A GENETIC INVESTIGATION OF SOME ADENINE-REQUIRING MUTANTS OF *ASPERGILLUS NIDULANS*

by

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CONTENTS.

GENERAL INTRODUCTION

Since the development of techniques for the production, isolation and use in genetic analysis of nutritional mutants of micro-organisms (Beadle and Tatum, 1941), these have been increasingly used as tools in genetic analysis. The range of micro-organisms that can be studied genetically has been greatly widened by the introduction of special techniques which overcome the difficulties presented by homothallic moulds (Pontecorvo, 1946; Pontecorvo, Roper, Hemmons and Bufton, 1953) and even by the complete absence of a meiotic stage in the life cycle (Pontecorvo and Roper, 1953; Pontecorvo, Roper and Forbes, 1953; Pontecorvo and Sermonti, 1953). Techniques similar to those employed for the homothallic moulds have also been employed for the genetic analysis of bacteria (Lederberg & Tatum, 1946; Lederberg, 1947) and viruses (Hershey and Rotman, 1946; Luria & Dulbecco, 1949)

For the study of certain fundamental problems in genetics, micro-organisms offer important advantages. In particular, the ease with which clonal material can be obtained in almost unlimited amounts, and the use of selective techniques, permits the detection and isolation of novel types almost irrespective of their rarity.

This property has been extensively exploited for studies on mutation (e.g. Luria and Delbrück, 1943; Witkin, 1947; Jensen, Kirk, Giles, 1951; Kolmark and Westergaard, 1951; Demerec, 1954). Less extensively has it been exploited for investigations into recombination between extremely closely linked loci (Roper, 1950; 1953; Giles, 1951; Lederberg, 1952; Demerec, 1954; Pritchard, 1954; Benzer, 1955).

The primary aim of the work reported in this thesis was an analysis of the linear relationships between a series of extremely closely linked adenine-requiring mutants of Aspergillus nidulans. The results of this analysis are presented in the first section of the thesis in the form of a published paper. Before this analysis was possible a considerable amount of routine genetic analysis was necessary. This led to the discovery of ascospores with diploid nuclei, ~~which is~~ described in the second section. The final sections include the genetic analysis of a rearrangement.

I MATERIAL AND METHODS.

1. Life cycle of Aspergillus nidulans.

A. nidulans (Eidam) Winter, is a homothallic ascomycete. Detailed descriptions of its structure and life cycle are available elsewhere (Thom and Raper, 1945; Pontecorvo et al, 1953) and only the salient features will be redescribed here.

The vegetative mycelium consists of branched, septate hyphae, the 'cells' of which are multinucleate. The mycelium forms a compact colony on solid media. Anastomoses occur between adjacent hyphae which may have genetically different nuclei. As a result of such anastomosis two or more kinds of genetically different nuclei may occur within a single hypha, which is termed heterokaryotic.

The asexual spores (conidia) are uninucleate and develop on differentiated aerial hyphae. They are produced in chains (Fig. 1), the members of a single chain having genetically identical nuclei. Different chains produced by a single heterokaryotic conidiophore may have genetically different nuclei.

The sexual phase of the life cycle takes place in cleistothecia (It is the practice in this laboratory to use the term perithecium rather than cleistothecium and this terminology will be adhered to). The perithecia are spherical, about 100-200 μ in diameter and have a tough wall which breaks only under considerable pressure.

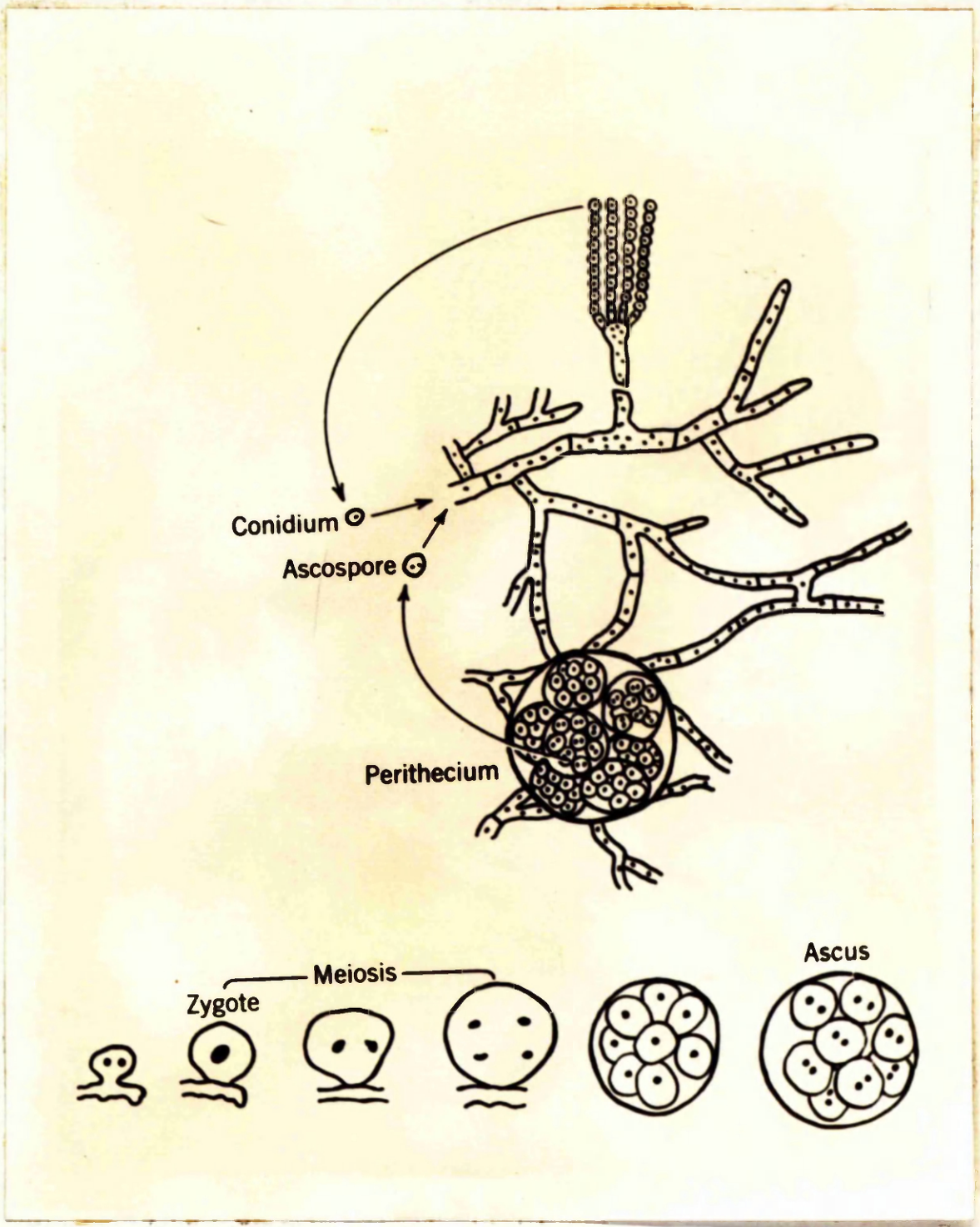

Fig. 1. The life cycle of Aspergillus nidulans (this photograph was previously published by Pontecorvo, Roper, Hemmons and Bufton - 1953).

A single perithecium may contain many tens of thousands of asci, each with eight ascospores which are easily liberated by rupture of the fragile ascus wall.

Cytological analysis of the events leading to ascospore formation is incomplete owing to the technical difficulties presented by the small size of the nuclei. It is certain, however, on both genetic and cytological grounds, that the eight ascospores of one ascus are derived from a single diploid nucleus. The eight spores represent the four haploid products of meiosis each duplicated by a single mitotic division.

Genetic analysis of the ascospores from asci of single perithecia borne by a heterokaryotic mycelium containing two types of nuclei (A and B) has shown (Hemmons, Pontecorvo and Bufton, 1952) that a single perithecium usually, but not inveriably, contains asci of one type only, either A xA, B xB, or A xB. Thus the many thousands of asci within one perithecium are usually derived ultimately from two original nuclei. These must either fuse early in the development of the perithecium, or undergo some kind of conjugate division prior to fusion in pairs in the ascus primordium. Cytological evidence (Pontecorvo, 1953; Elliot personal communication) is in favour of the latter alternative.

2. Media

Wild type A. nidulans will grow on media containing an organic source of carbon together with the usual mineral salts. Two types of medium are generally employed and full details of their composition have been published (Pontecorvo, 1953). Minimal medium (M.M.) contains only mineral salts and dextrose as organic carbon source. Complete medium (C.M.) consists of minimal medium supplemented with Difco Bacto Peptone, Yeast extract, casein hydrolysate, acid and alkali hydrolysates of yeast nucleic acid, peptic and tryptic casein digest, hydrolysed plasma, hydrolysed corpuscles, and B-vitamin solution. Both media are solidified with 1.5% agar when necessary.

Nutritional mutants are cultured on either complete medium or on minimal medium supplemented with appropriate growth factors.

3. Methods of culture.

Cultures are normally incubated at 35-37°C. Strains are usually maintained on slopes of complete medium and sub-cultured every six to twelve months. They are occasionally purified by isolation of single conidia or ascospores with a micro-manipulator, or by isolation from a single colony obtained from a plating of conidia on solid medium.

Suspensions of conidia for plating are made in sterile saline containing 1/10,000 calzolene oil as a wetting agent. The chains of conidia are broken up by sucking them up and down repeatedly in a Pasteur pipette, or by vigorous shaking in a screw-cap container when large volumes are involved. The density of the suspension is estimated from a haemocytometer count. After appropriate dilutions have been made, the suspension is spread in 0.1 ml. amounts over the surface of solid medium in petri dishes with a sterile glass rod. When platings with very high densities of conidia per dish are required, the suspension is added directly to melted agar medium cooled to 45-50°C, and this is then poured into dishes already containing agar medium, thus forming a thin top layer.

Two methods of preparing ascospore suspensions for plating are in use.

When a pooled sample of ascospores from several perithecia is required the perithecia are picked up with a platinum wire and transferred to a tube containing 1/1,000 calzolene oil. They are agitated with a Pasteur pipette to remove adhering conidia and other debris and then transferred to the inner wall of a testtube containing sterile saline, on which they are crushed with a sterile glass rod. Counting and plating is then similar to that for conidia. The washing in calzolene oil may be omitted when selective platings (Section I-5) are made.

When ascospores are needed from a single perithecium, this is first rolled on hard agar to remove conidia and Hulle cells. It is then transferred to the wall of a testtube containing not more than 0.3 ml. of saline on which it is crushed. Counting and plating are carried out as before.

Platings are made so as to give 30-50 colonies per dish when it is necessary to isolate each colony. When it is only necessary to make a colony count, or to classify the colonies for a morphological difference, up to 100 colonies per dish is manageable.

4. Methods of crossing.

To cross two strains of A. nidulans it is necessary for heterokaryotic hyphae containing nuclei from both strains to be obtained. Two techniques are used (see Pontecorvo, 1953, for full details).

'Balanced' heterokaryons between two strains can be synthesised provided that each has at least one growth factor requirement not common to the other. A dense, mixed suspension of conidia from the two strains is stabbed into or streaked on minimal agar medium, care being taken to carry over sufficient supplemented medium with the inoculum to allow the conidia to germinate.

Growth of the two strains is limited by the amount of supplemented medium carried over with the inoculum, but heterokaryotic hyphae, resulting from hyphal anastomoses between the two strains, are able to grow since each type of nucleus satisfies the growth factor requirement of the other. Provided the heterokaryotic hyphae can 'escape' from the surrounding mesh of parental hyphae, a balanced heterokaryon can be readily isolated and maintained on minimal medium by hyphal tip transfers.

An alternative method of obtaining the heterokaryon is to allow a mixed suspension of conidia to grow in liquid complete medium for 24 hours. The mycelium is centrifuged and washed before being teased out over the surface of minimal agar medium. Any patches of heterokaryotic growth which appear after further incubation of the teased out mycelium are isolated.

When one or both of the strains to be crossed has no growth factor requirement a balanced heterokaryon cannot be made. In such cases a 'mixed inoculum' cross (Pontecorvo, 1953) is made by plating a mixed suspension of conidia from the two strains on complete medium at a density of about 5×10^6 per dish. A thin second layer of complete medium (5 ml) is then poured on top. During growth of the two strains through this top layer many hyphal fusions will occur and give rise to heterokaryotic hyphae.

At least ten days incubation of the heterokaryon or mixed inoculum is necessary for the production of ripe ascospores.

5. Methods of genetic analysis.

Three methods have been employed in this work:-

i. Recombinant selection. (see Pontecorvo, 1953, for full details) Since Aspergillus nidulans is homothallic, a proportion of ascospores in any pooled sample from a number of perithecia will have been derived from asci produced by fusion of two genetically identical nuclei. Genetic analysis is possible only if ascospores derived from hybrid meiosis can be distinguished from those of selfed origin. This is done by selecting recombinants for two or more markers and following the segregation of other markers only among these.

The selection of recombinants is automatic if the two parent strains each have a different growth factor requirement and the ascospores are plated on medium deficient for both these factors. Only recombinants carrying the non mutant allele of each locus can grow and segregation of other markers is followed among these.

When selected recombinants have nutritional requirements platings must be made on complete medium and every colony isolated and classified, the segregation of other markers being followed only among the appropriate recombinant class.

Recombinants can be selected by inspection when morphological markers are used.

ii. Perithecium analysis. This is based on the discovery (Hemmons, Pontecorvo and Bufton, 1952; 1953) that the asci from a single perithecium tend to be of one type only: either selfed of one or other parental type, or crossed. A sample of ascospores from a perithecium of the latter type will be equivalent to a sample of gametes from a higher organism.

A sample of a suspension of ascospores from a single perithecium is plated on complete medium and the remainder stored in a refrigerator. After 48 hours the plates are examined. If the colonies show the correct allele ratio for one pair of markers (a morphological marker is always used to make classification by inspection possible) the remainder of the suspension is plated for complete analysis.

The discovery (Hemmons, Pontecorvo and Bufton, 1953) that crosses between certain strains yield largely, if not exclusively, 'crossed' perithecia has obviated the necessity of making preliminary sample platings from such crosses.

When a large number of isolates from a cross is to be tested for a single growth factor requirement a point inoculation of conidia from each isolate is made on a dish of minimal medium. Requirers

are recognised by their inability to grow. As many as 30 isolates can be tested on a single dish. When the isolates are segregating for two growth factor requirements they are inoculated at corresponding points on two dishes, one containing one growth factor and the other the second. Growth on both dishes indicates no requirements: growth on neither dish indicates requirement for both factors: and growth on one dish only indicates requirement for the factor present in that dish. When segregation for three requirements is being followed the isolates are inoculated at corresponding points on three dishes, each dish lacking one of the growth factors. Dishes are inspected for presence or absence of growth after not more than 36 hours incubation. Incubation for longer periods may make classification difficult owing to cross-feeding between adjacent isolates.

iii. Mitotic segregation Heterozygous diploid strains of A. nidulans (Roper, 1952) segregate spontaneously as a result of reduction to the haploid state and by mitotic crossing over (Pontecorvo and Roper, 1953; Pontecorvo, Tarr Gloor and Forbes, 1954; Pontecorvo and Kafer, 1954). These processes make genetic analysis of diploid strains possible (see the above references for details).

6. Origin of mutant strains

All strains used in this work are derived by mutation and recombination from a single wild type strain A. 69 (Yuill, 1939, 1950) and from a spontaneous mutant "alba" (A.70) derived from A.69 (Yuill, 1950). These two mutants are referred to as NRRL 194 and NRRL 195 respectively by Thom and Røper (1945).

X-rays or UV irradiation were used as mutagenic agents by Pontecorvo et al (1953) and the mutants isolated by one of two methods.

The "total isolation" technique involves plating of conidia on complete medium and the isolation and classification of each colony that appears for induced growth factor requirements.

The "starvation technique" (Mcdonald and Pontecorvo, 1953) is a selective technique which gives a very significant increase in the percentage of induced mutants among the survivors of irradiation and "starvation".

One of the most frequent types of mutant obtained by Mcdonald and Pontecorvo using the starvation technique following UV irradiation are those requiring adenine or related compounds for growth. Some 140 mutants of this type were available and 13 of these were selected for further study. In addition two UV-induced adenine requiring mutants obtained by total isolation, the only two available, and four X-ray-induced adenine requirers obtained by Pontecorvo and Roper (Pontecorvo, 1953) were used. The origin and designation of

of these mutants is given in Table 1. Other mutants used as genetic markers are listed in Table 2.

TABLE 1.

Origin and designation of the adenine-requiring mutants used.

Symbol of mutant.	Isolation number.	Strain irradiated.	Mutagen.	Method of isolation.
ad_1		y	X rays	Total isolation
ad_2		y	X rays	Total isolation
ad_3		y thi	X rays	Total isolation
ad_4		bi_1	X rays	Total isolation
ad_8	S5C2	bi_1	UV	Starvation
ad_9	S5D1	bi_1	UV	Starvation
ad_{10}	S5D2	bi_1	UV	Starvation
ad_{11}	S5D3	bi_1	UV	Starvation
ad_{12}	S5C3	bi_1	UV	Starvation
ad_{13}	S5C4	bi_1	UV	Starvation
ad_{14}	S5E3	bi_1	UV	Starvation
ad_{15}	S5E4	bi_1	UV	Starvation
ad_{16}	S5E5	bi_1	UV	Starvation
ad_{17}	S5E6	bi_1	UV	Starvation
ad_{18}	S5E7	bi_1	UV	Starvation
ad_{19}	S5E8	bi_1	UV	Starvation
ad_{20}	S6.2	bi_1	UV	Starvation
ad_{21}	S3A1	bi_1	UV	Total isolation
ad_{22}	S4A3	bi_1	UV	Total isolation

TABLE 2.

Mutants used as genetic markers.

Symbol of mutant.	Requirement determined by mutant.
bi_1	Biotin or desthiobiotin.
$paba_1$	p-aminobenzoic* acid.
$paba_6$	p-aminobenzoic* acid.
y	yellow conidia.
w	white conidia (epistatic to Y/y).
w_n	white conidia. (epistatic to Y/y)
pro_1	proline ornithine or arginine.
$pyro_4$	pyridoxin.
thi_1	aneurine or thiazole.
nic_2	nicotinic acid and several other compounds.
S 6	thiosulphate or sulphite.

* This is abbreviated to P.A.B.A. in the text and subsequent tables.

II THE LINEAR ARRANGEMENT OF A SERIES OF ALLELES OF ASPERGILLUS NIDULANS

Analysis of crosses involving each of the 15 UV-induced mutants indicated that 9 were located between the two loci y and bi and were extremely closely linked to y (see Fig. 1, this section). The positiom of these mutants permitted an analysis of their linear arrangement with respect to each other to be made. The results of this analysis are presented in the form of a published paper.

The Linear Arrangement of a Series of Alleles of *Aspergillus nidulans*

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I. INTRODUCTION

THE discovery of recombination between the two physiologically allelic mutants "*Star*" and "*asteroid*" in *Drosophila melanogaster* and the difference in phenotype between heterozygotes for the two mutants in *cis* and *trans* (Lewis, 1945) provided support for the conclusion of Raffel and Muller (1940), based on their analysis of the "*scute*" region of *Drosophila*, that definitions of the gene based on the tests of separate mutation, recombination and breakage, and the physiological test of non-allelism need not be coextensive. The number of reported cases of a similar nature from several organisms has now become so large as to lead to the suggestion (Pontecorvo, 1954, 1955) that the ability to recombine is a common rather than exceptional property of physiologically allelic mutants.

Two types of working model have been considered to account for this type of position effect (or "Lewis effect", see Pontecorvo, 1955). One (Raffel and Muller, 1940; Muller, 1947; Pontecorvo, 1952*a*, *b*) is that the unit of function, the gene, has several sites able to mutate independently and between which crossing over can occur. The other (Pontecorvo, 1950, 1952*a*, *b*, 1955; Lewis, 1951; Haldane, 1954) is that alleles between which crossing over occurs are mutants of functionally distinct chromosome segments controlling different steps of a reaction sequence which, owing to the nature of the reaction or reactants, can only take place by means of an "assembly line" process along the chromosome surface, and not between homologous chromosomes.

