
the aconidial appearance; four of them resemble certain hyperdiploid types, but since no photographs were taken, it is not possible to classify them with certainty (all aneuploids are extremely unstable, so that they cannot be transferred onto slants, and are only kept in the original or replated petri plates for a limited time). They might possibly carry one extra chromosome, which has lost its marker by mitotic crossing over. One additional case, which grew well but somewhat irregularly and produced very rare normal looking diploid sectors, showed several changes in the genotype, indicating the occurrence of a spontaneous aberration.

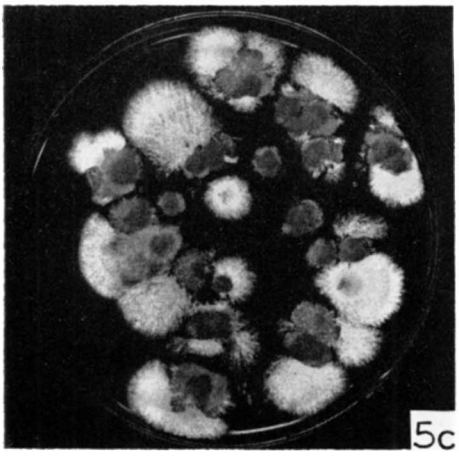
(c) *Hyperhaploids:* Sectoring segregants which give haploid sectors only are classified as hyperhaploids (type 7). In all cases they show the mutant phenotype of several chromosomes, for which they are presumably hemizygous. They always segregate for all other linkage groups, giving in the haploid sectors approximately a 1:1 ratio for the two homologues of each of these disomic linkage groups. Surprisingly few hyperhaploids were recovered (Table 6), but the few types found here correspond exactly to the hyperhaploids isolated with high frequency when conidial heads of mutant color were "needle plated" (KÄFER 1957, 1960). Again the number of extra chromosomes was usually low, four being the highest number of disomic chromosomes found. As found in hyperdiploids, hyperhaploids with increasing numbers of extra chromosomes are increasingly abnormal (Figure 5a–d) with n + 3 producing extremely tiny, practically aconidial centers. And, as found in the case of the 2n + 1, haploids with one extra chromosome have a specific growth type, according to which linkage group is disomic. Figure 5a shows a disomic for linkage group III (which produces sectors of two types, those carrying IIIb lighter than those carrying IIIa) with an appearance very similar to that of trisomics 2n + III (Figures 2b and 3b). Figure 5b shows another n + 1 segregant, disomic for linkage group IV, which forms considerably larger centers.

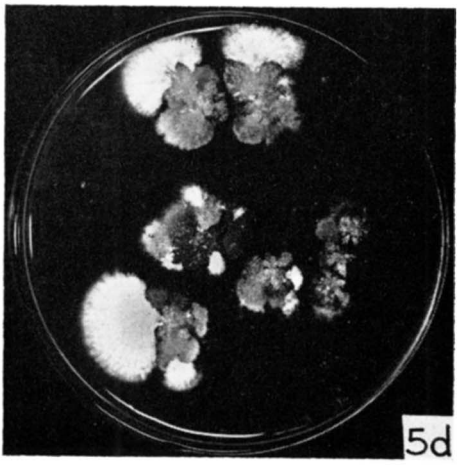
No attempts have been made to determine the frequency of the secondary segregation events in the aneuploids. The size of the aneuploid center and the number of conidia it produces obviously depend to a large extent on the growth rate of the abnormal type as well as on the chance occurrence of an early or late secondary segregation step.

(d) *Breakdown types:* A further type of unstable segregants (type 8) was found which produces a bewildering variety of different nondisjunctional as well as many haploid sectors. One of the four cases from diploid C is given in detail in Table 5b (only the genotypes of stable recovered sectors are listed). To permit simple tabulation of many different types, the symbol of each homologue is used to indicate homozygosity or hemizygosity of all corresponding markers (a or b from diploid R, c or d from diploid C, see Figure 1), while + indicates wild-type phenotype and presumably heterozygosity (? is used where epistasis does not permit distinction of the different types by simple tests).

Two cases from diploid R, which looked very similar, were both recovered as single colonies in secondary platings of hyperdiploid segregants. One of them was found in a replating of a 2n + Ib + IIIb intermediate type from the well analysed







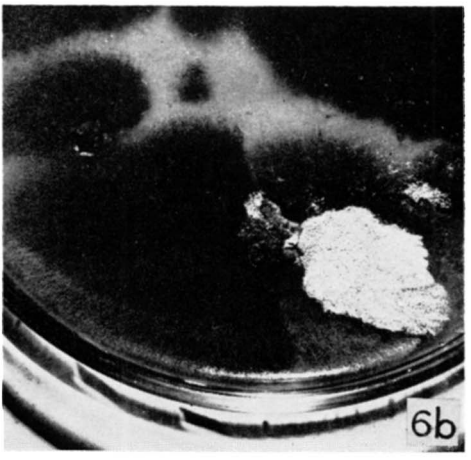


FIGURE 5.—Hyperhaploids: (a) and (b) disomic for one linkage group, (a) n + III; (b) n + IV; (c) n + 2; (d) n + 3.

FIGURE 6.—Breakdown types. (a) R 133.1, original isolate from R 133. (b) R 133.1.13, secondary isolate from R 133.1.

2n + 3 (R 133). The extremely abnormal colony grew to a large size, producing tiny conidiating sectors all over, and only one large, diploid sector was able to outgrow it on one side (segregant R 133.1, see Figure 6a). When conidial heads from different areas were replated, only one second order colony of the same type was recovered (segregant R 133.1.13, Figure 6b). All segregants recovered from both these colonies are listed in Table 5a. The haploids show that at least in R 133.1 all recessive markers were still present. Since only stable 2n and n sectors were isolated by transferring conidia from sectors, needle platings were made to recover possible intermediate steps. While several aneuploids were recovered in this way, it turned out that they all contained the same extra homologue (IIIb) present in the segregant of origin (R 133). This can therefore not be taken as evidence for a stepwise segregation as found in the aneuploids discussed above. In none of these cases was it possible to deduce the genotype from the recovered sectors. Whether a different process is operating, or whether special competitive conditions are present, which might be caused by an unusually high aneuploid chromosome number, is still a matter of speculation.

(e) *Lost unstable types (Table 1):* As mentioned above, extremely abnormal types are often lost, when they form stable sectors very early (type 9) or when normal colonies grow and conidiate freely close by (type 10). In most cases, where sectors but no centers were recovered, these were of at least two types, mostly diploids, nondisjunctional for one or two chromosomes; only in a few cases were haploid sectors present. It seems likely, therefore, that these segregants were highly unstable aneuploids, most of them of a hyperdiploid type. The small number of different sectors recovered did not permit the deduction of their genotypes.

(f) *Translocation segregants (from diploid R)*: A large number of sectoring segregants showed segregation patterns which were of a completely different type. Since in all cases the markers of the two translocation linkage groups (VI and VII) behaved differently from all others, it became obvious that the abnormal type of segregation obtained was due to the structural heterozygosity (type 11). Also, many of these segregants were directly comparable to segregants obtained from diploids heterozygous for another well-analysed translocation (and will therefore be discussed together with these and published elsewhere). These unstable segregants fall phenotypically into three main groups, some showing haploid sectors only, others showing diploid and haploid sectors, and a rare aconidial type giving diploid sectors only.

The observed frequency of such translocation segregants is surprisingly high (a total of 22 among about 9900 colonies from diploid R), but consistent with the hypothesis that these segregants arise mainly by mitotic crossing over in the structurally heterozygous arms of the linkage groups involved in the translocation.

DISCUSSION

The results presented above show that most mitotic segregants obtained from the two diploids studied are easily classified into the four categories of segregants which have been identified in earlier work: (1) diploid mitotic crossovers and

TABLE 5

Phenotype of all recovered sectors from "Breakdown" types

(a) Segregant R 133.1, recovered from plating of R 133 and segregant R 133.1.13, recovered from plating of R 133.1

Ploidy	Color	Linkage groups VIII	I	II	IV	V	VI	VII	III	Numbers 133.1 Transferred	133.1 Plated	133.1.13 Transferred
2n	dark green	a	b	+	+	+	+	+	+	5	.	1
	white	..	..	b	+	+	+	+	+	1	.	.
	dark green	..	..	+	a	+	+	+	a	.	.	4
2n + 1	dark green	..	..	+	+	+	+	+	+/b	.	3	.
n	yellow-chartreuse	b	a	a	a	b	a	a	b	1	1	.
n	white	a	b	b	a	a	a	a	a	2	.	2
		..	..	..	.	..	..	..	b	.	.	1
n + 1	grey	..	..	..	..	..	..	..	+	.	5	.
n	white	..	..	..	..	a	b	b	a	.	.	2
		..	..	..	..	..	..	..	b	1	.	.
		..	..	..	..	b	a	a	a	1	.	.
		..	..	..	..	b	b	b	a	.	.	2
		..	..	..	..	..	..	..	b	1	1	.
		a	b	b	b	a	a	a	b	.	1	.
n	dark green	a	b	a	a	a	a	a	a	.	1	.
		..	..	..	..	a	b	b	a	.	.	1
		..	..	..	..	b	a	a	b	.	.	1
		a	b	a	b	a	b	b	b	.	1	.
n + 1		..	..	..	..	b	b	b	+	.	1	.
n		..	..	..	..	b	a	a	b	.	1	1