The widespread occurrence of the Lewis effect makes it important to determine which, if either, of these two alternatives is correct. One approach to the problem would be to obtain an estimate of the number of sites of mutation and crossing over within a single chromosome segment behaving as a functional unit. If this turned out to be very large the second alternative would become less likely.

In the present work a start along these lines was made with a number of adenine-requiring mutants of *A. nidulans*. Infertility of crosses involving allelic adenine-requiring mutants unfortunately prevented an adequate number of pairs of alleles being tested against each other for recombination for this purpose. On the other hand, information was obtained which suggests that multiple exchanges within very short chromosome segments may occur with a much greater frequency than expected (Pritchard, 1954). These observations may help in understanding of the mechanism of crossing over.

2. ANALYSIS OF MEIOTIC RECOMBINATION

(i) *Material and preliminary experiments*

Unless otherwise stated the techniques and notations are those used for the genetics of *A. nidulans* (Pontecorvo, 1953). In the present work two recessive mutants are termed allelic, if a heterozygote in *trans* or heterokaryon between them is mutant in phenotype. The present work is primarily concerned with nine allelic adenine-requiring mutants located very close to the locus "*yellow*" (*y*) (fig. 1) and obtained by Macdonald and Pontecorvo (1953) following U.V.-irradiation of a biotin-requiring strain (bi_1). The mutants are designated as follows (isolation numbers in brackets) : ad_8 (S5C2) ; ad_{10} (S5D2) ; ad_{11} (S5D3) ; ad_{12} (S5C3) ; ad_{16} (S5E5) ; ad_{19} (S5E8) ; ad_{20} (S6.2) ; ad_{21} (S3A1) ; ad_{22} (S4A3). The first six were all derived from one irradiated suspension ; they could have represented repeated isolations from a single mutant clone. The genetic analysis reported in this paper, however, indicates that at least five were mutants of independent origin. With the exception of ad_{20}, which was selected for study because it was phenotypically distinguishable from all but ad_{16}, the nine mutants are a random sample of the available adenine-requiring mutants located immediately to the right of *y*. Markers at other loci used in this work are indicated in fig. 1. The map distances given were obtained by pooling data from a number of crosses (mainly unpublished data of other workers) giving homogeneous results.

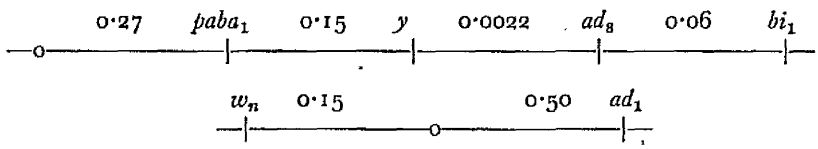


FIG. 1.—Linkage relationships of certain loci of two chromosomes. o : centromere. w_n (white) and *y* (yellow) : conidium colour mutants (wild type green). w_n epistatic to *y*/*Y*. ad_1, ad_6, $paba_1$, and bi_1 nutritional requirement mutants : *ad*, adenine ; *paba*, p-aminobenzoic acid (P.A.B.A.) ; *bi*, biotin. Two other markers not on the above chromosomes were also used : $pyro_4$ determining requirement for pyridoxin, and nic_2, determining requirement for nicotinic acid. The recombination fractions are in part based on work (unpublished) by other workers in the Department of Genetics, University of Glasgow. In the text, tables and subsequent figures the mutants w_n, $paba_1$, bi_1, $pyro_4$, and nic_2, are referred to without their subscripts for convenience. Wild type alleles (all dominant) are indicated with capital letters.

Estimates of the recombination fractions between *y* and each of the nine *ad* mutants were difficult to obtain owing to extremely close linkage and, with the exception of four, qualitative data only are available. Data concerning these four are given in table 1.

The nine *ad* mutants cannot be distinguished by the qualitative test of response to alternative growth factors, growth being supported in all cases by adenosine, adenine or hypoxanthine, and to a limited extent by 4.amino-5. imidazole carboxamidine (kindly supplied by Dr Nimmo-Smith) which exerts a marked sparing effect on adenosine. Two mutants, ad_{16} and ad_{20}, can grow to a limited extent on minimal medium (M.M.) on which they produce characteristic, slow-growing, aconidiate colonies and can therefore be distinguished from the rest. ad_{20} is distinguishable from all the others since it is specifically suppressed by a recessive mutant on the other arm of the same chromosome and more than 50 map units away (Pritchard and Kafer, unpublished).

Three methods have been used to establish that the *ad* mutants are allelic. Firstly, balanced heterokaryons between strains carrying ad_8 and strains carrying each of the other *ad* mutants were synthesised on adenine-supplemented medium and then transferred to adenine-deficient medium. The heterokaryons were balanced on other nutritional requirements. In no case was good growth maintained on transfer to adenine-deficient medium although repeated tests were made.

Secondly, diploids (Roper, 1952) heterozygous for three pairs of mutants (ad_{11} and ad_8; ad_{16} and ad_8; and ad_{19} and ad_8) were synthesised; all had mutant phenotypes, *i.e.* indistinguishable from that due to the "higher" allele. Finally, from crosses between those pairs of mutants which were fertile (*vide infra*) no *diploid* adenine-independent types were obtained from platings of large numbers of ascospores on adenine-deficient medium. Many would have been expected if the

TABLE 1

Recombination fractions between y *and four alleles of the* ad_8 *region*

Cross *	Selection †	Segregations					Recombination fractions
		y	*Y*	Total	Cross-overs	Total	
$\frac{w\ ad_1}{W\ AD_1}\ \frac{paba\ y\ AD_8\ BI}{PABA\ Y\ ad_8\ bi}$	$W\ AD_1\ AD_8$	1018	2	*1020*	6	2705	0·0022±0·00090
$\frac{paba\ y\ ad_8\ BI}{PABA\ Y\ AD_8\ bi}\ \frac{PYRO}{pyro}$	$AD_8\ PYRO$	4	1681	*1685*			
$\frac{w\ ad_1}{W\ AD_1}\ \frac{paba\ y\ AD_{10}\ BI}{PABA\ Y\ ad_{10}\ bi}$	$W\ AD_1\ AD_{10}$	471	2	*473*	12	3938	0·0030±0·00088
$\frac{paba\ y\ AD_{10}\ BI}{PABA\ Y\ ad_{10}\ bi}\ \frac{pyro}{PYRO}$	$AD_{10}\ PYRO$	3455	10	*3465*			
$\frac{w\ ad_1}{W\ AD_1}\ \frac{paba\ y\ AD_{11}\ BI}{PABA\ Y\ ad_{11}\ bi}$	$W\ AD_1\ AD_{11}$	54	0	*54*	9	2368	0·0038±0·0013
$\frac{paba\ y\ AD_{11}\ BI}{PABA\ Y\ ad_{11}\ bi}\ \frac{pyro}{PYRO}$	$AD_{11}\ PYRO$	2305	9	*2314*			
$\frac{w\ ad_1}{W\ AD_1}\ \frac{paba\ y\ AD_{16}\ BI}{PABA\ Y\ ad_{16}\ bi}$	$W\ AD_1\ AD_{16}$	216	0	*216*	1	2087	0·00048±0·00048
$\frac{paba\ y\ AD_{16}\ BI}{PABA\ Y\ ad_{16}\ bi}\ \frac{pyro}{PYRO}$	$AD_{16}\ PYRO$	1870	1	*1871*			

* Symbols above the fraction signs give the genotype of one parent, those below that of the other.

† Ascospores from each cross were plated on minimal medium supplemented with p-aminobenzoic acid and biotin only.

All apparent recombinants between *y* and *ad* were checked for diploidy. A few diploids did arise in most crosses: they are not included in the table.

mutants had been physiologically non-allelic since in *A. nidulans* about 1 in every 100 ascospores is unreduced (Pritchard and Pontecorvo, 1953; Pritchard, 1953 and unpublished).

(ii). *Crosses involving pairs of different* ad *alleles*

Attempts to improve the fertility of crosses involving allelic *ad* mutants by variation of a number of conditions were all unsuccessful. A slight improvement in fertility was usually observed, however, when crossing was carried out on "sporulating minimal medium" (Pontecorvo, 1953), and when the petri dishes containing the heterokaryons were partially sealed with "cellotape". All crosses between

strains with different *ad* alleles were therefore carried out under these conditions.

ad_8 and *ad_{11}*.—The procedure adopted for crossing strains carrying these two mutants and the detection and isolation of adenine-independent types will be given as an example of the procedure adopted in subsequent crosses.

A balanced heterokaryon between two strains *paba y ad_8* and *ad_{11}bi* was obtained by inoculating conidia from both strains together on minimal medium supplemented with adenine only. The heterokaryon was transferred to petri dishes containing sporulating minimal medium plus adenine. The dishes were sealed with " cellotape " and incubated for at least three weeks at 37°.

Large numbers of perithecia were collected from the heterokaryon and washed in a 1/1000 solution of detergent (" calzolene oil ") to remove as many conidia as possible. The washed perithecia were crushed and the ascospores suspended in saline.

The suspension contained $2{\cdot}13 \times 10^6$/ml. ascospores and approximately $7{\cdot}0 \times 10^6$/ml. conidia. The heavy conidial contamination was unavoidable owing to the relative infertility of the cross which necessitated collecting many thousands of perithecia, each with very few ascospores but with many conidial heads adhering to it. Complete removal of adhering conidia by washing was not possible. 3·6 ml. of the suspension was added to molten agar minimal medium supplemented with p-aminobenzoic acid and biotin. This was poured as a top layer on ten petri dishes already containing 10 ml. solidified medium with the same supplements. Each dish was therefore inoculated with about $7{\cdot}67 \times 10^5$ ascospores and $2{\cdot}52 \times 10^6$ conidia.

From this plating (table 2) 365 adenine-independent colonies were obtained of which 10 were green and 355 yellow. All the greens and a sample of 179 of the yellows were tested for nutritional requirements. One hundred and thirty-six of the tested yellows were crossovers between *y* and *bi* although the standard map distance between these loci is less than 6 units. There is clearly an association between the origin of adenine-independent colonies and crossing over between *y* and *bi*. All of the crossovers between *y* and *bi* have the genotype *y bi* ; there are no crossovers of the complementary type.

The simplest interpretation of these results is that *ad_{11}* and *ad_8* are mutants of different loci, the former located nearest to *y*, and that adenine-independent types can arise following an exchange between them. The absence of adenine-independent colonies with the genotype *Y BI* makes it unlikely that unequal crossing over is involved.

If the adenine-independent types arose exclusively as a result of crossing over between the alleles about 6 per cent. would be expected to have the parental association of markers *y BI* in the absence of interference (*i.e.* double crossovers with one exchange between the alleles and a second between *ad_8* and *bi*). In fact, over 24 per cent. of the yellows tested were of this type. Making the same assumptions,

no adenine-independent colonies with *Y bi* would be expected owing to the extremely close linkage between *y* and ad_{11}, but 10 were obtained.

Back-mutants of ad_{11} or ad_8 among the ascospores or conidia plated would also produce adenine-independent colonies with the

TABLE 2

Detection and estimation of recombinants from a cross involving ad_{11} *and* ad_8

Cross :— $\frac{paba \quad y \; AD_{11} \; ad_8 \; BI}{PABA \quad Y \; ad_{11} \; AD_8 \; bi}$

Spores plated on :	Selection		Spores plated		Colonies
			Total	Per dish	
M.M.+adenine . .	*PABA BI*	Ascospores	$9{\cdot}06\times10^4$	$9{\cdot}06\times10^3$	1000 (approx.)
M.M.+p-aminobenzoic acid+biotin	AD_{11} AD_8	Ascospores Conidia	$7{\cdot}67\times10^6$ $2{\cdot}52\times10^7$	$7{\cdot}67\times10^5$ $2{\cdot}52\times10^6$	365

Classification of adenine-independent colonies

		PABA BI	*paba BI*	*PABA bi*	*paba bi*	Total
Yellow . .	355 (179 tested)	6	37	22	114	179
Green . .	10	0	0	10	0	10

Estimation of the recombination fraction between ad_{11} and ad_8 (see text p. 349) :

n = No. of ascospores plated on minimal medium with adenine = $9{\cdot}06\times10^4$
a_1 = No. of colonies produced by n ascospores = 1000
$a_2 = (n-a_1)$
m = No. of ascospores plated on minimal medium with p-aminobenzoic acid and biotin = $7{\cdot}67\times10^6$
b_1 = No. of colonies obtained from m ascospores = 365
$b_2 = (m-b_1)$
x = Recombination fraction between *paba* and *bi*. A value of 0·2 has been used in this and subsequent calculations.
h = Fraction of ascospores viable and from hybrid asci = $2a_1/nx = 0{\cdot}11$
$S.E_h = \sqrt{(1/a_1+1/a_2).\, h^2(2-hx)^2/4} = \sqrt{h(2-hx)/nx} = 0{\cdot}0035$
q = Recombination fraction between ad_{11} and $ad_8 = nb_1x/ma_1 = 0{\cdot}00086$
$S.E^2 = \sqrt{q[nx(2-hq)+mq(2-hx)]/mnhx} = 0{\cdot}000053$

parental combinations *Y bi* and *y BI* and might thus account for the excess of these types obtained. Platings of large numbers of conidia from both parental strains (table 3), however, and also from the heterokaryon from which the ascospores had been obtained (table 3) failed to yield any adenine-independent colonies. The plating from the heterokaryon was made in view of the possibility that back-mutation during its growth could have produced a clone of non-mutant nuclei.

Clearly the excess of adenine-independent colonies with the parental combinations of markers *Y bi* and *y BI* could scarcely have

been due to back-mutation unless the mutation frequency were enormously greater in ascospores than conidia. Thus, even if the viability of ascospores was 100 per cent. (certainly an overestimate),

TABLE 3

Summary of available data regarding back-mutation of a number of alleles of ad_8

Origin of ascospores or conidia	Spores plated	Plated on	Total plated	Density per dish	Colonies
paba y ad_8 . .	conidia	M.M. with P.A.B.A.	$2{\cdot}03\times10^8$	Varied between $2{\cdot}03\times10^7$ and $5{\cdot}07\times10^6$	0
ad_{10} *bi* + *paba y* ad_8 (Heterokaryon)	conidia	M.M. with P.A.B.A. and biotin	$2{\cdot}16\times10^7$	$2{\cdot}16\times10^6$	0
		Complete medium	360		*Y* 182 *y* 96
ad_{10} *bi* . .	conidia	M.M. with biotin	$4{\cdot}92\times10^8$	$1{\cdot}23\times10^7$	0
ad_{11} *bi* . .	conidia	M.M. with biotin	$2{\cdot}63\times10^8$	Varied between $4{\cdot}83\times10^7$ and $4{\cdot}83\times10^5$	0
paba y ad_8 + ad_{11} *bi* (Heterokaryon)	conidia	M.M. with P.A.B.A. and biotin	$4{\cdot}80\times10^7$	$4{\cdot}80\times10^6$	0
		Complete medium	510		*Y* 376 *y* 36
ad_{12} *bi* . .	conidia	M.M. with biotin	$1{\cdot}58\times10^8$	See table 4	0
ad_{16} *bi* . .	conidia	M.M. with biotin	$3{\cdot}25\times10^7$	$5{\cdot}42\times10^6$	0
	ascospores + conidia	M.M. with biotin	$2{\cdot}15\times10^7$ + $6{\cdot}79\times10^6$	$1{\cdot}79\times10^6$ + $5{\cdot}66\times10^5$	0

and if 9 of the 43 *y BI* colonies are considered to be double crossovers, the frequency of back-mutation of ad_8 among ascospores would be nearly 10^{-4}. It is possible, however, that rare back-mutant nuclei in the heterokaryon would have a greater than random chance of participating in perithecium production and that the perithecia so produced would give rise to a greater number of ascospores than those originating from two *ad* nuclei.

That adenine-independence was not due to mutation at a non-linked or loosely-linked suppressor locus, except perhaps in a small proportion of colonies, was shown by outcrossing to wild type a number of adenine-independent colonies of each class with respect to their *Y*/*y* and *BI*/*bi* genotype. No adenine-requiring segregants were obtained from any of these crosses.

That colonies with the phenotypes *Y* or *BI* were not heterozygous diploids was shown by measuring the conidium diameter of all such types. None was outside the haploid range (Pontecorvo *et al.*, 1954).

Further discussion on the origin of the large number of adenine-independent colonies carrying the parental combinations of markers

will be deferred until the results obtained from similar crosses involving other pairs of alleles have been considered.

The recombination fraction between ad_{11} and ad_8 was estimated in the following manner (Roper, 1953). It was assumed in the first instance that all adenine-independent colonies were recombinants between the two alleles.

A dilution of the original suspension of ascospores was spread in 0·1 ml. amounts over the surface of 10 petri dishes containing solid minimal medium supplemented with adenine, and the number of colonies produced was counted. These are recombinants *PABA BI*. Taking the recombination fraction between *paba* and *bi* as 0·2, the

TABLE 4

Experiment to test for inhibition of an adenine-independent strain by conidia of an adenine-requiring strain

Conidia plated on :	Estimated conidia per dish from ad_{12} *bi*	Estimated conidia per dish from *y bi*	Colonies (all yellow)
M.M.+biotin	$9{\cdot}50\times10^4$	112	117
	$4{\cdot}75\times10^5$	112	123
	$9{\cdot}50\times10^5$	112	145
	$4{\cdot}75\times10^6$	112	129
	$9{\cdot}50\times10^6$	112	124
	$4{\cdot}75\times10^7$	112	141
	$9{\cdot}50\times10^7$ (two dishes)	112	237

number of colonies counted will represent one-half of this fraction, *i.e.* 0·1 of the viable ascospores plated of crossed origin. The proportion of ascospores of this type in the original suspension can therefore be estimated, and the recombination fraction between the two alleles calculated in the manner shown in table 2. I am indebted to Dr A. Durrant for the method of calculating the standard error of this estimate.

Calculation of the recombination fraction in this way is open to a number of sources of error such as inaccuracy of dilution and plating, differential viability of ascospores of different genotypes on different media, and at different density of plating (*cf.* Grigg, 1952), and probably most serious, variation of the recombination fraction between *paba* and *bi* from the standard value. In the present work, however, qualitative rather than quantitative information was required. The experimental data in table 4 indicate that inhibition of adenine-independent strains by high concentrations of adenine-requiring conidia (" Grigg effect ") does not occur at concentrations used in this work.

ad_8 and ad_{12}, ad_{16}, ad_{19}, ad_{20}.—These four crosses were carried out in exactly the manner previously described, the strain *paba y* ad_8 being crossed to ad_{12} *bi*, ad_{16} *bi*, ad_{19} *bi* and ad_{20} *bi*. The data are given in table 5.