(b) Segregant C 22

Ploidy	Color	Linkage groups I	II	III	IV	V	VI	VII	VIII	No.	
2n	green	+	+	+	+	+	+	+	+	19	
		..	..	..	..	..	c	+	+	9	
		..	..	..	..	..	+	+	d	1	
		+	+	+	d	+	+	+	+	1	
		+	+	+	d	+	c	+	+	1	
		+	+	c	+	+	c	+	+	1	
	dark green	d	+	+	+	+	+	+	+	1	
	white	+	d	+	+	+	+	+	+	1	
		..	..	..	..	..	c	+	+	1	35
n	yellow	c	c	d	c	c	c	d	c	1	
		..	..	d	d	c	d	d	c	1	
	white	c	d	d	c	c	c	d	c	1	
		..	..	c	d	c	c	c	c	1	
		..	..	c	d	d	?	d	c	1	
		d	d	d	c	c	c	c	c	1	
		..	..	d	c	c	c	d	d	1	7

Absence of a symbol for any linkage group indicates identity with the one above.

(2) diploids of a nondisjunctional type, (3) haploids and (4) aneuploids. While no more than random coincidence of mitotic crossing over with any of the other three types is found, it is confirmed that there is a connection between the latter three, in so far as aneuploid segregants regularly and with apparently high frequency produce either or both of the other two types, namely nondisjunctional diploid and haploid sectors (KÄFER 1960). At least two, and possibly only two, independent processes are therefore responsible for the appearance of mitotic segregants in *Aspergillus nidulans*: the first is mitotic crossing over, the second a process of chromosomal segregation.

(a) Mitotic crossing over

While the present analysis was designed to obtain information about the second of these processes, some new data on mitotic crossing over have also been obtained, due to the nonselective techniques used here. Mitotic crossing over occurs in the four strand stage of the mitotic division, with the result that two of the four strands are of a recombinant, two of a parental type. Segregation of the centromeres is normal, producing either two reciprocal segregant daughter nuclei each with one crossover and one parental strand, or one parental and one recombinant nucleus, the latter containing both crossover strands. This process was first elucidated by STERN (1936) in his brilliant analysis of somatic recombination in *Drosophila melanogaster*. There, cases of the first type of centromere segregation could be detected in the so-called "twin spots," which consist of two adjacent patches showing reciprocal mutant phenotypes, corresponding to two recessive markers originally in repulsion on the same chromosome arm. Such twin spots cannot be recovered easily with the markers available so far in *A. nidulans*, but in the present analysis two have been found: one showed a crossing over in linkage group I proximal to *paba*, the other in linkage group VIII, proximal to *ribo2*. The other type of centromere segregation, that produces a nucleus containing the two reciprocal crossover strands, has been identified in several cases in Aspergillus, all of them selected as mitotic crossovers between alleles of a single gene (ROPER and PRITCHARD 1955). In the present analysis two cases of a nucleus containing the two reciprocal products of a single mitotic exchange between genes were encountered: one showed a crossing over in linkage group VIII, which occurred very early in the purification of diploid C, the other a crossing over in linkage group II, which led to the formation of unexpected types of sectors in an aneuploid (R 21). These cases show conclusively that, as postulated by PONTECORVO *et al.* (1954) and supported by later evidence (ROPER and PRITCHARD 1955; PONTECORVO and KÄFER 1958), reciprocal mitotic crossing over followed by either of the two normal types of segregation of the centromeres is operating in *Aspergillus nidulans*.