TABLE 5

Detection and estimation of recombination in crosses involving ad_8 *and each of four other alleles*

Type of cross : $\frac{paba \quad y\ AD_x \quad ad_8\ BI}{PABA \quad Y\ ad_x \quad AD_8 \quad bi}$ *

Allele tested against ad_8		Spores on M.M.+ p-aminobenzoic acid +biotin (selecting AD_x AD_8)		Colonies	Ascospores on M.M.+ adenine (selecting *PABA BI*)	Colonies	Recombination fraction
		Total	Per dish				
ad_{12}	Ascospores	$3{\cdot}60\times10^6$	$4{\cdot}00\times10^5$	111			
	Conidia	$1{\cdot}44\times10^7$	$1{\cdot}60\times10^6$				
	Ascospores	$6{\cdot}00\times10^5$	$2{\cdot}00\times10^5$	28			
	Conidia	$2{\cdot}40\times10^6$	$8{\cdot}00\times10^5$				
	Total						
	Ascospores	$4{\cdot}20\times10^6$		139	$5{\cdot}11\times10^4$	282	$0{\cdot}0012\pm0{\cdot}00012$
	Conidia	$1{\cdot}68\times10^7$					
ad_{16}	Ascospores	$6{\cdot}34\times10^6$	$1{\cdot}58\times10^6$	65			
	Conidia	$1{\cdot}15\times10^6$	$2{\cdot}87\times10^6$				
	Ascospores	$3{\cdot}17\times10^6$	$7{\cdot}92\times10^5$	36			
	Conidia	$5{\cdot}75\times10^5$	$1{\cdot}44\times10^5$				
	Ascospores	$3{\cdot}17\times10^6$	$3{\cdot}96\times10^5$	38			
	Conidia	$5{\cdot}75\times10^5$	$7{\cdot}19\times10^4$				
	Total						
	Ascospores	$1{\cdot}27\times10^7$		139	$3{\cdot}34\times10^4$	52	$0{\cdot}0014\pm0{\cdot}00042$
	Conidia	$2{\cdot}30\times10^6$					
ad_{19}	Ascospores	$4{\cdot}20\times10^6$	$8{\cdot}40\times10^5$	28	$5{\cdot}20\times10^4$	56	$0{\cdot}0012\pm0{\cdot}00029$
	Conidia	$1{\cdot}20\times10^7$	$2{\cdot}40\times10^6$				
ad_{20}	Ascospores	$3{\cdot}94\times10^6$	$6{\cdot}56\times10^5$	80†	$9{\cdot}37\times10^4$	236	$0{\cdot}0016\pm0{\cdot}00021$
	Conidia	$1{\cdot}22\times10^6$	$2{\cdot}03\times10^5$				

Classification of adenine-independent colonies

Allele tested against ad_8		*PABA BI*	*paba BI*	*PABA bi*	*paba bi*	Totals	
ad_{12}	yellow	7	24	17	85	133	139
	green	1	0	4	1	6	
ad_{16}	yellow	7	24	16	86	133	139
	green	0	0	4	2	6	
ad_{19}	yellow	2	8	4	13	27	28
	green	0	0	1	0	1	
ad_{20}	yellow	2	10	24	35	71	79
	green	0	1	6	1	8	

* ad_x represents either ad_{12}, ad_{16}, ad_{19} or ad_{20}.

† One colony (*Y bi*) had a growth rate less than wild type and was stimulated by adenine. It probably carried a suppressor. It is not included in the lower table or in the estimate of the recombination fraction.

Adenine-independent colonies arose in each cross. In each case there was a clear association between adenine-independence and crossing over between *y* and *bi*, the simplest interpretation of the data being that ad_8 lies to the right of each of the other four alleles.

It is remarkable that the relative frequency of the four possible genotypes with respect to *y* and *bi* among the adenine-independent colonies is very similar in each cross (embarrassingly so in the case of the crosses to ad_{12} and ad_{16}). The data from all five crosses so far discussed involving ad_8 are in fact statistically homogeneous in this respect; the high proportion of colonies carrying the parental combinations of the markers *y* and *bi* being consistently obtained.

In view of the fact found later that reversion of ad_{20} strains due to suppressor mutation occurs frequently, it cannot be excluded that a proportion of the *Y bi* colonies obtained from the cross involving this mutant were of this type. In fact, one had a sub-normal growth rate and was stimulated by adenine. Two others, outcrossed to an adenine-independent strain, gave no *ad* progeny.

ad_8 and ad_{10}.—The proportion of adenine-independent colonies from this cross was extremely low (less than 10^{-5}) and the data in table 6 are the results of four separate platings of ascospores.

Out of 28 adenine-independent colonies obtained 14 were *Y BI*. This suggests that ad_{10} lies at a locus to the right of ad_8 representing a third locus in the series. However, the occurrence of 3 colonies with the complementary genotype (*y bi*) suggests that unequal crossing over may be involved in this case.

A high proportion of colonies with the parental combinations *y BI* and *Y bi* was again encountered. The massive numbers of ascospores and conidia involved in platings from this cross increased the possibility that back-mutants might be contributing to these classes, but platings of conidia from both parental strains and from the heterokaryon from which the ascospore suspension was obtained (table 3) indicated a very low back-mutation rate in conidia.

ad_{11} and ad_{16}.—With the exception of the cross involving ad_{16} and ad_{11}, attempts to cross together in pairs the four alleles located to the left of ad_8 failed due to infertility.

The technique in the cross involving ad_{16} and ad_{11} was slightly different from that previously used. The presence of an additional marker *pyro*, made possible a more reliable estimate of the percentage of viable ascospores from hybrid asci (table 7). In addition, exclusion of pyridoxin from the medium when selecting for adenine-independent types cut down the background growth caused by conidia from the ad_{16} parent and by ascospores from zygotes of selfed ad_{16} origin.

Adenine-independence was again associated with crossing over between *y* and *bi*, the data indicating that ad_{16} is located to the left of ad_{11}. The proportion of colonies with the parental combinations *y BI* and *Y bi* was again high although a low back-mutation rate for ad_{16} both in ascospores and conidia is indicated by the data in table 3.

(iii) *Discussion*

In each of the seven crosses so far analysed the origin of adenine-independent colonies was associated with recombination between *y*

TABLE 6

Detection and estimation of recombination in a cross involving ad_8 *and* ad_{10}

Cross : $\frac{paba \quad y\ ad_8\ AD_{10} \quad BI}{PABA \quad Y\ AD_8\ ad_{10} \quad bi}$

Plating		Spores on M.M.+ p-aminobenzoic acid +biotin (selecting $AD_8\ AD_{10}$)		Colonies	Ascospores on M.M.+ adenine (selecting *PABA BI*)	Colonies	Recombination fraction
		Total	Per dish				
1	Ascospores	$3{\cdot}00\times10^7$	$2{\cdot}73\times10^6$	11	$7{\cdot}74\times10^4$	875	
	Conidia	$1{\cdot}65\times10^7$	$1{\cdot}50\times10^6$				
2	Ascospores	$3{\cdot}85\times10^6$	$2{\cdot}14\times10^5$	1	$4{\cdot}34\times10^4$	41	
	Conidia	Not recorded					
3	Ascospores	$1{\cdot}12\times10^7$	$9{\cdot}35\times10^5$	3	$1{\cdot}18\times10^5$	488	
	Conidia	Not recorded					
4	Ascospores	$3{\cdot}09\times10^7$	$2{\cdot}06\times10^6$	13	$1{\cdot}07\times10^5$	705	
	Conidia	$5{\cdot}09\times10^6$	$4{\cdot}24\times10^5$				
	Total of 4 platings : Ascospores	$7{\cdot}59\times10^7$		28	$3{\cdot}46\times10^5$	2109	$0{\cdot}000012\pm0{\cdot}0000024$

Classification of adenine-independent colonies

	PABA BI	*paba BI*	*PABA bi*	*paba bi*	Total
Yellow	0	3	0	3	6
Green	12	2	8	0	22

and *bi*. Nevertheless the proportion of such colonies carrying the parental combinations *y BI* and *Y bi* was in every case much higher than expected on the assumptions that all adenine-independent types resulted from crossing over between alleles and that there was no interference. The first suggestion which comes to mind is that a proportion of the adenine-independent colonies originated by back-mutation. The following evidence, however, makes this interpretation unlikely.

First, the data summarised in table 3 indicate a very low back-mutation rate among conidia for all alleles so far tested. In one case,

ad_{16} (table 3), the incidence of back-mutation was also tested among ascospores: no back-mutant was present in $2{\cdot}15 \times 10^7$ ascospores. (It was not possible to make this test with other alleles owing to self-infertility.)

Second, the relative frequencies of the four genotypes *y bi*, *Y BI*,

TABLE 7

Detection and estimation of recombination in a cross involving ad_{16} *and* ad_{11}

Cross: $\frac{paba \quad y\ AD_{16}\ ad_{11} \quad BI \quad PYRO}{PABA \quad Y\ ad_{16}\ AD_{11} \quad bi \quad pyro}$

Spores plated on	Selection		Spores plated		Colonies	Rec. fract.*
			Total	Per dish		
M.M.+p-aminobenzoic acid+biotin	$AD_{16}\ AD_{11}\ PYRO$	Ascospores	$3{\cdot}71 \times 10^7$	$3{\cdot}09 \times 10^6$	57	$0{\cdot}00089 \pm 0{\cdot}00028$
		Conidia	$1{\cdot}57 \times 10^7$	$1{\cdot}31 \times 10^6$		
M.M.+adenine+biotin	*PABA PYRO*	Ascospores	$8{\cdot}19 \times 10^4$	$1{\cdot}36 \times 10^4$	141	

Classification of adenine-independent colonies

	PABA BI	*paba BI*	*PABA bi*	*paba bi*	Total
Yellow	1	5	4	36	46
Green	0	0	10	1	11

* In this cross the fraction of viable ascospores from hybrid asci is $4a_1/n$; the recombination fraction is nb_1/ma_1, and its S.E. is $\sqrt{q[n(4-hq)+mq(4-h)]/mnh}$. I am indebted to Dr O. Frydenberg for the calculation of this standard error. Symbols as in table 2.

y BI and *Y bi* among adenine-independent colonies from the five crosses involving ad_8 and the alleles to the left (tables 2 and 5) are statistically homogeneous. This would not be expected if back-mutants contributed appreciably to the two parental classes *y BI* and *Y bi* since back-mutant nuclei would have a clonal distribution and their frequency would be subject to considerable fluctuation in different crosses (Luria and Delbruck, 1943).

Finally, and most significant, is a comparison of the data from the crosses between ad_8 and the alleles to the left on the one hand, and between ad_8 and ad_{10} on the other. The frequency of adenine-independent colonies obtained from the former crosses is greater by a factor of nearly 10^2 than in the latter. If the majority of *y BI* colonies obtained in the former was due to back-mutation of ad_8, then in the latter cross the proportion of such types to crossover types should be very much higher. This was not found, however; the proportion

of the two types remains approximately the same. Such a result is inconsistent with the view that the majority of *y BI* colonies are back-mutants of ad_8.

Four other alternatives which might account for the observed results were therefore considered; unequal sister strand crossing over; the presence of rearrangements; negative interference; and some form of gene conversion or transformation.

Unequal sister strand crossing over might be capable of producing adenine-independent colonies with the parental combinations of the markers *y* and *bi*, but the argument against back-mutation applies equally well here, viz. that the frequency of such an event should be independent of the frequency of recombination between the alleles.

An inversion in one or both strains used to test two alleles against each other might account for the large proportion of adenine-independent colonies not apparently associated with crossing over between *y* and *bi* by elimination of a proportion of the single cross-overs between the alleles. It has proved impossible to construct a model for any simple system of rearrangements that will satisfactorily account for the results obtained from crosses involving pairs of alleles, taking into account that crosses involving each *ad* mutant and an adenine-independent strain gave no evidence of the presence of inversions. More conclusive evidence against this alternative was obtained from the results of mitotic analysis (see section 3).

Negative interference would need to be very intense to account for the observed results. Thus the greatest observed value for the recombination fraction between *y* and any *ad* mutant was 0·0038 (table 1). The smallest value for the same recombination fraction computed among crossovers between two *ad* mutants was 0·027 (from the cross involving ad_{11} and ad_8 in which there were 10 *Y bi* colonies among 365 adenine-independent). It should be realised that the term "negative interference" is merely a description, not an explanation, of the fact that a crossover betwen two *ad* alleles is associated with one or more further exchanges nearby more often than by chance.

A distinction between negative interference, mutation and gene transformation (or in other words, a high mutation rate in heterozygotes) could be made by means of tetrad analysis, but the low incidence of adenine-independence in crosses involving alleles and the infertility of these crosses makes this impracticable. On the other hand, the occurrence of mitotic crossing over in heterozygous diploids of *A. nidulans* makes the analysis of half-tetrads possible and at the same time allows the automatic selection of half-tetrads in which a change of phenotype from adenine-requirement to adenine-independence has occurred (Roper and Pritchard, 1955). An analysis of this type was therefore undertaken. It was based on the assumption that crossing over is substantially the same process at meiosis and at mitosis.

3. ANALYSIS OF MITOTIC RECOMBINATION

(i) *Introduction*

Following the synthesis of heterozygous diploid strains of *A. nidulans* (Roper, 1952) and the discovery of mitotic segregation in these (Pontecorvo and Roper, 1953), the analysis of the mechanisms of mitotic segregation has now progressed to the point where this process can be used for genetic analysis outside the sexual cycle (Pontecorvo, Roper and Forbes, 1953 ; Pontecorvo, Tarr Gloor and Forbes, 1954 ; Pontecorvo and Kafer, 1954).

The purpose of the experiments described in this section was threefold. Firstly, to test the possibility that " negative interference " was involved in the origin of adenine-independent colonies not associated with recombination in crosses involving pairs of alleles. Secondly, to explore the possibility of using mitotic crossing over as a method of genetic analysis of the linear relationships between alleles of ad_8, thereby circumventing the obstacle of infertility. Finally, to obtain recombinants carrying two *ad* alleles in coupling, thereby making it possible to follow the segregation of three alleles simultaneously. They would also make it possible to verify what had been assumed from the fact that all the *ad* alleles are recessive, namely that a strain heterozygous for two alleles in *cis* would be adenine-independent.

(ii) *Techniques*

In the following experiments three operations were involved. The first was to obtain a diploid heterozygous for two *ad* alleles. The second was to obtain adenine-independent diploid colonies from such a diploid. The third was to determine the genotype of these colonies.

Two diploids heterozygous for ad_8 and ad_{16} were synthesised (see Roper, 1952). These two alleles were chosen because they are phenotypically distinguishable which, as will become apparent, was a decisive advantage. The diploids had the following genotypes :

$$\text{I.}\quad \frac{W}{w}\ \frac{paba}{PABA}\ \frac{y\ AD_{16}\ ad_8\ BI}{Y\ ad_{16}\ AD_8\ bi}\ \frac{NIC}{nic}$$

$$\text{II.}\quad \frac{paba}{PABA}\ \frac{y\ AD_{16}\ ad_8\ BI}{Y\ ad_{16}\ AD_8\ bi}\ \frac{PYRO}{pyro}$$

As expected they were adenine-requiring and on minimal medium were intermediate in phenotype between ad_8 and ad_{16}.

When selecting adenine-independent colonies from these diploids doubly heterozygous in *trans*, it was important to avoid isolating from a single clone more than once. To this end, conidia from either diploid I or II were plated, about 10 per dish, on agar medium lacking adenine. The conidia gave rise to slow growing aconidiate colonies, and when these were about 1 cm. in diameter they were covered by a thin layer of the same medium. During further incubation very slow growth of the colonies occurred through the covering layer of medium. Hyphæ in which " adenine-independent nuclei " were present would have an enormous selective advantage over hyphæ with only " adenine-dependent nuclei " and would reach the surface first, giving rise to rapidly growing conidiate colonies. Such reversions were readily obtained and a single-conidium isolation made from each. Since only one adenine-independent strain was isolated from a single adenine-requiring colony, repeated isolation from a single clone was excluded. Only about 10 per cent. of

the colonies produced adenine-independent recombinants by this technique, indicating that the rate with which these arise is very low.

The techniques employed for the determination of the genotype of the adenine-independent diploids obtained from the diploids I and II were those in routine use in *A. nidulans* (Pontecorvo *et al.*, 1954; Pontecorvo and Kafer, 1954) and will not be redescribed here. The determination of the genotype with respect to ad_8 and ad_{16} requires explanation, however.

As is made clear in fig. 2, adenine-independent diploids of two types would be expected, presumably with equal frequency, following a single mitotic exchange between the two alleles. One carries the reciprocal products of a mitotic exchange between the two alleles, the other has one crossover and one non-crossover strand. A number of diploids apparently of the former type were obtained, *i.e.* they were prototrophs (a diploid of the latter type would be biotin-requiring), they had the

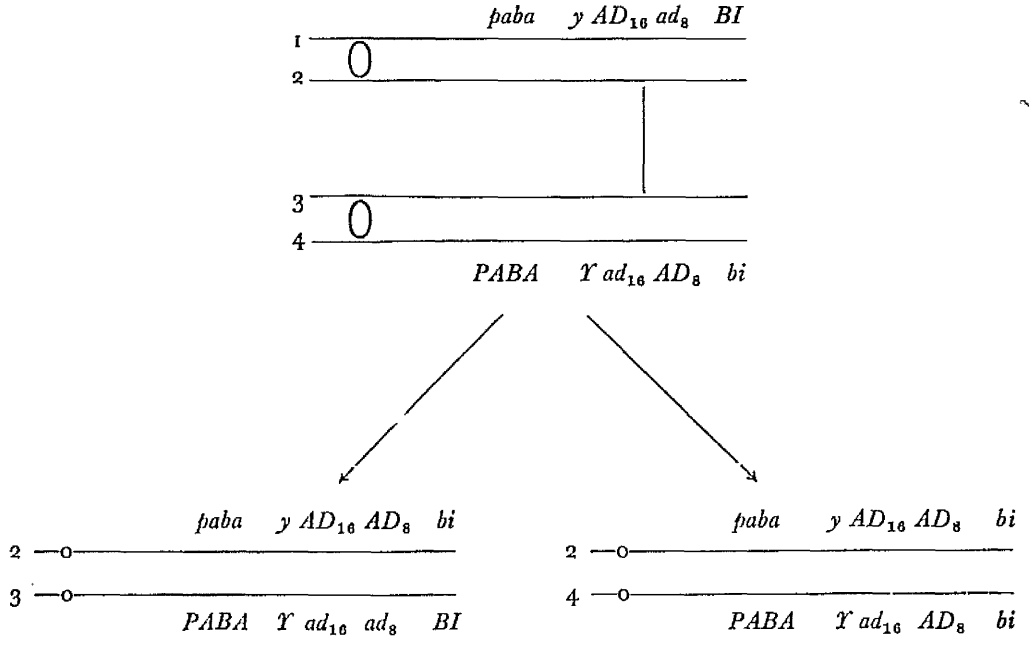


FIG. 2.—Types of diploid segregants produced following a mitotic exchange between ad_{16} and ad_8. In this and subsequent figures the parent diploid is shown with the two homologous chromosomes divided into chromatids joined at the centromere. The four chromatids are numbered. In the segregant diploids the two homologues are shown as single strands. Segregants not possessing chromatid 2 are not shown as they will be adenine-requiring.

constitution *y bi*/*Y BI* and therefore carried the complementary products of a mitotic exchange between *y* and *bi*, and the *y bi* strand carried the wild type alleles of ad_{16} and ad_8, suggesting that the mitotic exchange had taken place between the two alleles. Such diploids should carry the two alleles ad_{16} and ad_8 in *cis* on the other strand, *i.e.* that carrying *Y* and *BI*. Haploids carrying the *Y BI* strand from all diploids of this type were adenine-requiring and the majority had a phenotype indistinguishable from that of ad_8. In the parent diploid ad_8 was in coupling with *y* but it was now apparently in coupling with *Y*. This was again suggestive that the *Y BI* strand in segregant diploids of this type carried both ad_{16} and ad_8 and, as might be expected, that the phenotype of haploids carrying both these alleles in *cis* was identical with that of the more extreme of the two alleles (ad_8). Proof of this was obtained by outcrossing a haploid of this type to an adenine-independent strain and recovering the less extreme mutant (ad_{16}).

The technique for separating ad_{16} from ad_8 was as follows. Since recombination between the two alleles is rare, the technique had to be selective. From a suspected

double mutant with the genotype $(ad_{16})ad_8$ *pyro*, a recombinant $(ad_{16})ad_8$ *bi* was obtained. This was outcrossed to a strain *paba y*. Ascospores were plated on minimal medium supplemented with adenine and any green colonies which arose were tested for adenine-requirement (table 8). These colonies (*Y BI*) are all crossovers between *y* and *bi*. Since the ratio of the distances between ad_{16} and ad_8 on the one hand and ad_8 and *bi* on the other is about 1 : 50, about 2 per cent. of the colonies tested should be crossovers between ad_{16} and ad_8 and carry the single mutant ad_{16} if it is present. In fact 3 ad_{16} AD_8 recombinants were obtained out of 129 colonies tested. The presence of ad_{16} in the double mutant was therefore proved.

The result shows beyond doubt that a heterozygote with ad_{16} and ad_8 in *cis* is wild type while the heterozygote in *trans* is mutant, and the Lewis effect is therefore established in this case.

TABLE 8

Recovery of ad_{16} *from a double mutant* ad_{16} ad_8

Cross : $\frac{PABA \quad Y \; ad_{16} \quad ad_8 \quad bi}{paba \quad y \; AD_{16} \; AD_8 \quad BI}$

Ascospores plated on :	Selection	Segregation			Total
		AD_{16} AD_8*	ad_{16} AD_8	AD_{16} ad_8 or ad_{16} ad_8	
M.M.+adenine	*PABA Y BI*	1	3	125	129

* Diploids (6) with this phenotype were also obtained.

(iii) *Results*

A total of 43 adenine-independent diploids was obtained in the manner already described from one or other of the parent diploids. The genotype of all but two of these was determined by further analysis. They fell into 13 classes with respect to their phenotype and genotype (table 9) and the origin of each class will be considered in turn. In what follows the chromosome of a diploid carrying *y* will be called the yellow strand and that carrying *Y*, the green strand.

Class I.—The origin of diploids of this type has already been discussed. They carry the reciprocal products of a mitotic exchange between the alleles. The genotype ad_{16} ad_8 has been inferred from the fact that a haploid carrying the green strand, or a diploid homozygous for this strand, has a phenotype identical with that of ad_8, since this allele was in coupling with *y* in the parent diploid. In one case the presence of ad_{16} has been proved by outcrossing as described before (table 8).

Class II.—Diploids of this type have the genotype expected following a single mitotic exchange between the alleles and inclusion of one crossover and one non-crossover product in the same daughter nucleus (see fig. 2).

It should be noted that whereas 16 diploids of Class ii were obtained, there were only 8 in Class i. On the assumption that normal mitotic segregation of the centromeres occurs after a mitotic exchange,

equal numbers of these types were expected. Although the deviation is not significant, it is possible that the two chromatids involved in one mitotic crossover tend to segregate to opposite poles with greater than random frequency.

TABLE 9

The phenotype and genotype of adenine-independent diploids obtained from the two parent diploids heterozygous for ad_{16} *and* ad_8

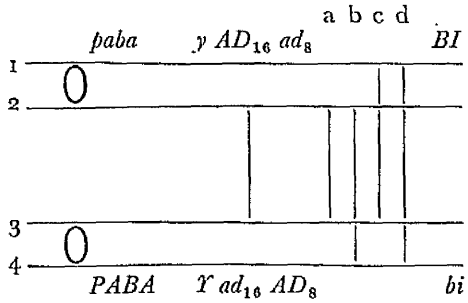
Class no.	Phenotype	Genotype *	No. obtained
i	wild type	$\frac{paba \quad y\ AD_{16}\ AD_8 \quad bi}{PABA \quad Y\ ad_{16}\ ad_8 \quad BI}$	8
ii	biotin-requiring	$\frac{paba \quad y\ AD_{16}\ AD_8 \quad bi}{PABA \quad Y\ ad_{16}\ AD_8 \quad bi}$	16
iii	wild type	$\frac{paba \quad y\ AD_{16}\ AD_8 \quad BI}{PABA \quad Y\ ad_{16}\ ad_8 \quad bi}$	1
iv	wild type	$\frac{paba \quad y\ AD_{16}\ AD_8 \quad bi}{PABA \quad Y\ ad_{16}\ AD_8 \quad BI}$	2
v	wild type	$\frac{paba \quad y\ AD_{16}\ AD_8 \quad BI}{PABA \quad Y\ ad_{16}\ AD_8 \quad bi}$	5
vi	biotin-requiring	$\frac{paba \quad y\ ad_{16}\ AD_8 \quad bi}{PABA \quad Y\ AD_{16}\ AD_8 \quad bi}$	1
vii	wild type	$\frac{paba \quad y\ ad_{16}\ AD_8 \quad BI}{PABA \quad Y\ AD_{16}\ AD_8 \quad bi}$	1
viii	wild type	$\frac{paba \quad y\ AD_{16}\ ad_8 \quad BI}{PABA \quad Y\ AD_{16}\ AD_8 \quad bi}$	4
ix	biotin-requiring	$\frac{paba \quad Y\ AD_{16}\ AD_8 \quad bi}{PABA \quad Y\ ad_{16}\ AD_8 \quad bi}$	1
x	wild type	$\frac{paba \quad Y\ AD_{16}\ AD_8 \quad BI}{PABA \quad Y\ ad_{16}\ AD_8 \quad bi}$	1
xi	wild type	$\frac{paba \quad y\ AD_{16}\ AD_8 \quad BI}{PABA \quad Y\ AD_{16}\ AD_8 \quad bi}$	1
xii	yellow	Not analysed	1
xiii	yellow, P.A.B.A., pyridoxin	Not analysed	1

* All were heterozygous for *W/w* and *NIC/nic*, or for *PYRO/pyro* except type xiii.

Classes iii, iv and v.—Diploids in each of these classes have genotypes expected following the simultaneous occurrence of two mitotic exchanges, one between the alleles and a second between ad_8 and *bi*. No other simple explanation can account for the genotype of Classes iii and iv, but Class v diploids would also be produced following back-mutation of ad_8. In fig. 3 the eight possible types that can be produced following a two-, three- or four-strand double exchange of this type are indicated.

The Class iii diploid requires a two-strand double exchange and inclusion of both crossover products in the same daughter nucleus. The presence of ad_{16} on the green strand in coupling with ad_8 was verified by outcrossing in the manner already described.

The two Class iv diploids can only be produced following one type of three-strand double, or a four-strand double. On the assumption that the four types of double exchange occur with equal frequency



Type of double	Chromatids 2 and 3 to daughter nucleus	Class	Chromatids 2 and 4 to daughter nucleus	Class
a	$\frac{paba \quad y\ AD_{16}\ AD_8\ BI}{PABA \quad Y\ ad_{16}\ ad_8\ bi}$	iii	$\frac{paba \quad y\ AD_{16}\ AD_8\ BI}{PABA \quad Y\ ad_{16}\ AD_8\ bi}$	v
b	$\frac{paba \quad y\ AD_{16}\ AD_8\ bi}{PABA \quad Y\ ad_{16}\ ad_8\ bi}$		$\frac{paba \quad y\ AD_{16}\ AD_8\ bi}{PABA \quad Y\ ad_{16}\ AD_8\ BI}$	iv
c	$\frac{paba \quad y\ AD_{16}\ AD_8\ BI}{PABA \quad Y\ ad_{16}\ ad_8\ BI}$		$\frac{paba \quad y\ AD_{16}\ AD_8\ BI}{PABA \quad Y\ ad_{16}\ AD_8\ bi}$	v
d	$\frac{paba \quad y\ AD_{16}\ AD_8\ bi}{PABA \quad Y\ ad_{16}\ ad_8\ BI}$		$\frac{paba \quad y\ AD_{16}\ AD_8\ bi}{PABA \quad Y\ ad_{16}\ AD_8\ BI}$	iv

FIG. 3.—The 8 possible types of adenine-independent diploid produced by two mitotic exchanges, one between ad_{16} and ad_8 and a second between ad_8 and *bi*. Daughter nuclei carrying chromatid 1 are adenine-requiring and are not shown.

and segregation of the pairs of chromatids is random, diploids Class iv are expected to arise twice as frequently as diploids Class iii. The actual numbers are 2 and 1.

The five Class v diploids require a two- or three-strand double exchange and repeating the same assumptions would be expected to occur with a frequency equal to that of Class iv. They could also occur as a result of back-mutation of ad_8 since they have a genotype identical with that of the parent except that ad_8 has been replaced by its wild type allele. There is no way of distinguishing between these two alternatives but the fact that back-mutation of ad_8 had not been previously encountered, and that diploids of this type produced by a double mitotic exchange were expected with a frequency at least equal to that of Class iv diploids, suggested that back-mutation was the least likely of the two alternatives.

The occurrence of possible back-mutants made it desirable to make a test for back-mutation of ad_8 in a diploid homozygous for this mutant. From such a diploid $3{\cdot}42 \times 10^8$ conidia were tested and 6 reversions were obtained, of which 5 were due to back-mutation of ad_8 on the green strand and 1 on the yellow strand (table 10). The first 5 may have been repeated isolates from a single clone, but the results indicate that at least 2 back-mutants were picked up. The origin of the Class v diploids cannot therefore be decided.

CLASSES VI and VII.—The occurrence of these two diploids was extremely significant and their origin must be considered at some length. Both carried a strand with *y* and ad_{16} in coupling whereas in the parent diploids these were in repulsion. This can only be

TABLE 10

The genotype of adenine-independent mitotic segregants obtained from two diploids, one homozygous for ad_8 *and the other for* ad_{16}

Parent diploid	Conidia plated		Genotype of adenine-independent colonies	No. obtained
	Total	Per dish		
$\frac{paba \quad y\ ad_8\ BI}{PABA \quad Y\ ad_8\ bi}$	$3{\cdot}42 \times 10^8$	$5{\cdot}70 \times 10^7$	$\frac{paba \quad y\ ad_8\ BI}{PABA \quad Y\ AD_8\ bi}$	5
			$\frac{paba \quad y\ AD_8\ BI}{PABA \quad Y\ ad_8\ bi}$	1
			$\frac{PABA \quad Y\ AD_8\ bi}{}$ *	1
$\frac{W}{w}\ \frac{paba \quad y\ ad_{16}\ BI}{PABA \quad Y\ ad_{16}\ bi}\ \frac{NIC}{nic}$	$2{\cdot}00 \times 10^8$	$1{\cdot}00 \times 10^7$	$\frac{W}{w}\ \frac{paba \quad y\ AD_{16}\ BI}{PABA \quad Y\ ad_{16}\ bi}\ \frac{NIC}{nic}$	3

* This haploid could be a contaminant or represent a case of back-mutation followed by haploidisation.

satisfactorily accounted for it there has been an exchange *between y and* ad_{16}. As a result of this exchange the yellow strand should carry *bi* and this is found to be the case for diploid vi, but on the contrary diploid vii carries *BI* on this strand. Unless the extremely improbable assumption is made that back-mutation of *bi* has occurred, a second exchange must have occurred *between* ad_8 *and bi* in this diploid. The green strand of both diploids carries the parental markers except that ad_{16} has been replaced by its wild type allele. There are two possibilities that will account for this. Either back-mutation of ad_{16} has occurred or a further mitotic exchange between the alleles.

If the former assumption is made then back-mutation of ad_{16} has on two occasions been accompanied by a mitotic exchange between *y* and ad_{16}. This can scarcely be due to coincidence since the recombination fraction between these two loci is not greater than 0·002.

The results of meiotic analysis of crosses between alleles indicated that reversion to adenine-independence was associated with crossing

over between *y* and *bi*. It was an extrapolation from the data, not necessarily correct, that the exchange occurred *between* the alleles. It might be argued that an exchange close to the right or left of the alleles might also result in an adenine-independent strand. If this were true it might be argued that the exchange between *y* and ad_{16} in diploids vi and vii, and the reversion of ad_{16} are but two aspects of one event. Such an assumption is still unable to explain the genotype of diploids vi and vii, however, since the strand in which

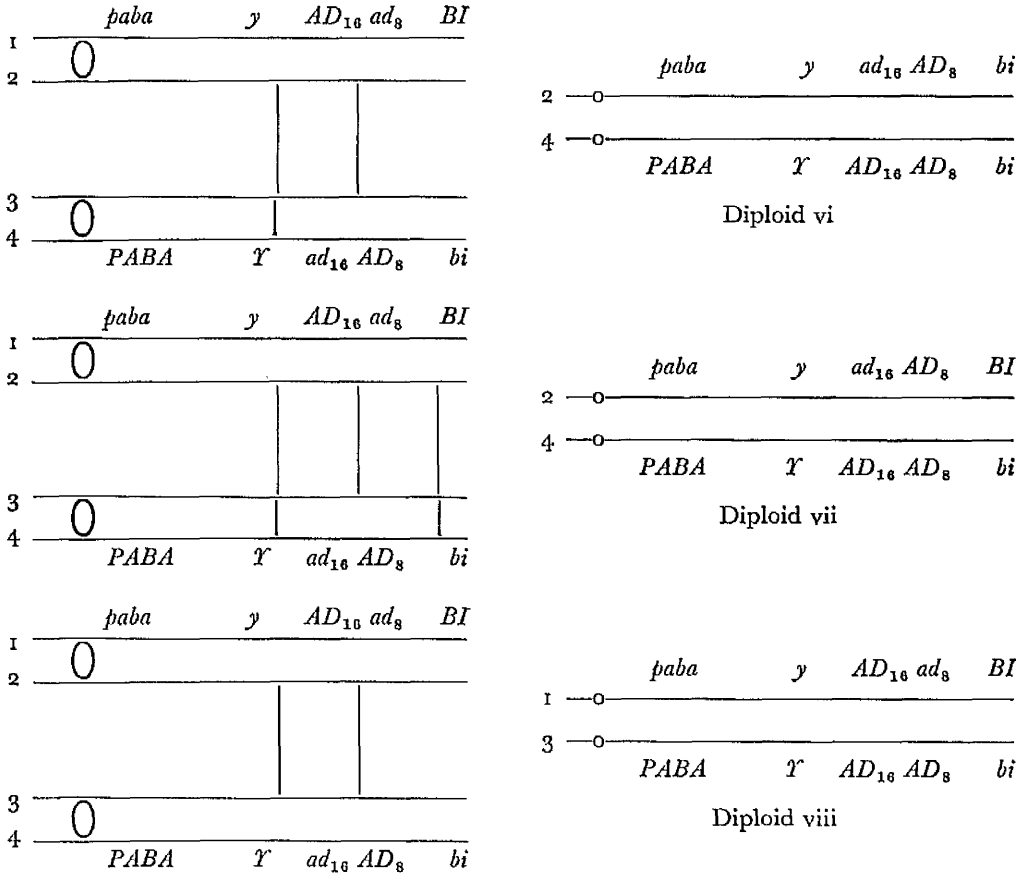


FIG. 4.—Types of mitotic exchange which may produce diploids of classes vi, vii and viii. The parent diploid is shown on the left.

reversion of ad_{16} to AD_{16} could have occurred cannot be either of those involved in the exchange between *y* and ad_{16}. One would have to assume that an exchange between two strands results in reversion of ad_{16} on a *third*.

On the other hand, if it is assumed that an exchange between the two alleles greatly increases the probability of a second exchange nearby, as is strongly suggested by the results of meiosis in crosses involving pairs of alleles, then the genotypes of diploids vi and vii offer no difficulty. In vi an exchange between the alleles has been accompanied by a second exchange between *y* and ad_{16}, as indicated in fig. 4, and in vii an exchange between ad_8 and *bi* has occurred in addition (fig. 4).

Since the conclusion that multiple exchanges have occurred to give rise to vi and vii is based on the fact that diploids of both these classes possess a strand with *y* and ad_{16} in coupling, it should be pointed out that these diploids could not be contaminants. No strain, haploid or diploid, with these two mutants in coupling was in existence when these diploids were recovered. Moreover, vi was obtained from diploid II and was heterozygous *W/w* and *NIC/nic* and vii, from diploid I, was heterozygous *PYRO/pyro*.

Class viii.—The four diploids in this class are identical with the parent diploids except that ad_{16} has been replaced by its wild type allele. The absence of ad_{16} from the yellow strand was in each case verified by outcrossing to an adenine-independent strain. Back-mutation of ad_{16} or certain types of double mitotic exchange between *y*, ad_{16} and ad_8 (see fig. 4) will both produce diploids of this type. Since Class vi and vii diploids indicate that double exchanges of this type do occur, and since back-mutants of ad_{16} were obtained from a homozygote ad_{16}/ad_{16} (table 10), the origin of these diploids cannot be determined.

Classes ix and x.—These two diploids were similar in that both were homozygous *Y/Y*. Back-mutation of *y* to *Y* can be rejected as extremely improbable. There must therefore have been a mitotic exchange between *paba* and *y* followed by segregation of one crossover and one non-crossover strand to the daughter (fig. 5). If there had been no further exchanges, both diploids would be homozygous *bi/bi*. Diploid xi, however, is heterozygous *BI/bi* and a further exchange must have taken place between *y* and *bi* unless mutation is again invoked. Both are, of course, adenine-independent. Either back-mutation of ad_{16} or two further mitotic exchanges are required.

Thus two explanations to account for diploid x are possible. Either back-mutation of ad_{16} has been accompanied by a mitotic exchange between *paba* and *y*, or three mitotic exchanges have occurred simultaneously. Diploid xi similarly requires either back-mutation of ad_{16} together with two mitotic exchanges, or four mitotic exchanges.

The occurrence of an exchange between *paba* and *y* simultaneously with or subsequent to back-mutation of ad_{16} in two diploids out of 41 analysed can scarcely be due to chance, since the former event occurs with very low frequency. Thus from a plating of approximately 500 conidia from both parent diploids no yellow colonies were obtained, although one-quarter of the mitotic exchanges between *y* and the centromere would result in colonies of this type. On the other hand, it is difficult to understand the nature of an association between mutation and crossing over certainly at a different locus (the locus *y* intervenes between the sites of mutation and crossing over). The latter of the two alternatives would therefore seem the more reasonable.

Class xii.—The extraordinary aspect of the genotype of this diploid was that it possessed no *ad* allele at all. Either simultaneous back-mutation of both ad_{16} and ad_8 are required or four mitotic

exchanges, two of which must be between the alleles (fig. 5). It is unlikely that this diploid was a contaminant since it was heterozygous *PYRO/pyro*.

(iv) *Discussion*

Before discussing the significance of the results of mitotic analysis, it should be recalled (Pontecorvo *et al.*, 1954 and personal communication) that mitotic crossing over occurs with low frequency and that coincidence of two, let alone more, mitotic exchanges in the arm of the chromosome studied here is a rare event.

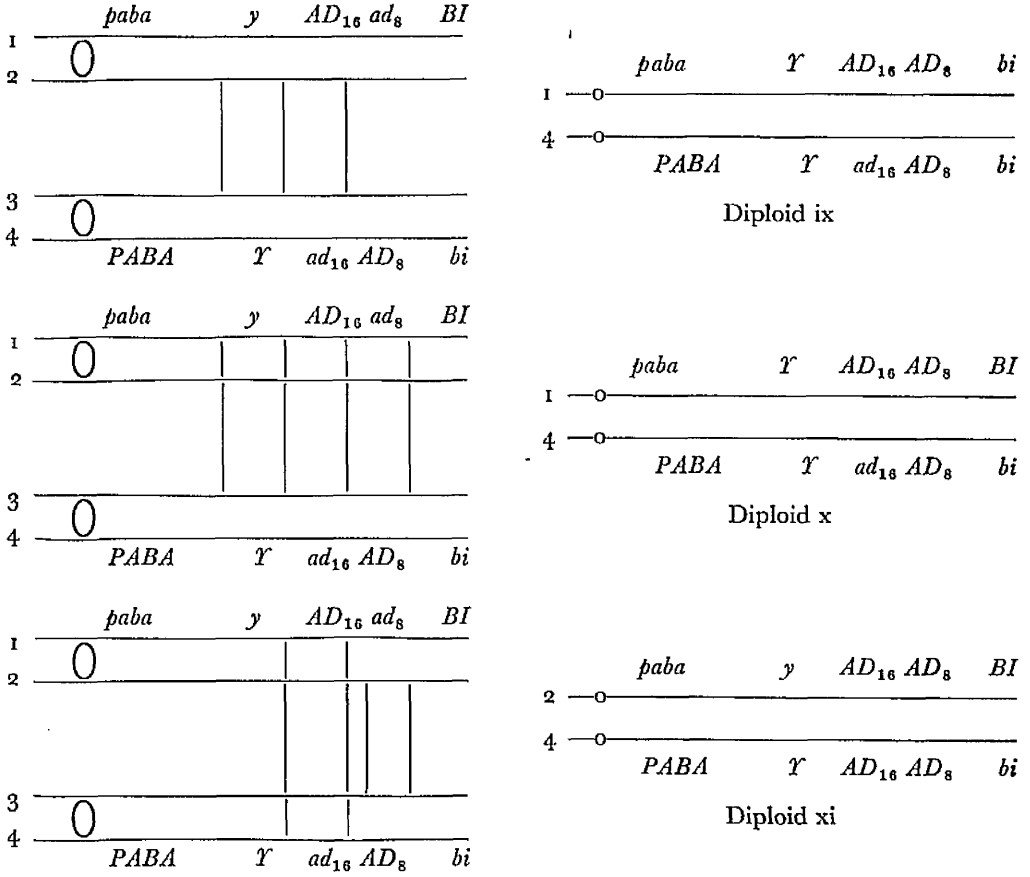


FIG. 5.—Types of mitotic exchange which may produce diploids of classes ix, x and xi. Only one of several possible types of strand arrangement is shown.