Under standard conditions, in structurally homozygous arms, mitotic crossing over shows a regular and characteristic distribution. Compared to meitotic maps it appears to be relatively more frequent in proximal parts of the chromosome arms (in Drosophila as well as in Aspergillus) and may actually correspond

more closely to the physical length of the chromosomes. Absolute frequencies of mitotic crossing over in different arms, therefore, are not much influenced by the exact position of a fairly distal marker and the average frequency of mitotic crossing over per marked chromosome arm is a meaningful value which may be used to compute the total incidence of mitotic crossing over per mitosis. An average value of 0.07 percent (Table 2) was obtained for the frequency of mitotic crossovers homozygous for a single color marker and of about 0.05 percent for the "requiring" markers, which are expected to be less viable. These frequencies are slightly higher than the values obtained earlier, when an average of 0.04 percent for "requiring" segregants (7800 colonies tested from a diploid with four heterozygous nutritional markers, KÄFER, unpublished) and an average of 0.03 percent of segregants homozygous for a single color marker (y or w) were detected (PONTECORVO *et al.* 1954). In the two diploids studied here it was found that relatively more segregants were recovered when the density in the original platings was decreased. The difference between the two diploids R and C (Tables 1, 2 and 6), as well as the differences between the present and the earlier experiments, are therefore considered to be due to differences in experimental procedure rather than to strain differences. For the estimate of the over-all incidence of mitotic crossing over in *A. nidulans* (with at least 14 genetically fairly long chromosome arms in the haploid set) a value of about 2 percent is obtained ($14 \times 2 \times 0.07$ percent, assuming that both types of centromere segregation are equally frequent); that is, on the average one in 50 mitotic divisions show such an event.

No more than random coincidence of mitotic crossing over in different chromosomes is observed, as has also been found earlier for coincidence in different intervals of the same chromosome. This is in great contrast to the results obtained when selection is applied within a small segment. Negative interference and/or nonreciprocal exchanges are found to a very large extent in the latter case (PRITCHARD 1960), as has been observed also in yeast (ROMAN and JACOB 1958).

Results from the segregation in diploid R indicate that mitotic crossing over in structurally heterozygous chromosome arms leads to the formation of unbalanced, sectoring types, which look like aneuploids produced by chromosomal segregation. They are presumably monosomic and trisomic for translocated pieces (details will be published elsewhere). The absolute frequency of these translocation segregants was found to be about 0.2 percent (Table 1). This frequency, presumably the frequency of mitotic crossovers homozygous for either one of the three markers on the involved chromosome arms (comparable to the value of 0.05–0.07 percent observed for single markers in other arms) is about half the frequency of all identified aneuploid chromosomal types from the same diploid (0.4 percent, Table 1). This shows how structural heterozygosity, even if of a simple type, can greatly influence the proportion of the different segregant types. Many similar and some more extreme segregation abnormalities have been encountered in the preparation for this analysis, when various irradiated mutant strains were checked for chromosomal aberrations.

The high frequency of very aberrant segregation patterns in the same test diploids (R and C) encountered after treatment of conidia with mutagenic agents

will be discussed in detail elsewhere; for example, after high doses of gamma radiation it is found that the competitive, often haploid, products of segregation show a high coincidence of mitotic crossing over and chromosomal segregation, and many stable products appear to be permanent aneuploids (KÄFER, unpublished). It is not surprising, therefore, that in *Penicillium chrysogenum* results from spontaneous and nitrogen mustard induced segregation differ widely (MUCHNIK 1961). It seems likely that the high frequency of segregation after treatment with agents which produce a high rate of chromosome breaks is only apparent, since nuclei which had lost the damaged chromosomes and contained a haploid set of normal ones would be at a selective advantage and produce sectors. Segregation induced by such agents would therefore be a mechanism of recovery different in each differently damaged cell and not be very useful for the mapping of new markers. More specifically acting substances, however, may perhaps affect one of the segregation processes more directly (e.g. it is claimed that para-fluoro-phenylalanine increases chromosomal segregation only; MORPURGO 1961; LHOAS 1961; the evidence is, however, mostly based on the recovery of haploid sectors, which cannot decide this question).

So far, mitotic crossing over has been identified with certainty only in a very few organisms which show somatic segregation: in addition to Drosophila it is assumed to occur in yeast as a reciprocal process (ROMAN and JACOB 1958). It also seems to occur in the endosperm of maize (MCCLINTOCK 1932; JONES 1937) but other processes producing variegation appear to be much more frequent (MCCLINTOCK 1941, 1956; JONES 1944; BRINK 1958). Some evidence for mitotic crossing over has also been obtained in other fungi: in *Penicillium chrysogenum* (SERMONTI 1957) and in *Aspergillus niger* (HUTCHINSON 1958) and some kind of mitotic exchange seems to occur in the basidiomycete *Schizophyllum commune* (CROWE 1960). In many other cases where variegation occurred, mitotic crossing over does not appear to provide a likely explanation. It seems possible, however, that some cases of regular mitotic crossing over are not recognized because the segregation patterns are distorted in the presence of aberrations. For example a case of an unstable diploid of *Aspergillus nidulans* has been reported by MORPURGO and SERMONTI (1960); it showed sectors produced by mitotic crossing over in linkage group I and an unusual type of segregation in II (interpreted as breakage fusion cycle) which could well be due to the presence of an aberration. Also, the rejection of mitotic crossing over as an explanation of twin spots in tomatoes, on the grounds that no somatic pairing is observed (ROSS and HOLM 1960), does not seem justified until more is known about the relationship of visible pairing and mitotic crossing over.