Mitotic analysis has shown that there is an unquestionable association between reversion to adenine-independence and crossing over between *y* and ad_{16}, or between ad_8 and *bi*. In most cases it was also clear that the change from adenine-dependence to independence was due to or associated with crossing over between the alleles, but in some cases the results could also be interpreted as due to the simultaneous occurrence of back-mutation of one or other allele and mitotic crossing over elsewhere. The ambiguity of these types was due to the fact that if multiple exchanges indeed occurred, they were 3- or 4-strand doubles, or multiples, in which more than two strands were involved. The reciprocal products in multiples of this type

cannot be recovered mitotically by our technique, though they could by tetrad analysis in meiosis. Alternatively the non-reciprocal products of a 2-strand multiple were recovered.

Coincidence of back-mutation of ad_8 or ad_{16} with mitotic crossing over is unlikely owing to the rarity of the two events. On the other hand, an association between mutation and crossing over cannot be ruled out, particularly in view of the recent work of Mitchell (1955) with *Neurospora*. While we have no evidence at present that would favour an interpretation of this type there is positive evidence of the occurrence of additional mitotic exchanges following an exchange between alleles, as for example in Classes iii and iv. It therefore seems reasonable at present to conclude that possible cases of mutation associated with mitotic crossing over are instances of multiple mitotic crossing over in which, for the reasons already stated, both products of every exchange have not been recovered.

If the results of mitotic analysis have been interpreted correctly a multiple mitotic exchange rate greater than expected at *meiosis* has indeed been observed. Thus types iii, iv, vii, x and xi, a total of 6, all have an exchange between ad_8 and *bi* in addition to one between the alleles. The fraction of doubles is 6/36 or 16·7 per cent. (the 5 Class v diploids are excluded from this calculation since they are either back-mutants or doubles of the type considered here). Even if types vii and x are excluded as possible cases of back-mutation associated with crossing over and class xi as a case of simultaneous back-mutation of both ad_8 and ad_{16}, the frequency of doubles (9·1 per cent.) remains not only enormously higher than expected from chance coincidence of two mitotic exchanges, but also higher than expected at meiosis.

Similarly there were at least two (vi and vii), and possibly three more (ix, x and xi), diploids in which crossing over had taken place between *y* and ad_{16} although these loci are extremely closely linked (see table 1).

It is clear from the mitotic data that inversions cannot be responsible for the excess of multiple exchanges observed since the majority of these are not 2-strand doubles.

4. GENERAL DISCUSSION

Two questions are considered in this section : the bearing of the results obtained here on the problem of allelism, and the meaning of the intense negative interference discovered.

The crosses involving pairs of alleles in what will be called the "ad_8 region" identify at least four mutational sites separable by crossing over and arranged in the linear order : ad_{16}, ad_{11}, ad_8 and ad_{10}. The data also indicate that ad_{12}, ad_{19} and ad_{20} are mutants of different sites from ad_8 and ad_{10} although their relationships to each other and to ad_{16} and ad_{11} have not yet been worked out. The recombination fractions between *ad* alleles and between *y* and different

ad alleles are for the most part consistent with each other (see fig. 6, which gives all recombination fractions so far measured).

With the exception of the cross involving ad_8 and ad_{10} there is no evidence from either meiotic or mitotic analysis that unequal crossing over is involved in the origin of adenine-independent types, and even in this cross the results may be due to multiple exchanges in view of the results of mitotic analysis.

Infertility has so far prevented the test of all the available alleles in the ad_8 region against each other. This would have provided an estimate of the probable number of sites of mutation separable by crossing over in this region. But other evidence from *A. nidulans* alone already suggests that the frequency of independent re-occurrences of the " same " mutation, *i.e.* not distinguishable from another one by a test of crossing over, is small, and consequently that the number of

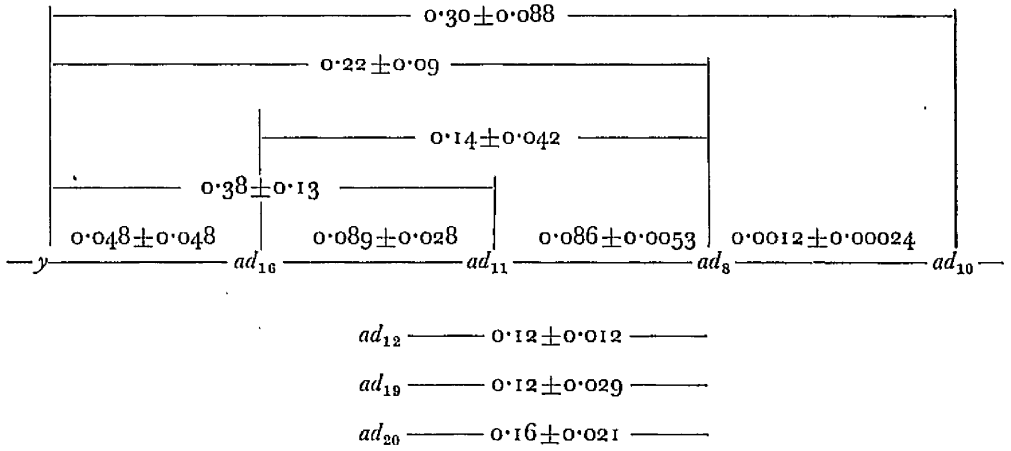


FIG. 6.—Linkage map of the ad_8 region giving all recombination fractions ($\times 10^2$) so far measured.

sites of mutation within chromosome segments behaving as functional units is large. Thus, tests between over twenty alleles in five regions have in no case failed to yield recombinants in experiments with a resolving power of 10^{-5} (Roper, 1953 ; Pontecorvo, 1955 ; Calef, unpublished ; Forbes, unpublished).

Explanations of the Lewis effect in terms of sequences of reactions and localised gene products therefore seem less satisfactory than at first supposed by Pontecorvo (1950, 1952*a*, 1952*b*). On the other hand, the occurrence of suppressors specific for some but not others of an allelic series (Giles and Partridge, 1953 ; Yanofsky, 1953 ; Green 1954)—and ad_{20} is a further example—and the separation of " suppressible " and " non-suppressible " alleles by crossing over, has been considered evidence that the two types of allele are mutants of " different genes with distinctive loci and functions " (Green, 1954). It is equally reasonable to suppose, however, that mutation at different sites, or different types of mutation at one site, within a single functional unit can result in qualitatively or quantitatively distinct modifications of a single normal gene product. Some types of modification, probably

the less extreme, might give rise to a mutant phenotype under certain intracellular conditions but not in others. A change of *p*H, for example, produced by a suppressor mutation might suppress a mutant which produced an enzyme with an altered *p*H optimum, but not one which completely prevented the synthesis of the enzyme.

Clearly distinction between alternative explanations of the Lewis effect must await more critical information. In particular, we need more information as to the probable number of sites of mutation within chromosome segments behaving physiologically as a unit, and as to qualitative differences between alleles.

The occurrence of negative interference associated with recombination between *ad* alleles raises a number of questions. How far does the influence of a crossover between alleles extend? Is negative

TABLE 11

Recombination fractions between paba *and* y, y *and* ad, *and* ad *and* bi *calculated only among crossovers between* ad *alleles (from the data of tables 2, 5, 6 and 7)*

ad alleles involved in the cross	Recombination fraction between *paba* and *y*	Probability of deviation from 0·15	Recombination fraction between *y* and *ad*	Recombination fraction between *ad* and *bi*
8 and 10	2/28 = 0·071	not sig.	6/28 = 0·21	11/28 = 0·39
8 and 11	28/179 = 0·16	not sig.	10/365 = 0·027	43/179 = 0·24
8 and 12	25/139 = 0·18	not sig.	6/139 = 0·043	32/139 = 0·23
8 and 16	25/139 = 0·18	not sig.	6/139 = 0·043	31/139 = 0·22
8 and 19	6/28 = 0·21	not sig.	1/28 = 0·036	10/28 = 0·36
8 and 20	28/79 = 0·35	0·001	8/79 = 0·10	13/79 = 0·16
11 and 16	6/57 = 0·10	not sig.	11/57 = 0·19	6/57 = 0·10

interference associated only with crossing over between alleles or between any closely linked mutants? Is it a general property of recombination between alleles or closely linked mutants in all organisms or is it confined to *A. nidulans* or even to the ad_8 region.

The recombination fraction between *paba* and *y* among crossovers between *ad* alleles from all available crosses has been calculated in table 11. On the same table these values are compared with 0·15, the standard recombination fraction between these two loci. In five of these crosses this fraction among crossovers was greater than 0·15 (significantly so only in one). There is therefore some indication that negative interference associated with an exchange between alleles extends beyond *y*.

The data in table 11 also suggest that the degree of negative interference associated with recombination between alleles is different in different crosses and shows no apparent correlation with the position in the ad_8 region of the alleles involved. It has been assumed in the table, however, that all *AD* types showing no recombination for *y* and *bi* were double exchanges with one exchange between two alleles and a second between *y* and *bi*. The possibility cannot of course be excluded that some have originated by mutation.

In order to answer the question of the dependence of negative interference on crossing over between any two closely linked loci, all available data from crosses involving different *ad* mutants and an adenine-independent strain were re-examined (table 12). The recombination fraction between an *ad* mutant and *bi* (or between *y* and *bi* which will have approximately the same value) can be compared with the same fraction among crossovers between the *ad* mutant and *y*. The number of crossovers obtained between any one *ad* mutant and *y* is too small to give statistically significant information, but the results as a whole strongly suggest that exchanges between *y* and an *ad* mutant also increase the probability of a second exchange nearby, *i.e.* between *ad* and *bi*.

A similar comparison of the recombination fraction between *paba* and *y* among crossovers and non-crossovers between *y* and different *ad* mutants (table 12) gives little evidence of negative interference, but in view of the distance between *paba* and *y* much more extensive data would be needed for significant information.

In connection with the question of how general the properties described here are, there are a number of important observations. In *Aspergillus*, similar effects are associated with recombination between alleles of *paba* (Roper, unpublished), and with recombination between closely linked mutants of the ad_9 region (Calef, unpublished). In *Neurospora*, the results obtained by Giles (1951) from a cross between two inositol alleles showing recombination were strikingly similar to those obtained here. The frequency of inositol independent cultures with parental combinations of markers was considerably greater than expected from the known distance between these markers, but platings of ascospores from crosses between strains carrying the same inositol allele indicated that the results could probably not be attributed to back-mutation. The results of Weijer (1954) from crosses involving allelic tryptophan ("*td*") mutants suggest that negative interference is associated with recombination between alleles here as well, although less reliance can be placed on these results since markers were available on one side of the "*td*" region only.

In *Drosophila* on the other hand, published data on crossing over between very closely linked mutants give no evidence of negative interference. On the contrary, the results of Green and Green (1949) and Lewis (1945) suggest that crossing over between alleles is associated with positive interference. Certain so-called mutations of "*Bar*" (Sturtevant, 1925 ; Bonnier, Nordenskiöld and Bågman, 1943) could have been produced by multiple crossing over within a very short chromosome segment, but unequal sister-strand crossing over would also produce the same types. It was suggested to me by Prof. C. D. Darlington that "reciprocal crossing over" in the sex chromosomes of *Drosophila* (Darlington, 1934) might be an evolutionary exploitation and modification of a system of negative interference of the type encountered here.

TABLE 12

Comparison of the recombination fractions between paba *and* y, *and* ad *and* bi *among crossovers and non-crossovers between* y *and three* ad *mutants*

Cross	Selection	Segregations			Rec. fract. between *paba* and *y*	Rec. fract. between *ad* or *y* and *bi*
		PABA *BI*	*bi*	*paba* *BI*	*bi*	Total		
$\frac{w\ ad_1}{W\ AD_1}\ \frac{paba\ y\ AD_8\ BI}{PABA\ Y\ ad_8\ bi}$	*W Y AD*	6	1	2	0	*9*	0·22±0·14	0·11±0·10
	W y AD	26	1	165	14	*206*	0·13±0·023	0·072±0·018
$\frac{paba\ y\ ad_8\ BI}{PABA\ Y\ AD_8\ bi}\ \frac{PYRO}{pyro}$	*y AD PYRO*	0	3	1	16	*20*	0·15±0·080	0·050±0·049
			Y		*y*			
	BI PYRO		28		515	*543*	...	0·052±0·0095
		PABA *BI*	*bi*	*paba* *BI*	*bi*			
$\frac{w\ ad_1}{W\ AD_1}\ \frac{paba\ y\ AD_{10}\ BI}{PABA\ Y\ ad_{10}\ bi}$	*W Y AD*	4	1	0	1	*6*	0·16±0·15	0·33±0·19
	W y AD	9	1	47	7	*64*	0·16±0·033	0·12±0·041
$\frac{paba\ y\ AD_{10}\ BI}{PABA\ Y\ ad_{10}\ bi}\ \frac{pyro}{PYRO}$	*Y AD PYRO*	11	3	5	0	*19*	0·26±0·10	0·16±0·084
	y AD PYRO	10	1	61	3	*75*	0·15±0·041	0·053±0·026
			Y		*y*			
	BI PYRO		17		258	*275*	...	0·062±0·014
		PABA *BI*	*bi*	*paba* *BI*	*bi*			
$\frac{paba\ y\ AD_{11}\ BI}{PABA\ Y\ ad_{11}\ bi}\ \frac{pyro}{PYRO}$	*Y AD PYRO*	6	1	2	0	*9*	0·22±0·14	0·091±0·050
		BI		*bi*				
	PABA Y AD PYRO	22		2		*24*		
		Y		*y*				
	BI PYRO	27		509		*536*	...	0·050±0·0094
		PABA		*paba*				
	y BI PYRO	36		165		*201*	0·18±0·027	...

Any model to account for negative interference of the type found here must take into account that negative interference is not observed when recombination between loosely linked mutants is followed (extensive data from many crosses in *A. nidulans* involving *paba*, *y* and *bi* give no evidence of interference, either positive or negative). One possibility is that " effective " pairing (that is pairing that can lead to crossing over—not necessarily identical with pairing observed cytologically) is confined to short chromosome segments, at any one point the homologues usually remaining " effectively " unpaired. Positive interference would occur if " effective " pairing of one segment reduced the probability of " effective " pairing of neighbouring segments. Within " effectively " paired segments, however, negative interference would be apparent (Rothfels, 1952).

If a model of this type is correct an important question is the length of " effectively " paired segments. The association of negative interference with recombination between *ad* alleles would not be of fundamental importance if the apparent close linkage between the ad_8 region and *y* were simply due to suppression of crossing over by pairing failure. On the other hand if the chromosome segment between the ad_8 region and *y* is of the same order of length as the ad_8 region itself, as the map distances suggest, then multiple crossing over within a chromosome segment of the same size as that occupied by one gene has been observed. In *Aspergillus*, where cytological localisation of mutants is not possible, these alternatives cannot be distinguished until the segregation of three alleles has been followed simultaneously, but the latter alternative raises the possibility that single crossovers detected either cytologically or genetically are frequently the net result of two or more exchanges so close together as to be detectable only by the use of extremely closely linked mutants, or possibly in tetrad analysis as an excess of non-parental ditypes. The ease with which multiple exchanges of this type could be detected would depend on the length of " effective " pairing segments. Variation of this might account for the difference between *Drosophila* and *Aspergillus* with respect to the occurrence of negative interference.

Present information does not warrant the building of a precise working model for the basis of negative interference and of its connections with crossing over and chromosome re-duplication. The data so far give only a clear indication that analysis of crossing over within very short chromosome segments may reveal novel modalities.

5. SUMMARY

1. The first four out of a group of nine physiologically allelic adenine-requiring mutants of *Aspergillus nidulans* were found to be mutants at different, but very closely linked, loci separable by crossing over. Three others were found to be mutants at loci different from two of the first four, but their location with respect to the other two and to each other has not been determined.

2. The results provide evidence additional to that already available in four other chromosome regions of the species and from other organisms, that physiologically allelic mutants are not usually the result of re-occurrence of mutation at the same locus. Within a chromosome segment behaving functionally as a unit there are several, probably very many, sites capable of independent mutation and likewise several sites of crossing over.

3. Two suppressors specific for one but not others of these alleles have been found.

4. Meiotic analysis suggested that a crossover between alleles, and perhaps between any very closely linked mutants, is associated with *negative interference*. Analysis of half-tetrads following mitotic crossing over has provided further evidence to support this.

5. There is evidence that negative interference of this type occurs in other organisms. This raises the possibility that single crossovers detected cytologically or genetically may be the net result of clusters of exchanges so close together as to be detectable only under special conditions.

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III THE OCCURRENCE OF DIPLOID ASCOSPORES, DISCOVERED AS A RESULT OF AN ANALYSIS OF THE RELATIONSHIP BETWEEN THE MUTANTS *ad*$_1$ and *ad*$_3$.

1. Introduction.

It had been shown (Pontecorvo, 1953) that rare prototrophs could be obtained from crosses between *ad*$_1$ and *ad*$_3$ and it had been tentatively assumed that these were crossovers with the constitution *AD*$_1$*AD*$_3$. It was uncertain whether the two mutants were allelic. The results of heterokaryon tests to check this point had been inconclusive owing to the ability of both mutants to grow on minimal medium at very reduced rates.

Preliminary work (Pontecorvo, 1953) had also given some evidence that the two mutants were linked to the loci *w* and *y*. In most linkage tests *W*/*w* and *Y*/*y* segregated independently from *ad*$_1$ and *ad*$_3$, but among the prototrophs obtained from crosses between these mutants they no longer gave 1:1 segregations (Crosses 1 and 2, Table 3). These results suggested that the loci were linked in the order shown in Table 3.

That evidence of linkage was obtained only among apparent crossovers between *ad*$_1$ and *ad*$_3$ was considered to be due to interference

TABLE 3.

Segregation of W/w, Y/y, and THI/thi in crosses involving ad_1 and ad_3.

Cross 1:- $\frac{w \quad ? \quad ad_1\ AD_3 \quad ? \quad THI^*}{W \qquad AD_1\ ad_3 \qquad thi}$

2:- $\frac{ad_1\ AD_3 \quad ? \quad Y \quad ? \quad THI}{AD_1\ ad_3 \qquad y \qquad thi}$

Cross	Selection	Segregation of unselected markers.				
1	AD_1 AD_3 THI ø	W 29	w 9			Total 38
2	AD_1 AD_3 THI ø	Y 35	y 14			49
	AD_1 AD_3 ⊠	Y THI 41	Y thi 14	y THI 9	y thi 11	75
	Perithecium analysis (all colonies either ad_1 or ad_3)	41	54	59	55	209

* Symbols above the fraction signs indicate the alleles derived from one parent; those below the line, the alleles from the other parent. Linked loci have common fraction signs.

ø Data of G. Pontecorvo.

⊠ Data of the author.

resulting from the crossover between these loci reducing the effective distance between them and w and y.

The work reported in this section was undertaken to verify the occurrence of recombination between ad_1 and ad_3, to obtain an estimate of the recombination frequency, and to obtain more critical evidence for the linkage of these loci to w and y.

During the course of these experiments the discovery was made that ascospores with diploid nuclei, and probably also with aneuploid nuclei, occur with low frequency in Aspergillus nidulans and are detectable under certain selective conditions. An attempt has been made to ascertain the origin of these ascospores.

2. Experimental.

The analysis of cross 2 was repeated and the segregation of THI/thi followed. The majority of adenine independent isolates were THI Y (Table 3), confirming the previous results with respect to the segregation of Y/y, but in addition suggesting linkage of y and thi. No evidence of such linkage was obtained from perithecium analysis however (Table 3).

Perithecium analysis and selection of adenine independent types from two further crosses (Tables 4 and 5) suggested that the

linkage between w, y, and thi, and the ad loci was spurious. Irrespective of the coupling arrangements of the different markers involved, an excess of the dominant, wild type, alleles was always obtained among the adenine-independent types. This is most clearly illustrated in cross 4 (Table 5). In this cross the markers y and bi were in repulsion, but although these two loci are not more than 6 units apart, the majority of adenine independent colonies were phenotypically Y BI; i.e. apparent crossovers between y and bi.

This immediately suggested that some at least of what had been considered to be crossovers between ad_1 and ad_3 (AD_1AD_3) might in fact be diploids or polysomics heterozygous for these loci (ad_1AD_3/AD_1ad_3) and for the other markers as well.

The properties of diploids produced by rare fusion of nuclei in heterokaryons of A. nidulans are well known (Pontecorvo and Roper, 1953; Pontecorvo, Tarr Gloor and Forbes, 1954; Pontecorvo and Kafer, 1954) following their discovery by Roper (1952). They can be readily distinguished from haploids in several ways. Their conidia have approximately, and very constantly, twice the volume of haploid conidia; they undergo mitotic segregation and reduction; and they produce some asci with 16 spores.

The conidium diameter of a number of green prototrophs from cross 4 was measured with an eyepiece micrometer and found to have a

TABLE 4.

Comparison of recombinant selection and perithecium analysis of a cross involving ad_1 and ad_3.

Cross 3:- $\frac{w_n}{W_n}$ $\frac{ad_1\ AD_3}{AD_1\ ad_3}$ $\frac{paba_1\ y}{PABA_1\ Y}$ $\frac{THI}{thi}$

Selection			Segregations * PABA THI	PABA thi	paba THI	paba thi	Total
perithecium analysis. (Ascospores plated on m.m. + adenine aneurine and P.A.B.A)	white	ad_1	24	20	17	12	73
		ad_3	26	35	16	17	94
	yellow	ad_1	1	1	20	21	43
		ad_3	2	4	21	12	39
	green	ad_1	24	24	3	5	56
		ad_3	29	35	1	1	66
	Total		106	119	78	68	371
AD_1AD_3 (Ascospores plated on m.m. + aneurine + P.A.B.A)	white		20	10	6	5	41
	yellow		4	2	4	5	15
	green		68	22	2	3	95
	Total		92	34	12	13	151

* It is possible to distinguish between ad_1 and ad_3 colonies since the latter grow slowly at 37°C to produce characteristic aconidiate colonies.

TABLE 5.

Recombinant selection and perithecium analysis from a further cross involving ad_1 and ad_3.

Cross 4:- $\frac{w_n \quad AD_1\ ad_3 \quad PABA_1\ Y\ bi}{W_n \quad ad_1\ AD_3 \quad paba_1\ y\ BI}$

Selection			Segregations PABA BI	PABA bi	paba BI	paba bi	Total
Perithecium analysis. (Ascospores plated on M.M. with adenine P.A.B.A. and biotin)	white	ad_1	3	17	19	7	46
		ad_3	5	16	18	2	41
	yellow	ad_1	4	0	25	0	29
		ad_3	1	0	12	0	13
	green	ad_1	0	27	0	1	28
		ad_3	0	14	0	4	18
	Total		13	74	74	14	175
AD_1AD_3 (Ascospores plated on M.M. with P.A.B.A. and biotin)	white		13	23	30	2	68
	yellow		4	0	16	0	20
	green		75	28	5	2	110
	Total		92	51	51	4	198

value characteristic for diploids. A careful examination of these isolates showed all of them to possess a few heads of yellow or white conidia (or of both types) indicating that they were heterozygous for one or both colour markers and segregating mitotically.

Following this discovery the conidium diameter of each of the 198 adenine independent isolates from cross 4 was measured. The standard procedure (Pontecorvo et al, 1954) is to obtain a mean value for the conidium diameter based on the length of 20 chains of five conidia. In most cases, however, it was only necessary to measure a few chains in order to establish into which class an isolate belonged and since large numbers of colonies had to be examined this method was used as it gave a considerable saving of labour. When there was any doubt, the full 20 chains were measured. The conidium diameter of all isolates measured by the standard method is given in Table 6.

The adenine independent isolates fell into two distinct and non-overlapping classes, those with a conidium diameter characteristic of diploids (144 in number) and those with a diameter characteristic of haploids (54). A single colony of each green and yellow isolate was examined for the presence of white and yellow conidial heads (white only in the case of yellow isolates). Of the 93 'diploid' greens, 70 showed a few heads of white or yellow conidia.

TABLE 6.

Conidium diameter of parent strains and some adenine-independent isolates from cross 4.

Isolate number.	Phenotype.	Conidium diameter(μ) *	Somatic Segregants.	Inferred ploidy.
parent	$w_n ad_3$ bi	2.63	-	haploid
parent	ad_1 paba y	2.79	-	haploid
17	green	3.70	white and yellow	diploid
18	white	3.72	-	diploid
22	green	3.61	yellow	diploid
26	green	3.72	yellow	diploid
31	green paba bi	2.76	-	haploid
28	white paba	3.13	-	haploid
54	green paba bi	2.87	-	haploid
59	white paba bi	2.85	-	haploid
81	white	3.13	-	haploid
82	green bi	3.68	-	diploid
208	green	3.63	yellow	diploid
230	green paba	3.61	yellow	diploid
237	yellow	3.00	-	haploid
242	green bi	3.70	-	diploid
249	green	3.52	white and yellow	diploid
264	yellow	3.61	white	diploid

* Haploid range 2.52 - 3.62μ; diploid range 3.50 - 4.14μ. (from Pontecorvo, Tarr Gloor and Forbes, 1954). Diameter given is the mean obtained by measurement of 20 chains of 5 conidia.

On the 15 haploid greens no spots of either yellow or white were found. Of the 12 'diploid' yellows, 8 possessed a few white heads, but none could be found on the 8 haploid yellows.

There was thus evidence on two counts, conidium diameter and mitotic segregation of w and y, that the majority of adenine independent isolates from cross 4 were either diploids or polysomics. In Table 7 the isolates have been divided into two dasses, haploid and not haploid. The majority of isolates with the dominant phenotype for y, paba and bi, including most of the apparent crossovers between y and bi, fell into the diploid class suggesting that they were heterozygous for these markers.

There is little doubt that the haploid adenine independent isolates were crossovers between ad_1 and ad_3. Conceivably they could have been back mutants of one or other of these mutants but this is unlikely since, in the first place, platings of several million conidia from both ad_1 and ad_3 strains and also from the heterokaryon of cross 4, gave no reversions (Table 8), although the frequency of adenine independent isolates from cross 4 would require a back mutation rate of at least 10^{-4}. Admittedly the back mutation rate may be much higher among ascospores than conidia, but a plating of approximately 2×10^4 viable ascospores from hybrid asci from a cross between two ad_3 strains (Table 8) gave no adenine independent colonies.

TABLE 7.

Separation of the adenine-independent isolates from cross 4 into two classes on the basis of conidium diameter.

HAPLOIDS.

	PABA		paba		Total
	BI	bi	BI	bi	
White	0	11	18	2	31
yellow	1	0	7	0	8
green	1	12	0	2	15
Total	2	23	25	4	54

DIPLOIDS. *

	PABA		paba		Total
	BI	bi	BI	bi	
white	13	12	12	0	37
yellow	3	0	9	0	12
green	74	16	5	0	95
Total	90	28	26	0	144

* The symbols refer to phenotype, not to genotype.

TABLE 8.

Tests for back mutation of ad_1 and ad_3.

Origin of conidia or ascospores	Spores plated	Spores plated on:	No. of spores plated	Colonies
ad_1	conidia	M.M. with adenine	520	483
		M.M.	4.8×10^6	0
ad_3 y	conidia	M.M. with adenine	330	345
		M.M.	3.3×10^6	0
w_n ad_3 bi + ad_1 $paba_1$ y (Heterokaryon)	conidia	Complete medium	420	404 (y 12, Y 392)
		M.M. with biotin and P.A.B.A.	1.4×10^6	0
w_n ad_3 bi + y thi ad_3 (Heterokaryon)	ascospores	M.M. with adenine	1.04×10^4	97
		M.M. with biotin and aneurin	5.4×10^5	0

Unfortunately partial sterility of crosses between two $\underline{ad}_3$ strains prevented plating greater numbers of ascospores. In the case of crosses between two strains carrying $\underline{ad}_1$ the sterility was almost complete and prevented a test for back mutation among ascospores from being made.

Among the haploid crossovers between $\underline{ad}_1$ and $\underline{ad}_3$ from cross 4 (Table 7) and also from a second cross (cross 3, Table 9) the other markers segregated independently. There was thus no evidence of linkage between the ad loci and w, paba, y, bi or thi. A study of patterns of mitotic segregation from heterozygous diploids however (Pontecorvo, Tarr Gloor and Forbes, 1954) has more recently shown that w and the ad loci are in fact located on the same chromosome, although more than 50 ~~ellorgans~~ units apart, (Section IV, Fig. 2) and that this chromosome is different from the one carrying y.

Two estimates of the recombination fraction between $\underline{ad}_1$ and $\underline{ad}_3$ have been obtained. The indirect method described in Section II was used, the relevant data being given in Table 10. The estimates from the two crosses are in reasonable agreement and a combined estimate works out at 0.00059 ± 0.000060.

The next point to be investigated was the genotype of the putative diploids with respect to $\underline{ad}_1$ and $\underline{ad}_3$. Are they heterozygous for these loci ($\underline{ad_1AD_3}/\underline{AD_1ad_3}$) or recombinants ($\underline{AD_1AD_3}$; $\underline{AD_1AD_3}/\underline{AD_1}ad_3$; $\underline{AD_1AD_3}/\underline{ad_1AD_3}$ or $\underline{AD_1AD_3}/\underline{ad_1ad_3}$).

TABLE 9.

Adenine-independent colonies obtained from a further plating from cross 3, separated into haploid and diploid on the basis of conidium diameter.

Cross 3:- $\frac{w_n\ ad_1\ AD_3}{W_n\ AD_1\ ad_3}$ $\frac{paba_1\ y}{PABA_1\ Y}$ $\frac{THI}{thi}$

HAPLOIDS

	PABA		paba		Total
	THI	thi	THI	thi	
White	7	3	2	5	17
yellow	1	0	1	3	5
green	5	6	0	1	12
Total	13	9	3	9	34

DIPLOIDS*

	PABA		paba		Total
	THI	thi	THI	thi	
White	20	7	5	1	33
yellow	7	1	16	0	24
green	227	35	0	0	262
Total	254	43	21	1	319

* Symbols refer to phenotype, not genotype.

TABLE 10.

The recombination fraction between ad_1 and ad_3, and the minimum frequency of diploids, estimated from crosses 3 and 4.

Ascospores plated on:	Phenotypes selected.	No. of ascospores plated.	Colonies.	Percent recombination and diploid frequency.
Cross 3:- $\frac{w_n\ ad_1\ AD_3}{W_n\ AD_1\ ad_3}$ $\frac{paba_1\ y}{PABA_1\ Y}$ $\frac{THI}{thi}$ *				
M.M. with adenine	THI PABA	6.4×10^3	148	
M.M. with aneurin and P.A.B.A.	AD_1 AD_3	8.64×10^5	Haploids 34 Diploids 262 Diploids from dwarfs 57	0.085 ± 0.05 0.40
Cross 4:- $\frac{w_n\ AD_1\ ad_3}{W_n\ ad_1\ AD_3}$ $\frac{PABA_1\ Y\ bi}{paba_1\ y\ BI}$ *				
M.M. with adenine biotin and P.A.B.A.	W Y	2.9×10^3	340	
M.M. with biotin and P.A.B.A.	AD_1 AD_3	3.36×10^5	Haploids 40 Diploids 71 Diploids from dwarfs 43	0.051 ± 0.027 0.072

* w_n has been arbitrarily placed to the left of ad_1. It may equally well lie to the right.

It has been shown (Pontecorvo, Tarr Gloor and Forbes, 1954) that from the phenotype of diploid and haploid mitotic segregants derived from a diploid, and from the phenotype of diploid and haploid colonies derived from ascospores of this diploid, it is possible to deduce the genotype of the diploid.

Four green adenine independent isolates from cross 4 with conidium diameters in the diploid range, were selected for further analysis. Two of these isolates were biotin requiring, one of which did not give either yellow or white mitotic segregants, and the other only white segregants. The other two were prototrophs, one giving both yellow and white segregants and the other only yellow. Thus for further analysis the isolates chosen were of various types with respect to phenotype and mitotic segregation.

Each isolate was purified by single conidium isolation with a micromanipulator and a sample of conidia from each strain was plated on complete medium. The colonies which came up were examined for yellow and white conidium heads and these, if found, were isolated, purified and tested for nutritional requirements and conidium diameter. Not more than one segregant of either colour was isolated from any one colony to avoid isolating from the same segregant clone more than once. When necessary, samples of ascospores from the purified strains were plated on complete medium and the colonies produced were classified

for nutritional requirements and conidium diameter.

The results of this analysis are presented in Table 11. From each of the four strains analysed in this way it was possible to isolate haploid segregants which had the phenotype of $\underline{ad}_1$ and $\underline{ad}_3$. (It is possible to distinguish between these two mutants since $\underline{ad}_3$ will grow slowly at 37°C and is adenine independent at room temperature. $\underline{Ad}_1$ will not grow at the higher temperature but grows slowly at the lower). No haploid adenine independent segregants were obtained from any of the four strains analysed. These results proved that each strain was heterozygous for the two ad mutants in trans. It is therefore clear that the two mutants are complementary.

As far as these diploids are concerned there is also no connection between recombination between $\underline{ad}_1$ and $\underline{ad}_3$ and the formation of diploids. It was not practicable to test every putative diploid for its genotype with respect to $\underline{ad}_1$ and $\underline{ad}_3$, but it is unlikely that any were recombinant for these markers since this would have required the simultaneous occurrence of rare recombination and rare heterozygosity.

Whether all the remaining chromosomes were also duplicated in the $\underline{ad}_1/\underline{ad}_3$ heterozygotes could not be proved since only one of these was marked. That the marked chromosome was duplicated in the majority of the heterozygotes which were green was indicated by the occurrence of yellow mitotic segregants. The green heterozygotes which gave no yellow segregants could have been either homozygous $\underline{Y}/\underline{Y}$ or haploid

TABLE 11.

Determination of the genotype of adenine-independent strains with conidium diameters in the diploid range, obtained from a cross involving ad_1 and ad_3.

Cross from which strains obtained:- $\frac{w_n \ AD_1 \ ad_3}{W_n \ ad_1 \ AD_3}$ $\frac{PABA_1 \ Y \ bi}{paba_1 \ y \ BI}$

Strain number	Phenotype	Segregants obtained from:	Ploidy of segregants	Phenotype or genotype of segregants	Number obtained	Inferred genotype of strain
22	Green prototroph	Conidia	diploid	y	5	
				y paba	15	
			haploid	ad_1 y	1	
				ad_1 paba y	6	$\frac{W_n \ ad_1 \ AD_3}{W_n \ AD_1 \ ad_3}$ $\frac{paba \ y \ BI}{PABA \ Y \ bi}$
				ad_3 paba y	3	
		Ascospores	haploid	ad_1 y	1	
				ad_1 bi	4	
				ad_1 paba y	5	
				ad_3 bi	1	
				ad_3 paba bi	1	
				ad_3 paba y	3	
154	Green bi	Conidia	diploid	w bi	12	
			haploid	w ad_3 bi	11	
		Ascospores	diploid	bi	5	

TABLE 11 (Contd.)

Strain number	Phenotype	Segregants obtained from:	Ploidy of segregants	Phenotype or genotype of segregants	Number obtained	Inferred genotype of strain
			haploid	ad_1 bi	3	$\frac{w_n\ AD_1\ ad_3}{W_n\ ad_1\ AD_3}$ PABA Y bi
				w ad_1 bi	2	
17	Green prototroph	Conidia	diploid	w	17	
				y bi	5	
				paba y bi	13	
				w paba bi	1	
			haploid	ad_1 paba y bi	4	$\frac{w_n\ AD_1\ ad_3}{W_n\ ad_1\ AD_3}$ paba y bi / PABA Y BI
				w ad_3	11	
				w ad_3 paba bi	3	
242	Green bi	Ascospores	haploid	ad_1 bi	4	
				ad_1 paba bi	4	$\frac{W_n\ AD_1\ ad_3}{W_n\ ad_1\ AD_3}$ paba Y bi / PABA Y BI
				ad_3 bi	3	
				ad_3 paba bi	6	

for this chromosome. Two of the green adenine independent isolates analysed in detail (Table 11) were of this type. One of these (No. 242) was heterozygous PABA/paba and must therefore have been Y/Y in genotype. The other (No. 154) was either homozygous for all three markers on the chromosome carrying y or haploid for this chromosome. The fact that the conidia of this strain had a diameter (3.92) near the upper limit of the diploid range suggest that the former alternative is correct.

Since the majority, if not all, the ad_1/ad_3 heterozygotes had the other marked chromosome duplicated, although no selection was imposed that would favour such duplication, it is not unreasonable to assume that the remaining unmarked chromosomes were also duplicated and that the heterozygotes were in fact diploids. Morphologically they were indistinguishable from known diploids; this would not be expected if they were aneuploids.

An estimate of the incidence of diploids among ascospores can be made by a calculation similar to that for obtaining the recombination fraction between ad_1 and ad_3. It will be an underestimate of the total incidence since it takes no account of ad_1/ad_1 and ad_3/ad_3 homozygotes which are undetected. That diploids of this type occur was shown in the following way.

Diploid green colonies can by experience be distinguished from haploid greens, both by being paler in colour (particularly when

heterozygous for one or both colour markers) and by a distinct morphological difference of the conidial heads. Ascospores from crosses 3 and 4 were plated in minimal medium with adenine only and the resulting colonies were searched for pale greens which were isolated and tested for diploidy by measurement of conidium diameter. From cross 3, two pale greens were obtained and both were diploid and adenine requiring (one with $\underline{ad}_1$ phenotype and one $\underline{ad}_3$). Both gave yellow mitotic segregants. From cross 4 seven diploids were obtained of which one was adenine requiring ($\underline{ad}_1$).

These experiments did not give a quantitative estimate of the frequency of $\underline{ad}_1/\underline{ad}_1$ and $\underline{ad}_3/\underline{ad}_3$ homozygotes since white and yellow diploids, and any which might have been dark green, were not detected.

The frequency of heterozygotes for the ad mutants from crosses 3 and 4 (including those recovered from dwarfs - vide infra) works out at 0.4% and 0.072% respectively of the viable ascospores from hybrid asci (Table 10). The marked discrepancy between the two estimates is in contrast with the fair agreement found for the recombination frequency between the two ad mutants and probably results from a real difference in the frequency of occurrence of diploids in the two crosses.

Mention has been deferred until now of a type of colony, the nature of which was puzzling, which was obtained when selecting

for adenine independent types from crosses between $\underline{ad}_1$ and $\underline{ad}_3$. These colonies which occurred with greater frequency than either haploid recombinants or diploid heterozygotes possessed the following properties:-

(i) They were slow growing on all media and growth was not stimulated by adenine. Some were biotin requiring and some P.A.B.A. requiring (cross 4).

(ii) They produced few or no conidia, but when these were present they were generally green. There were rarely sufficient to make an adequate measurement of diameter and in most cases this was extremely variable. Conidial heads of more than one colour were often present.

(iii) When repeatedly transferred on adenine deficient medium a small proportion sooner or later produced one or more sectors of normal appearance and growth rate. These sectors usually had conidia of the diploid size (Table 12) and gave yellow and white mitotic segregants. These segregants were always adenine independent and in most cases were prototrophic. Some were either P.A.B.A. or biotin requiring (Table 12).

(iv) When repeatedly transferred on complete medium, the majority sooner or later gave one or more sectors of normal

growth rate and appearance. These sectors invariably had conidia with the haploid diameter. A single dwarf might give rise to normal growing haploid sectors of more than one genotype (Table 12) but they were invariably adenine requiring.

The ~~incidence~~ frequency of these dwarfs was difficult to estimate since many were only pin-point colonies which were rapidly overgrown by colonies with normal growth rate. Some of them also gave rise to normal growing diploid sectors so rapidly that the parent dwarf could not be isolated or kept.