(b) Chromosomal segregation

The present analysis shows that in *Aspergillus nidulans* segregants of a second type are produced by some kind of chromosomal segregation. It has been postulated that a single process would produce all these "chromosomal" segregants—aneuploids, nondisjunctional diploids and haploids (KÄFER 1960). The present

results support this hypothesis. This process is assumed to be essentially a two step mechanism. The first step happens in the original (heterozygous) diploid: as a rare event of misdistribution of a small number ($x = 1$ or 2) of chromosomes it produces two reciprocal aneuploids, one trisomic the other monosomic for the chromosomes in question ($2n + x$ and $2n - x$). Such a process would be called nondisjunction by definition. The unbalanced types produced would show reduced viabilities and the recovery of the two aneuploids would depend on their abilities to form conidia. The second step would be produced by further (possibly independent) occurrences of the same process in the aneuploid segregants. The chance of such secondary nondisjunction would depend on the number of divisions produced by the aneuploid nucleus in competition (or symbiosis, see below) with the normal nuclei. Wherever a secondary step of segregation would restore the diploid or produce a haploid chromosome number a normally viable segregant would be formed.

This two step mechanism is suggested by the following main observations: (1) That the primary event is likely to be nondisjunction of one or two chromosomes is deduced from the fact that the majority of primary aneuploids from the diploid are of the type $2n + 1$ or $2n + 2$ (56 out of the 78 identified cases, Table 6). The corresponding $2n - 1$ and $2n - 2$ types are not found, however, and further possibilities will be discussed below. (2) The stepwise secondary process of loss of single chromosomes is directly observed in all hyperdiploids and hyperhaploids with more than one extra chromosome and intermediate types are easily recognized and isolated in all cases (for hyperdiploids see Table 4).

(1) *The primary event of chromosomal segregation in the diploid:* Since the frequencies of the different types of aneuploid segregants (Table 6) do not obviously agree with the postulated mechanism of nondisjunction (e.g. no $2n - 1$ and $2n - 2$ are found) the two other most plausible processes of chromosomal segregation which have been found to operate in tissues of higher organisms are also considered here. The most obvious one is complete breakdown of mitosis resulting in random distribution of chromosomes (first discussed as a possible mechanism of haploidisation by PONTECORVO *et al.* 1954). Such a mechanism would produce all different types of aneuploids as well as haploid, triploid and tetraploid segregants. The other is somatic reduction or genome segregation (HUSKINS 1948; GLÄSS 1957; STERN 1958), which produces four haploids or near-haploids from a dividing diploid nucleus. The latter has been briefly discussed earlier (KÄFER 1960) and is not supported by any evidence from the present results. It would mainly produce haploids and hyperhaploids, which were both observed so rarely (among a total of 13,500 conidia from the two diploids R and C two haploids and nine hyperhaploids were found, the latter being about 1/7 of all aneuploids), that this process could not contribute more than a very small fraction of all segregants.

The two main mechanisms which have to be considered for the primary segregation event in the diploid are, therefore, nondisjunction and random distribution of chromosomes. Both would produce the types which were most frequently recovered ($2n + 1$, $2n + 2$) as well as others which were not found (nondisjunc-

TABLE 6

Frequency of the different types of aneuploid segregants

Type		Diploid R		Diploid C		Frequency R	Frequency C
Hyperdiploids							
(a) Identified:							
2n + 1			26		12	..	..
2n + 2	2n + 1, homozygous for 1	5		6		..	..
	2n + 2	4	9	3	9	..	..
2n + 3	2n + 2,homozygous for 1	0		3		..	..
	2n + 3	1	1	2	5	..	..
2n + 4	2n + 2, homozygous for 2	.	.	.	1	..	..
(b) Segregating for unmarked chromosomes			5?		12?	..	..
Total			41		39	0.4%	1.1%
Hyperhaploids							
n + 1			2		1	..	..
n + 2			1		1	..	..
n + 3			1		2	..	..
n + 4			0		1	..	..
Total			4		5	0.04%	0.15%
Breakdown types*							
Hyperdiploids (?), giving haploids			2		4	0.02%	0.1%
Total of analysed aneuploids			47		48	0.46%	1.3%

* Several of these were second order segregants.

tion mainly 2n − 1 and 2n − 2, while random distribution would also produce many others). Thus in either case it has to be assumed that some of the types are too inviable to be recovered. The reduction in viability shown by the different aneuploid types recovered supports these assumptions. While the most viable aneuploids, 2n + 1 and n + 1, grow to a fair size (the central part of the colonies in Figures 2, 3, 5a and 5b) before they are outgrown by their respective 2n or n sectors, neither is able to compete when grown in mixture with the sector types. As expected, aneuploids with two or three extra chromosomes grow even more slowly and are very poorly conidiating (see the very central parts in the colonies of Figures 4, 5c and 5d). Even segregants which are probably monosomic for only part of a chromosome show reduced growth rate and conidiation.

The problem of deducing the frequency of events from the observed incidence of certain surviving, permanently changed, products has been excellently discussed by PUCK (1960) in connection with his attempts to measure the dose of radiation which produces an average of one primary chromosome break per cell in tissue cultures of human cells and to compare it with similar earlier observations. In the present case growth rate and especially formation of conidia are the main factors influencing the recorded frequency of the different primary aneuploid products, and recovery depends on especially favorable circumstances (which explains why no aneuploids were recorded among the 13,000 inspected colonies from conidial platings with a higher density than used here, PONTECORVO *et al.* 1954).

The most frequent and fairly viable class of 2n + 1 hyperdiploids is therefore used separately to compare with the predictions of the hypothesis of random distribution of chromosomes. Among the total of 49 recovered 2n + 1 types, 38 were heterozygous for all seven disomic linkage groups and the other 11 were homozygous for one and none for more than one. On the basis of a random distribution of chromosomes, less than one case is expected to be of the first kind and about four of the second, while the rest (44) should be nondisjunctional (homozygous) for more than one linkage group. Thus, there is no likelihood at all that random distribution of chromosomes could produce the observed proportion of the two kinds of 2n + 1 hyperdiploids, nor is this process likely to be responsible for most of the rarer types. However, its rare occurrence cannot be excluded. Nondisjunction, on the other hand, is expected to produce mainly 2n + 1 types which show no segregation for all the disomic linkage groups. Consequently it is assumed that the 2n + 1 types, homozygous for a second linkage group, are not primary products but result from secondary loss of a chromosome in a 2n + 2.

(2) *The secondary process of stepwise loss of chromosomes:* Only a small number of aneuploids other than 2n + 1 and n + 1 have been recovered and identified (14 hyperdiploids and six hyperhaploids, Table 6). All these produce sectors of many different kinds, aneuploid with fewer extra chromosomes and euploid. The former type is still "abnormal," usually fairly small and situated close to the center, and regularly produces euploid sectors at the periphery in a further step of segregation (Figures 4, 5c and 5d). That is, loss of single chromosomes is found in all cases, but it is not possible to decide whether occasionally two or three chromosomes may not be lost in a single event. The regularity and randomness with which one of the three homologues is lost in a 2n + 1 aneuploid is indicated by the almost perfect 2:1 ratio, among the 2n sectors, of wild type (heterozygous):nondisjunctional (homozygous) type (see Table 3). Nondisjunctional diploid types are therefore produced with high frequency from hyperdiploids and in no case has origin from a 2n − 1 been observed.

While there is no doubt that a stepwise loss of single chromosomes occurs in isolated aneuploids, it is not possible to tell whether the process or its frequency are the same in the mycelium of an original colony where diploid and aneuploid nuclei divide in the same cytoplasm. It seems possible that aneuploids show a higher rate of division under such heterokaryotic conditions and certain types are able to divide which are not recovered when conidia are plated. This would explain how haploids could be formed by the stepwise loss of single chromosomes from intermediate products (2n − 1 = n + 7, n + 6 and n + 5) which have never been recovered (contrary to optimistic statements by PONTECORVO in CAVALLI-SFORZA *et al.* 1959).