The characteristics of dwarf colonies presented above suggest that they were aneuploids which were heterozygous ~~and~~ for the ad mutants. The fact that some gave rise to what are almost certainly diploids suggests that these dwarfs had more than the diploid number of chromosomes. Cytological examination would be necessary before the nature of these dwarfs can be established with certainty but the difficulties inherent in this material preclude such an analysis at the present time. More recently, however, Dr. E. Kafer (unpublished) has shown that diploid strains frequently give rise to aneuploid strains and that these have properties very similar to the dwarfs described here. Some of the dwarf colonies were completely stable never giving rise to haploid segregants. Unless they had less than the haploid number of chromosomes~~number~~, such

TABLE 12.

Types of segregant obtained from some dwarf colonies from cross 4.

Cross 4:- $\frac{w_n\ AD_1\ ad_3}{W_n\ ad_1\ AD_3}\ \frac{PABA_1\ Y\ bi}{paba_1\ y\ BI}$

No. of isolate	Phenotype	Phenotype of non-dwarf segregants	Ploidy of segregant	Conidium* diameter (μ
23	prototroph	a) y ad_1 paba	haploid	
		b) w_n ad_3 bi	haploid	
		c) ad_1 bi	haploid	
41	prototroph	a) bi	diploid	
		b) y ad_1 paba	haploid	
125	prototroph	a) prototroph	diploid	3.61
		b) w_n ad_1 paba	haploid	2.97
133	bi	a) ad_3 bi	haploid	
		b) w_n ad_1 bi	haploid	
134	paba	a) y ad_1 paba	haploid	
		b) w_n ad_3 paba	haploid	
159	y	a) y	?	3.33ø
162	paba	a) paba	?	3.30ø
152	prototroph	a) prototroph	diploid	3.61
154	bi	a) bi	?	3.92⌘
156	prototroph	a) prototroph	diploid	3.86
160	prototroph	a) prototroph	diploid	3.77
240	prototroph	a) prototroph	diploid	
		b) w_n ad_3 bi	haploid	
253	prototroph	a) ad_1 bi	haploid	2.87

* Conidium diameter given only where the full 20 chains of 5 were measured.

ø Both rather low for diploids but both give somatic segregants; may be aneuploids.

⌘ Conidia very variable in size, a few single conidia having a diameter over 5.6μ.

stability would not be expected. On the other hand nuclei with less than n chromosomes would probably be lethal. It was suggested by Dr. Pontecorvo that some of these might be heterokaryons between complementary aneuploids, one nucleus being disomic for one chromosome and nullisomic for a second, the other nucleus having the complementary chromosome constitution. Such a heterokaryon would be stable since both component nuclei would be lethal.

3. Discussion

The following conclusions have been drawn from the facts presented in this section. A small proportion of any sample of ascospores from a cross between ad_1 and ad_3 are heterozygous for these markers and a small proportion are recombinants between them. Neither heterozygotes nor recombinants will be recovered when non-selective platings of ascospores are made, owing to their rarity. On the other hand, when platings are made on adenine deficient medium the only colonies which will arise will be one or other of these types, and their relative frequencies will depend on 1) the recombination fraction between the two loci and 2) the frequency of the process leading to heterozygosity at both loci. The heterozygotes must be disomic for the chromosome carrying the ad loci, but may or may not have the remaining chromosomes

in duplicate.

Subsequent work has made it clear that the production of diploids and of probable aneuploids with appreciable frequency is a phenomenon of general occurrence in A. nidulans. Diploids have been found in all crosses in which conditions were such as to make their isolation possible viz:- in crosses between any two closely linked recessive mutants in repulsion in which recombinants with the non-mutant phenotype for both markers are selected (e.g. Tables 16, 18 and 19).

Estimates of the incidence of diploids from a large number of crosses range from about 1.0 to 0.01%, but in every case are underestimate since diploids of certain genotypes are undetected.

An attempt has been made to ascertain the mechanism or mechanisms by which diploid ascospores are produced. Several mechanisms have been considered which might operate alone or simultaneously. They are as follows:-

(i) That fusion of nuclei prior to or during perithecium development is followed by a second fusion in the ascus primordium of either two diploid, or one diploid and one haploid nucleus. The tetraploid or triploid nucleus then undergoes a meiotic division.

(ii) That the diploid zygote nucleus in the ascus primordium

fails to undergo a meiotic division and gives rise directly to one or more diploid ascospores. A mechanism of this type would produce diploids of one phenotype only and cannot, therefore, be the only mechanism operating. Neither could it account for the production of aneuploids. This mechanism will be termed ameiosis.

(iii) That non-disjunction of every bivalent occurs at the 1st meiotic anaphase, as a result of spindle or other mechanical failure, and gives rise to an interphase nucleus with the diploid number of centromeres and the tetraploid number of chromatids. The interphase nucleus then undergoes a normal 2nd meiotic division and gives rise to two diploid nuclei. Such a mechanism would give rise to aneuploid nuclei if not all the bivalents underwent non-disjunction or the non-disjunctional bivalents did not go to the same pole.

(iv) That segregation fails at the 2nd meiotic division with the result that a single diploid restitution nucleus is formed instead of two haploid nuclei. Aneuploids could be produced as in (iii).

(v) That fusion between two haploid products of meiosis occurs before ascospore formation. A mechanism of this type could not account for the production of aneuploid nuclei.

A clue to the mechanisms involved was provided by the observation that the phenotype ratios among diploids with respect to two pairs of markers did not seem to be independent, even when the two pairs were unlinked. Homozygous recessives for one pair of markers tended to be homozygous recessive for the second as well, and dominants for one pair dominant for the second. This is made clear in Table 13 which gives data from three crosses.

In cross 3 the phenotype ratio W : w was 270 : 27 among PABA diploids, but among paba diploids it was 16 : 6. The difference between these ratios is statistically significant at the 2.5% level. A similar effect is observed if the W : w and THI : thi ratios are compared although in this case the difference is not significant. The same type of relationship is observed in crosses 4 and 10 between unlinked markers (Table 13) although the lack of independence is not significant in all cases.

The only exception to this type of relationship was found when the PABA/paba and THI/thi phenotypes were compared. PABA diploids tended to be THI and vice versa. This type of correlation would be expected between linked markers in repulsion. Linkage between paba and thi has on several occasions been suggested by segregation ratios in crosses involving these markers and the results obtained here may be due to linkage.

TABLE 13.

Lack of independence of phenotype with respect to both linked and unlinked Markers in diploids of meiotic origin and heterozygous for ad_1 and ad_3.

Cross*	Segregations$^{\emptyset}$ and heterogeneity					
		PABA	paba	Total	χ^2*	P(2.5%)
3 $\frac{w_n\ ad_1AD_3}{W_n\ AD_1ad_3}$ $\frac{paba\ y}{PABA\ Y}$ $\frac{THI}{thi}$	W	270	16	286	5.47	sig.
	w	27	6	33		
	Total	297	22	319		
	THI	254	21	275		
	thi	43	1	44		
	Total	297	22	319		
		W	w			
	THI	250	25	275	2.47	not sig.
	thi	36	8	44		
	Total	286	33	319		
4 $\frac{w_n\ ad_1AD_3}{W_n\ AD_1ad_3}$ $\frac{paba\ y\ BI}{PABA\ Y\ bi}$	PABA	93	25	118	5.71	sig.
	paba	14	12	26		
	Total	107	37	144		
	BI	91	25	116	4.30	sig.
	bi	16	12	28		
	Total	107	37	144		

TABLE 13 (Contd.)

10	$\frac{w_n\ ad_1}{W_n\ AD_1}$	$\frac{AD_9\ paba\ y\ BI}{ad_9\ PABA\ Y\ bi}$		BI	bi	Total	4.56	not sig.
			W	117	10	127		
			w	9	4	13		
			Total	126	14	140		

* This table has been constructed from data already presented in full in Tables 9 (cross 3), 7 (cross 4) and 16 (cross 10).

ø Symbols refer to phenotype in all cases.

⁂ Yates' correction for continuity has been applied in the calculation of the χ^2.

The correlation of phenotypes of the type observed can be explained if it supposed that at least two mechanisms are responsible for the production of diploid ascospores; ameiosis of the zygote nucleus (giving rise to diploids heterozygous for all markers) and one or more unknown mechanisms.

If the diploids are divided into two classes, dominants (class I) and recessives (class II) for one pair of markers, then diploids of ameiotic origin must fall in class I. Considering now a second pair of markers unlinked to the first, the proportion of homozygous recessives will be greater in class II than in class I since the latter contains all diploids of ameiotic origin which have the dominant phenotype for all markers.

From a consideration of the phenotype ratios for different pairs of markers (using only those diploids homozygous recessive for a second unlinked marker) it is possible to show whether or not any of the mechanisms already outlined can be responsible for the production of the majority of diploids not produced as a result of ameiosis.

Consider a zygote with the genotye B/b. If diploids are produced as a result of non-disjunction at anaphase I, homozygons bb diploids can only arise from zygotes in which recombination has occurred between b and the centromere. At anaphase II, with random segregation of the chromatids of the two homologous chromosomes, $\frac{1}{4}$ of the resulting diploids will be homozygous bb. The fraction of

homozygotes of this type will thus be

$$\tfrac{1}{4}\, y \text{ ---------- (1)}$$

where y is the 2nd division segregation frequency of the locus b and may have a value between 0.0 and 0.67 (Mather, 1938).

If the diploids are produced as a result of non-disjunction at anaphase II, bb homozygotes will only occur following 1st division segregation of b, in which case $\frac{1}{2}$ of the resulting diploids will be homozygous bb. The fraction of homozygotes of this type will, therefore, be

$$\tfrac{1}{2}\,(1-y) \text{ ---------- (2)}$$

In the case of diploids produced by random fusion of the haploid products of meiosis, the phenotype of a diploid with respect to one pair of markers B/b will not be independent of that with respect to a second, unlinked pair C/c. This is shown in Table 14, in which the frequencies of the four possible phenotypes of diploids with respect to two pairs of markers B/b and C/c are given. The frequencies are only given for diploids heterozygous for a third pair of markers A/a, unlinked to the other two pairs, since in the present case all diploids were heterozygous for $\underline{ad}_1$ and $\underline{ad}_3$ (or in the case of those arising in cross 10, heterozygous for $\underline{ad}_9$ and $\underline{paba}_1$).

Unfortunately expressions cannot be derived for diploids produced as a result of triploid or tetraploid meiosis since the zygote nucleus may have any one of a number of possible genotypes

TABLE 14.

Phenotype ratios expected among diploids produced by randon fusion of the haploid products of meiosis.

Cross:- $\frac{A}{a}\frac{B}{b}\frac{C}{c}$ x, y and z are the 2nd division segregation frequencies of a, b and c respectively.

Phenotype (only A/a diploids considered)	Frequency	Fraction b/(B+b)	Fraction bc/(Bc+bc)
BC	$\frac{1}{48}\cdot(32-12x-8y-8z+8xy+8xz+2yz+3xyz)$		
Bc	$\frac{1}{48}\cdot(4x+8z+4xy-8xz-2yz-3xyz)$		
bC	$\frac{1}{48}(4x+8y-8xy+4xz-2yz-3xyz)$		
bc	$\frac{1}{48}(4x-4xy-4xz+2yz+3xyz)$	$\frac{1}{8}x(2-3y)+\frac{1}{4}\cdot y$ ------(3)	$\frac{\frac{1}{4}x(4-4y-4z+3yz)+\frac{1}{2}yz}{x(2-3z)+2z}$ ----(4)

and the proportion of each type is unpredictable. It is known however, that diploid strains of A. nidulans are highly sterile, and it is therefore unlikely that a mechanism of this type could account for more than a very small fraction of the diploids obtained.

Estimates of the 2nd division segregation frequencies of ad_1, $paba_1$, and w are now available (Strickland, unpublished) and have values of 0.67, 0.521 and 0.334 respectively. This makes it possible to compare the observed phenotype ratios W : w and PABA : paba (among recessives for an unlinked marker to exclude all diploids of ameiotic origin) with the ratios expected on the basis of expressions (1), (2) and (4).

From Table 15 it will be seen that of the five available W : w ratios, three are significantly different from that expected on the basis of (1) and the other two do not fit very closely. None are significantly different from that expected on the basis of (2). Only two ratios can be compared with that expected on the basis of (4), one being significantly different.

Of the two available PABA : paba ratios one is significantly different from the ratio calculated from expression (1) but neither is different from expectation on the basis of (2) and (4).

Thus the observed ratios in all cases but one give poor agreement with those expected if non-disjunction at anaphase I were responsible for the production of the majority of diploids not produced

TABLE 15.

Observed ratios of PABA:paba and W:w among diploids from three crosses, compared with those expected according to three possible modes of origin.

Cross	Homozygotes among which ratio calculated	Observed ratio			Mode of origin	Expected ratio		χ^2 *	P
		PABA	paba	Tot.					
3	w	27	6	33	Non-disjunct.AI	28.7	4.3	0.012	0.8
					Non-disjunct.AII	25.1	7.9	0.188	0.5
					Random fusion	26.2	6.8	0.020	0.9
4	w	25	12	37	Non-disjunct.AI	32.2	4.8	10.7	0.01
					Non-disjunct.AII	28.1	8.9	1.03	0.3
					Random fusion	29.3	7.7	2.38	0.1
		W	w	Tot.					
3	paba	16	6	22	Non-disjunct.AI	20.2	1.8	7.97	0.01
					Non-disjunct.AII	14.7	7.3	0.140	0.7
					Random fusion	17.4	4.6	0.248	0.5

TABLE 15 (Contd.)

3	thi	36	8	44	Non-disjunct.AI	40.3	3.7	4.35	0.05-0.02
					Non-disjunct.AII	29.4	14.6	3.87	0.05-0.02
4	paba	14	12	26	Non-disjunct.AI	23.8	2.2	43.7	0.01
					Non-disjunct.AII	17.3	8.7	1.40	0.2
					Random fusion	20.6	5.4	8.77	0.01
4	bi	16	12	28	Non-disjunct.AI	25.7	2.3	39.2	0.01
					Non-disjunct.AII	18.7	9.3	0.761	0.3
10	bi	10	4	14	Non-disjunct.AI	12.8	1.2	5.07	0.05-0.02
					Non-disjunct.AII	9.3	4.7	0.008	0.9

* Yates correction for continuity has been applied in calculating the χ^2.

following ameiosis. On the other hand, they are consistent both with the hypothesis that diploids not produced by ameiosis result from non-disjunction at anaphase II and from random fusion of the haploid products of meiosis.

Random fusion of the haploid products of meiosis could not produce aneuploid nuclei. The high frequency of occurrence of these suggests, therefore, that non-disjunction at anaphase II is the probable mechanism by which most diploids not of ameiotic origin are produced.

A clear-cut distinction between the various possibilities must await the availability of loci with low 2nd division segregation frequencies since the expected proportions of homozygous recessives would in this case be very different on the basis of the different hypotheses.

The production of diploid and aneuploid nuclei at meiosis is certainly not unique to A. nidulans. Mitchell, Pittenger and Mitchell, (1952) and Pittenger (1954) have shown that a proportion of prototrophs obtained from zygotes heterozygous for very closely linked markers in Neurospora crassa are heterozygous for these markers and are probably disomics. This suggests that quantitative, if not qualitative, differences exist between the two organisms with respect to the relative frequency of diploid and aneuploid ascospores. This difference may be more apparent than real since aneuploids of Neurospora

are apparently fully viable in contrast to those of Aspergillus.

In plants, particularly in Angiosperms, diploid and aneuploid gametes have been repeatedly reported (cf. Darlington, 1937). Unreduced gametes are particularly frequent in species hybrids (eg. Karpechenko, ~~1924;~~ 1927; Winge, 1933) and it would seem that this is largely due to inviability and selection against reduced gametes, which will generally be unbalanced, rather than to an increase in the frequency of non-reduction although this is certainly a contributary factor. If this is so it provides an interesting analogy with Aspergillus in which diploid ascospores are only detected when there is strong selection against reduced gametes.

The ease with which polyploids and aneuploids of meiotic origin can be obtained in Aspergillus may provide a useful tool for the study of mechanisms of breakdown of meiosis and its frequency under different environmental conditions. In most organisms such problems can only be attacked cytologically owing to the inviability of abnormal gametes, and such methods are laborious owing to the rarity of spontaneous abnormal meioses. The problem can now be attacked genetically.

4. Summary

1. The mutants ad_1 and ad_3 have been shown to be

non-allelic although they are extremely closely linked.

2. No evidence of linkage ~~to~~ with any other mutant could be obtained by formal genetic analysis.

3. It has been shown that diploid and probably aneuploid ascospores occur regularly with low frequency in A. nidulans and can be readily detected and isolated under suitable selective conditions.

4. The available evidence suggests that both complete absence of meiosis of the zygote and non-disjunction at the 2nd meiotic division are the mechanisms primarily responsible for the production of diploid ascospores.

IV LOCALISATION OF NINETEEN ADENINE-REQUIRING MUTANTS AND THEIR ALLELIC RELATIONSHIPS.

1. Localisation and allelism.

It had been found that nine of the UV-induced mutants were located very close to the locus y and were physiologically allelic with each other. The properties and linear relationships of these mutants, belonging to the "ad_8 region", have been described in detail in Section II.

Four of the remaining UV-induced mutants were located extremely close to the locus $paba_1$ (Fig. 2). Heterokaryon tests between ad_9 and the other three mutants of this group were made as follows. A heterokaryon between a strain carrying ad_9 and one carrying one of the other three mutants was synthesised on medium containing adenine. The heterokaryon was "balanced" on other nutritional requirements. It was transferred to minimal medium. In no case did appreciable growth of the heterokaryon take place on minimal medium although repeated tests were made. The three mutants were therefore allelic with ad_9 on the basis of this test, and were assumed to be allelic with each other. More recently, however, it has been shown (Calef, unpublished) that ad_{15} and ad_{17} are complementary. The physiological relationships between the mutants in this region,

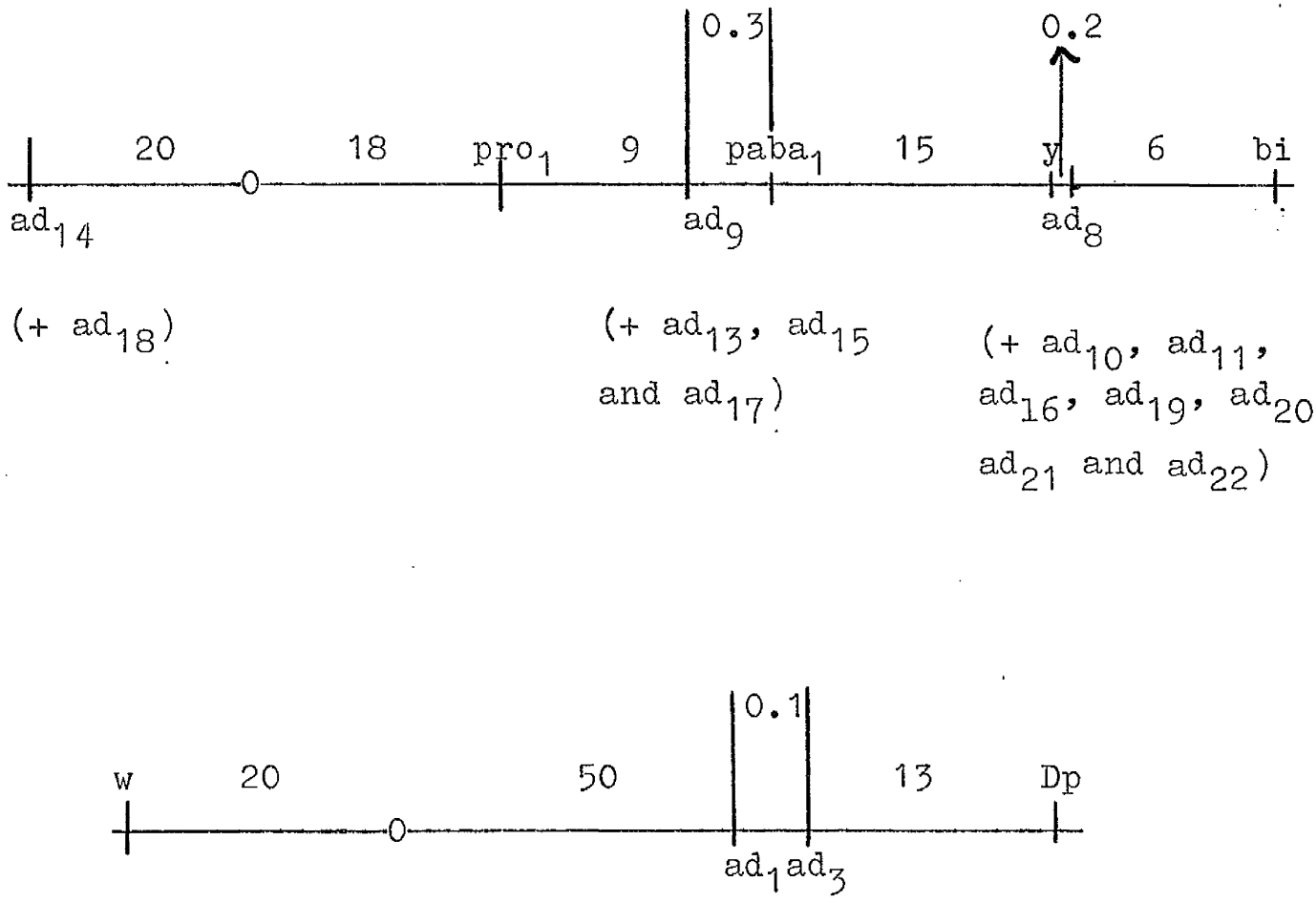


Fig. 2. Linkage map showing the position of the ad mutants. Thd positions of pro$_1$, paba$_1$, y, bi, w and the centromeres were not determined by the author. The order of ad$_1$ and ad$_3$ is unknown; the positions on the map are chosen arbitrarily. For an explanation of Dp, see Section V.