That most haploids are produced from intermediate aneuploid types has been found consistently in all well analysed diploids. Using the specially favorable method of isolating conidial heads showing mutant color by "needle plating," it was possible to identify such unstable aneuploid precursors in most of the 73 recovered cases of haploids (KÄFER 1960). In the present analysis, however, a

rare type of segregant was recovered which, while being at least 2n, frequently produced haploids without much evidence of intermediate steps (breakdown types, Tables 5 and 6). The segregation seems to be of a chromosomal type, but details of the process or the exact constitution of the breakdown types are not understood. They form a thin, aconidial mycelium which competes extremely well with the numerous conidiating normal segregants it produces (Figure 6), in contrast to hyperdiploids and hyperhaploids, which are regularly outgrown by their sectors.

The mechanism of the secondary process of loss of single chromosomes is assumed to be nondisjunction, but could equally well be lagging of single chromosomes as frequently observed in tissues of higher organisms, or both processes might be operating.

(3) *Frequency of nondisjunction:* It can be concluded that in diploids of *A. nidulans*, in accordance with the present hypothesis, haploids originate in two steps as secondary products from hyperhaploids, and similarly nondisjunctional diploids are formed by the hyperdiploids. The frequency of aneuploid segregants present among conidia of a diploid colony is sufficiently high (at least 1.5 percent, Table 6) to account for the production of all stable haploid (frequency less than 0.02 percent) and nondisjunctional diploid segregants (0.3 percent, extrapolating from the color segregants, Table 2). Thus the incidence of primary nondisjunction is likely to be of the order of two percent and about as high as the incidence of mitotic crossing over; mitotic segregation of either type would occur in *A. nidulans* in at least one out of every 25 mitoses.

Still unsettled is the question whether the frequency of this easily observable secondary process in the aneuploids is any higher than the one of primary nondisjunction in diploids. In view of the extremely different growth rates in these various types it seems impossible to measure and compare such frequencies. It is felt that the main difference is one of competition: missegregation of a chromosome in the 2n produces inferior products, while the reverse is true for all observed aneuploids, since sectors of lower ploidy always conidiate better and outgrow the original. Lagging of chromosomes, if occurring in addition to nondisjunction, would produce a competitive product only in aneuploids; on the other hand, segregation in the aneuploid is of an advantage only if it occurs in the linkage group for which an extra homologue is present.

Only a few rare segregants do not fit into the postulated pattern; some are of an extremely aconidial type (from diploid R) and produce conidial sectors of various sizes but no segregation of markers. One of them has been tentatively classified as a translocation segregant, others which showed no change in the genotype as cytoplasmic segregants. It is hoped that further analysis will clarify their segregation patterns.

(4) *Conclusion:* It appears likely, therefore, that the second main process producing mitotic segregants in diploid *A. nidulans* is a process of misdistribution of single (or few) homologues in mitosis, generally called mitotic nondisjunction. Since hyperdiploids with more than one (usually two) extra chromosomes were relatively frequent (over 20 percent of the hyperdiploids), it must be postulated

that coincidence of nondisjunction in more than one chromosome pair is much higher than random. Similar findings for meiotic nondisjunction in Oenothera, where double nondisjunction seems to be a fairly frequent occurrence, are described by CATCHESIDE (1936) and more than random coincidence was also found by FROST (1961) in Drosophila. Meiosis in *A. nidulans* also produces aneuploids with fairly high frequency (PRITCHARD 1954), but these have not yet been analysed at all. A direct comparison is therefore not possible and further discussion will be restricted to mitotic nondisjunction even though it is felt that the striking parallels in mitotic and meiotic processes (crossing over as well as nondisjunction) may well be due to common features in the underlying mechanisms.

A similar process of mitotic nondisjunction seems to occur in *Penicillium chrysogenum*, where several unstable segregants have been found which give diploid segregant sectors of a single type (the small number of mapped markers made analysis of the responsible mechanism impossible, BALLIO and SERMONTI 1961); these are likely to be hyperdiploids. In higher organisms mitotic abnormalities seem to be more frequent in certain tissues (possibly those with higher ploidy, like endosperm or liver, GLÄSS 1957). It has been suggested that a triploid tissue of a normally diploid organism may show and tolerate a higher frequency of abnormal mitosis and exert less stringent control over the process of mitosis (BRINK and COOPER 1947). The same might then be true for an abnormally diploid strain of Aspergillus, which is a haploid organism. The high frequency of abnormal chromosome distribution in the diploid would then be a phenomenon due to the unusual ploidy (supported by the relatively higher frequency of chromosomal segregation observed among mitotic color segregants in triploids, ELLIOTT 1956, and personal communication). If this is the case, it could not be expected that a similar frequent process of nondisjunction would be operating in tissues of higher organisms.

The main question with respect to the general occurrence of this process of mitotic nondisjunction, however, is the general occurrence of a true mitosis itself. While there seems to be increasing agreement that an unorthodox type of mitosis without a conventional spindle is present in yeasts (ROBINOW 1961), details of mitosis in Neurospora are interpreted very differently by different workers (SOMERS, WAGNER and HSU 1960; BAKERSPIEGEL 1959). No analysis of mitosis in *Aspergillus nidulans* has been carried out so far. Clearly, if the control of vegetative division of nuclei in fungi is of a type different from that in higher organisms, it would also determine the related mechanism of missegregation of chromosomes. Consequently, it is felt that speculations on the actual mechanism will be more profitable when this question is settled (as well as discussions about a suitable term for this type of segregation, for which "mitotic" might be even more misleading than the less specific term "somatic").

Even if a true mitosis is found in Aspergillus and therefore a corresponding mechanism of nondisjunction might be expected in higher organisms, it is likely that its frequency varies, not only between widely different groups of organisms, but even between different strains of the same species as well as between different tissues of the same individual. Such differences would be caused by environ-

mental as well as genetic factors. For example SAX and PASSANO (1961) found that in human cells in tissue culture a nongenetic factor, the age of the culture, was one of the determining factors when the frequency of spontaneous chromosome aberrations was determined, and an interesting case of a single gene controlling the production of mosaic wings in a moth, most likely by loss of chromosomes, has been described by KÜHN (1960).

Differences with respect to the frequency of the various segregant types may therefore be found in either case, whether the processes of mitosis and of nondisjunction are the same or not in any two different organisms which are analysed, usually by different methods.

Very little is known about the process of primary nondisjunction in higher organisms. Aneuploids, on the other hand, are known in many species. Some differences appear to exist between fungi and higher organisms with respect to the frequency of the secondary essentially regulatory events in the aneuploids. While disomics in haploid Neurospora (PITTENGER 1954) and tetrasomics in yeast (Cox 1960) are as unstable as aneuploids in *A. nidulans*, trisomics in many higher plants have been found to be exceedingly stable and may even be transmitted through the female gamete (e.g. in Datura, BLAKESLEE 1921; Oenothera, GATES and THOMAS 1914; or maize, MCCLINTOCK and HILL 1930). Similarly many aneuploid strains of human cells in tissue culture seem to show surprising viability and stability, while euploid primary cultures often show increase or decrease by one chromosome even in the first transfer (CAVALLI-SFORZA *et al.* 1959). On the other hand instability of aneuploid types (2n + 1) and secondary nondisjunction leading to a more stable 2n type comparable to the process found here, have been postulated to explain human mosaics (GRIBOFF and LAWRENCE 1960). The observed differences and parallels in these results therefore encourage comparison of evidence from different organisms, which will be profitable as long as the possibility is kept in mind that the same results may be produced by different mechanisms, as well as that different results are produced by the same mechanism under different conditions.

SUMMARY

Most mitotic segregants recovered under optimal conditions from two well-marked diploids of *Aspergillus nidulans* appear to be produced by two main processes: (1) mitotic crossing over and (2) a process of misdistribution of small numbers of chromosomes (mitotic nondisjunction). Both processes seem to be about equally frequent and mitotic segregation of either kind is estimated to occur in at least one out of every 25 vegetative divisions.

(1) Mitotic crossovers were found to be about twice as frequent as previously reported. The complementary products of a single event were recovered in four cases, twice in a single nucleus, twice as twin spots. One of the analysed strains was a translocation heterozygote which produced 0.2 percent unstable segregants of a specific type, probably products of mitotic crossing over in the structurally heterozygous arms.

(2) Of the "chromosomal" segregants, unstable aneuploid types showed the

highest frequency (1–2%). Most of them were hyperdiploid, hyperhaploids being found only rarely. The most frequent types were $2n + 1$; they showed a recognizable phenotype characteristic for each trisomic linkage group. All aneuploids are unstable, that is, they are always outgrown by the rare, more vigorous diploid or haploid sectors which they regularly produce. The sectors from a $2n + 1$ are in $\frac{2}{3}$ of the cases of parental, in $\frac{1}{3}$ of nondisjunctional type with respect to the trisomic linkage group. Hyperdiploids as well as hyperhaploids show a stepwise loss of the extra chromosomes, presumably by the same process that produces aneuploids from diploids (but lagging of chromosomes could equally be the cause). Nondisjunctional diploid segregants are estimated to have a total frequency of about 0.3 percent, haploids of less than 0.02 percent. A few unstable segregants seem to be produced by other processes, some possibly by cytoplasmic segregation.

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