"the $\underline{ad}_9$ region", are therefore probably complex.

Estimates of the recombination fraction between members of the $\underline{ad}_9$ region and $\underline{paba}_1$ have been obtained only in the case of $\underline{ad}_9$. The relevant data are given in Table 16. Owing to close linkage between the two loci, heterozygous diploids were obtained when selection was made for $\underline{AD}_9$ $\underline{PABA}_1$ recombinants. Among recombinants of this type the ratio of green to yellow colonies was 29:7. This established the linear order of $\underline{ad}_9$, $\underline{paba}_1$ and $\underline{y}$ shown in the table. The recombination fraction and the incidence of heterozygous diploids was obtained by the indirect method already described in Section II.

The remaining two UV-induced mutants were found to be physiologically allelic and were located about 30 units from $\underline{paba}_1$ (see Fig. 2). Subsequent work by Pontecorvo and Kafer (unpublished) has shown that $\underline{ad}_{14}$ is located on a different arm of the chromosome from $\underline{paba}_1$ as indicated in Fig. 2.

2. The mutant $\underline{ad}_2$.

This mutant was X-ray induced. Heterokaryon tests indicated that it belonged to the $\underline{ad}_9$ group but preliminary tests by Pontecorvo (1953) had indicated that it was located midway between $\underline{paba}_1$ and $\underline{y}$.

TABLE 16.

Localisation of ad_9.

Cross 10:- $\frac{w_n\ ad_1}{W_n\ AD_1} \quad \frac{AD_9\ paba_1\ y\ BI}{ad_9\ PABA_1\ Y\ bi}$

Ascospores plated on:	Recombinants selected	Ascospores plated	Colonies
m.m + p.a.b.a + biotin	AD_1AD_9	2.4×10^4	1200 (approx.) (193 classified - all paba)
m.m. + biotin	$AD_1AD_9PABA_1$	4.0×10^5	208* {Haploid 68 Diploid 140

Classification of $AD_1AD_9PABA_1$ recombinants:-

		white	yellow	green	Total
Haploid	BI	8	7	3	18
	bi	24	0	26	50
	Total	32	7	29	68
Diploid	BI	9	3	114	126
	bi	4	0	10	14
	Total	13	3	124	140

Recombination fraction $paba_1$-ad_9:- = 0.0034

Frequency heterozygous diploids:- = 0.0017

* Many dwarfs similar to these obtained in crosses 3 and 4, also obtained.

The preliminary localisation of $\underline{ad}_2$ has been found to be incorrect but its position remains uncertain.

The strain $\underline{y}$ $\underline{ad}_2$ was crossed to a second $\underline{paba}_1$ $\underline{bi}$, and also to a strain $\underline{paba}_6$ $\underline{bi}$ ($\underline{paba}_1$ and $\underline{paba}_6$ are allelic - Roper, 1952). Perithecium analysis of both crosses was carried out.

Among a total of 960 colonies classified from the two crosses (Table 17) there were 11 apparent crossovers between $\underline{ad}_2$ and $\underline{paba}$. Unfortunately these were not tested for diploidy since at the time they were obtained, the occurrence of diploid ascospores was unknown. Nine of the eleven were green prototrophs, however, and must, therefore, have been double crossovers between $\underline{paba}$, $\underline{y}$ and $\underline{bi}$ in addition to being crossovers between $\underline{ad}_2$ and $\underline{paba}$ if they were not diploids. This together with the fact that there were no colonies in the complementary class suggests they were diploids. One of the apparent crossovers between $\underline{ad}_2$ and $\underline{paba}_1$ was biotin requiring and could equally well have been a recombinant or a diploid, but the remaining possible crossover required both adenine and p.a.b.a. and must, therefore, have been a recombinant between the two loci.

The single certain crossover between $\underline{ad}_2$ and $\underline{paba}$ carried the markers $\underline{Y}$ and $\underline{BI}$. If the order of the loci is $\underline{ad}$ $\underline{paba}$ $\underline{y}$ $\underline{bi}$ then it was a double crossover with one exchange between $\underline{ad}_2$ and $\underline{paba}$, and a second between $\underline{y}$ and $\underline{bi}$. If the order is $\underline{paba}$ $\underline{ad}$ $\underline{y}$ $\underline{bi}$, then it was a triple crossover. The first order thus seems the least improbable

TABLE 17.

Perithecium analysis of two crosses to localise ad_2.

Crosses:- 11. $\frac{ad_2\ PABA_1\ y\ BI}{AD_2\ paba_1\ Y\ bi}$; 12. $\frac{ad_2\ PABA_6\ y\ BI}{AD_2\ paba_6\ Y\ bi}$

(I II)

Classification:-

Combinations		Cross 11* AD	Cross 11* ad	Cross 11* Tot.	Cross 12 AD	Cross 12 ad	Cross 12 Tot.
Parental	PABA y BI	0	161	307	0	292	601
	paba Y bi	146	0		~~301~~ 299?	0	
Crossovers I	PABA Y bi	0	3	7	1ø	10	23
	paba y BI	4	0		12	0	
II	PABA y bi	0	3	6	0	2	4
	paba Y BI	2	1		2	0	
I II	PABA Y BI	4ø	2	7	5ø	0	5
	paba y bi	1	0		0	0	
	Total	157	170	327	329	304	633

Recombination fractions:-

ad_2 - paba	=	0.001 ± 0.001
I paba - y	=	0.034 ± 0.0058
II y - bi	=	0.014 ± 0.0038
I II observed	=	0.0032 ± 0.0018
expected	=	0.00048

* Pooled data from 3 perithecia, one of which gave excess of one parental class (38 : 18) due to proportion of selfed asci. The data have been corrected by equating the two parental classes from this perithecium.

ø Probable diploids.

and is supported by the allelism between $\underline{ad}_2$ and $\underline{ad}_9$.

The other notable feature of the results from both crosses is the great reduction in crossing over between paba and bi compared with that usually found. On the other hand the frequency of double crossovers between paba, y and bi was much greater than expected from the frequency of singles in the two regions, and was in fact nearly half what would have been expected had the frequency of recombination between the three loci been that usually found (0.15 between paba and y, and 0.06 between y and bi). This suggested the reduction in crossing over was due to an inversion in the strain $\underline{ad}_2$ y covering all the marked region and extending beyond it, some of the apparent single crossovers being doubles in which one exchange occurred outside the marked region.

The fact that the single recombinant between $\underline{ad}_2$ and paba was a multiplThe fact that the single recombinant between $\underline{ad}_2$ and pabaed.

More recently another cross involving $\underline{ad}_2$ was made and analysed to verify the high incidence of diploids implied in the previous two crosses. The incidence of heterozygous diploids was found to be nearl; one per cent (Table 18) which is much greater than that usually found.

3. The mutant $\underline{ad}_4$

This mutant was found to be allelic with members of the $\underline{ad}_8$

TABLE 18.

A cross involving ad_2 showing the high incidence of diploid ascospores and reduction in crossing over.

Cross:- $\frac{w_n \quad ad_1}{W_n \quad AD_1} \quad \frac{AD_2 \quad paba_1 \quad y \quad BI}{ad_2 \quad PABA \quad Y \quad bi}$

Selection:- AD_1AD_2 (ascospores plated on m.m. + biotin and p.a.b.a.)

Classification:-

	PABA BI	PABA bi	paba BI	paba bi	Total
white	0	0	38	2	40
yellow	0	0	60	0	60
green	3*	0	0	3	6
Total	3	0	98	5	106

Frequency heterozygous diploids:- $3/106 \times 4 = 0.0071 \pm 0.0041$

Recombination fractions:-

$paba_1 - ad_2 = 0.00$

$paba_1 - y = 0.048 \pm 0.027$

$y - bi = 0.00$

$paba_1 - bi = 0.048 \pm 0.021$

* All diploid.

regions but had been provisionally mapped about 0.7 units ~~to~~ distal to bi by Roper (see Pontecorvo, 1953).

This suggested that a rearrangement might be involved as in the case of ad_2 and analysis of a cross involving ad_4 (Table 19) confirmed this.

Analysis of this cross was made difficult by low viability of ascospores, by the occurrence of large numbers of dwarf colonies following platings of ascospores, and by the high incidence of diploids (1.3%). The data also indicate the following points:-

(a) That recombination between paba and y was greatly reduced relative to the standard map distance.

(b) That there was an excess of white colonies suggesting linkage of ad_4 and w.

(c) That out of five crossovers between ad_4 and bi, at least two were multiple crossovers irrespective of the relative positions of the two loci.

A rearrangement is strongly suggested by the data and the high incidence of diploids and dwarfs (aneuploids?), and the irregular segregation at the w locus, suggest that a translocation may be involved. A translocation would result in a high frequency of unbalanced and inviable ascospores, hence the high frequency of diploid ascospores which was found might well have been caused by low viability of non-diploid ascospores rather than by an increase in the

TABLE 19.

Cross showing irregular segregation and recombination values in crosses involving ad_4.

Cross:- $\frac{w_n\ ad_1}{W_n\ AD_1} \quad \frac{paba_1\ y\ BI}{PABA\ \ Y\ bi} -\!- \frac{AD_4}{ad_4}$

Selection:- AD_1AD_4 (ascospores plated on m.m. with biotin and p.a.b.a.)

Classification:-

	PABA BI	PABA bi	paba BI	paba bi	Total
white	2	1	71	1	75
yellow	1	0	34	1	36
green	0	2	0	0	2
Total	3	3	105	2	113

The following diploids were obtained:- white prototroph 2 ; green prototroph 4.

Recombination fractions:-

paba - y = 0.026 ± 0.026 (1/38)

y - bi = 0.026 ± 0.026 (1/38)

paba - bi = 0.044 ± 0.019 (5/113)

ad_4 - bi = 0.044 ± 0.019 (5/113)

y - ad_4 = 0.053 ± 0.036 (2/38)

Frequency heterozygous diploids:- 6/113 x 4 = 0.013.

incidence of diploid formation at meiosis.

4. Discussion.

The nineteen adenine-requiring mutants have been found to fall into five complementary groups. Heterokaryons between strains carrying mutants from different groups are adenine-independent. Heterokaryons between strains carrying mutants from the same group are adenine-requiring. More recently Calef (unpublished) has shown that the mutants in the $\underline{ad}_9$ region belong to at least two complementary groups, making six groups in all.

The two complementary groups represented by $\underline{ad}_1$ and $\underline{ad}_3$, and the complementary mutants in the $\underline{ad}_9$ region (e.g. $\underline{ad}_{15}$ and $\underline{ad}_{17}$; Calef, unpublished), are extremely closely linked. Very close linkage between complementary mutants with similar effects would be expected to occur occasionally, even were their distribution along the chromosome random. The number of reported instances of close linkage between mutants of this type has now become so large as to suggest that their distribution may not always be random. Example may be cited from Maize, the A mutants (Laughnan, 1952) and the R mutants (Stadler and Nuffer, 1953); from the Mouse, the t mutants (Dunn, 1954); from Neurospora; pyr mutants (Mitchell, Pittenger and Mitchell, 1952) and

pdx *pyr* mutants (Mitchell, 1955); from *Drosophila* ; *bx* and *bxd* (Lewis, 1951); and from *Aspergillus*; *pro* mutants (Forbes, 1955).

Two possibilities which might account for very close linkage between complementary mutants with similar effects, and perhaps controlling related processes are :-

(a) that selection favours close linkage between genes controlling related processes.

(b) that duplication of a gene has been followed by evolutionary divergence of function of the two products of duplication. Horowitz (1945) has used a model of this type in an attempt to explain the evolution of biological syntheses.

The basis of close linkage between complementary mutants with similar effects maybe quite different from that between physiologically allelic mutants. In the case of mutants of the latter type, the mutant loci are component parts of a larger physiological unit, the gene (Pontecorvo, 1952 *a*, *b*; Section II, this thesis). On the other hand, as pointed out by Pontecorvo (1955), it may turn out that separation of a number of closely linked mutants with similar effects into complementary and non-complementary groups may not always be possible, and in addition, intermediate conditions may be found.

An observation of Lewis (1951) may be important in this connection. He found that of three closely linked mutants of Drosophila, bx, Bxl and bxd, the first and third were complementary but Bxl was non-complementary with both and is located between them. Bxl does not seem to be a deficiency covering both the other loci since recombination was reported to occur between it and the other two.

5. Summary.

1). Nineteen mutants requiring adenine for growth have been mapped and tested for allelism. They fell into five groups, the mutants of each group being allelic. Those of different groups were complementary.

2). Two of the complementary groups, each represented by a single mutant (ad_1 and ad_3), were extremely closely linked.

3). Two mutants, both isolated following treatment with X-rays, were found to be associated with a rearrangement.

V A DUPLICATION INVOLVING PART OF THE "bi" CHROMOSOME.

1. Introduction

Reversions of strains carrying $\underline{ad}_{20}$ to adenine-independence can be readily obtained. Two of these were investigated and found to carry suppressors of $\underline{ad}_{20}$. A third (a partial reversion with a growth rate intermediate between that of $\underline{ad}_{20}$ and wild type and stimulated by adenine) was found to carry a duplication of part of the "bi" chromosome attached to a second chromosome. The evidence for the presence of a duplication and some of its properties are described below.

2. Experimental.

The reversion was obtained in a strain $\underline{ad}_{20}$ bi. The reverted strain was purified by isolation of a single conidium, crossed to an adenine-independent strain and a single perithecium analysed (Table 20).

Two types of adenine-dependent colony were obtained, one with a phenotype identical to that of $\underline{ad}_{20}$, the other similar to the reverted $\underline{ad}_{20}$; designated $\underline{ad}_{20}$ (R). Mutation of $\underline{ad}_{20}$ was not,

TABLE 20.

A strain carrying a reversion of ad_{20} crossed to an adenine-independent strain.

Cross 13:- $PABA_1$ Y ad_{20}(R) bi // $paba_1$ y AD_{20} BI

	green			yellow	Total
	ad_{20}	ad_{20}(R)	AD	AD	
PABA bi	18	9	0	0	27
paba BI	0	1	17	17	35
PABA BI	0	0	2	4	6
paba bi	3	1	2	0	6
Total	21	11	21	21	74

therefore, the cause of reversion.

All $\underline{ad}_{20}$ and $\underline{ad}_{20}$(R) types were green, as expected from the closeness of linkage with y. On the other hand about half of the phenotypically AD colonies were also green (i.e. apparent crossovers between $\underline{ad}_{20}$ and y). The majority of these colonies carried the parental combination paba BI (i.e. they were not recombinant in the paba-bi interval). This made it unlikely that phenotypically Y AD types were crossovers.

There was also an excess of green colonies, although other markers segregated 1 : 1. This excess was largely accounted for by the Y AD class.

The results suggested that reversion of $\underline{ad}_{20}$ was due to mutation at an independent suppressor locus, the suppressor suppressing y in addition to $\underline{ad}_{20}$. On this hypothesis the Y AD colonies obtained from cross 13 should have the genotype yAD su. Outcrossing a strain of this type to a strain carrying $\underline{ad}_{20}$ should give identical classes and frequencies with those found in cross 13. The results of perithecium analysis of such a cross (cross 14, Table 21) were as expected.

In both crosses 13 and 14 there was a deficiency of the Y $\underline{ad}_{20}$(class.

Analysis of further crosses has subsequently shown that the

TABLE 21.

A strain of the supposed genotype y AD_{20} su crossed to a strain carrying ad_{20}.

Cross 14:- $\frac{y \quad AD_{20} \quad BI}{Y \quad ad_{20} \quad bi} \quad \frac{su}{SU} \quad \frac{pyro_4}{PYRO_4}$ (phenotype green)

	green			yellow	Total
	ad_{20}	ad_{20}(R)	AD	AD	
PYRO BI	3	1	7	9	20
pyro BI	0	1	14	13	28
PYRO bi	10	7	0	1	18
pyro bi	13	5	0	2	20
Total	26	14	21	25	86

"suppressor" is in fact a duplication of part of the "bi" chromosome including the loci y, ad_{20} and bi, and that this duplication is attached to the chromosome carrying w and ad_1 about 13 units from ad_1. The crosses on which these conclusions are based are shown in Tables 22 and 23.

Table 22 gives the results of perithecium analysis of a cross between two strains, one of which carried the duplication (strains carrying the duplication are now indicated by the symbol ~~of~~ Dp followed by the genotype of the duplication in brackets). In the first part of the table the classification with respect to ad_{14}, ad_{20}, y, bi and Dp is given. Classification for presence or absence of the duplication is made possible by the fact that colonies carrying it are usually morphologically distinguishable from those which do not, having a sharper boundary and smaller, more uniform conidial heads. In addition green colonies carrying y on the "bi" chromosome and Y on the duplication show small patches of yellow conidial heads due, presumably, to crossing over at mitosis between the duplication and the "bi" chromosome.

Neither of the parent strains of cross 15 (Table 22) was bioti requiring but biotin requiring colonies occurred among the progeny. This indicated the presence of the recessive allele bi in the strain with the duplication and was in fact the first evidence that a

TABLE 22.

Cross providing evidence that the bi locus was duplicated and that recombination occurs between duplication and the homologous segment of the "bi" chromosome.

Cross 15:- $\frac{AD_{14}{}^{I}PRO_{1}{}^{II}PABA_{1}y\ AD_{20}BI}{ad_{14}\ pro_{1}\ \ paba_{1}y\ AD_{20}bi} \cdot Dp(yad_{20}bi)\ \frac{pyro_{4}}{PYRO_{4}}$

(a) Classification for ad, bi, colour and Dp.

		yellow		green		Total
		Dp	+	Dp	+	
ad_{14}	BI	7	30	22	0	59
	bi	0	3	1	3	7
ad_{20}	BI	0	0	0	0	0
	bi	0	0	0	3	3
$ad_{20}(R)$	BI	0	0	0	0	0
	bi	0	0	1	0	1
AD	BI	6	23	28	0	57
	bi	0	0	0	0	0
Total		13	56	52	6	127

TABLE 22 (Contd.)

(b) Classification for $\underline{ad_{14}}$, pro and paba.

Combinations:-

Parental	AD_{14} PRO PABA	47	97
	ad_{14} pro paba	50	
Crossovers I	AD_{14} pro paba	11	24
	ad_{14} PRO PABA	13	
II	AD_{14} PRO paba	1	2
	ad_{14} pro PABA	1	
I II	AD_{14} pro PABA	2	4
	ad_{14} PRO paba	2	

(c) Recombination fractions:-

I	ad_{14}	- pro	=	0.22 ± 0.037
II	pro	- paba	=	0.047 ± 0.019
I II	observed		=	0.032 ± 0.015
	expected		=	0.010

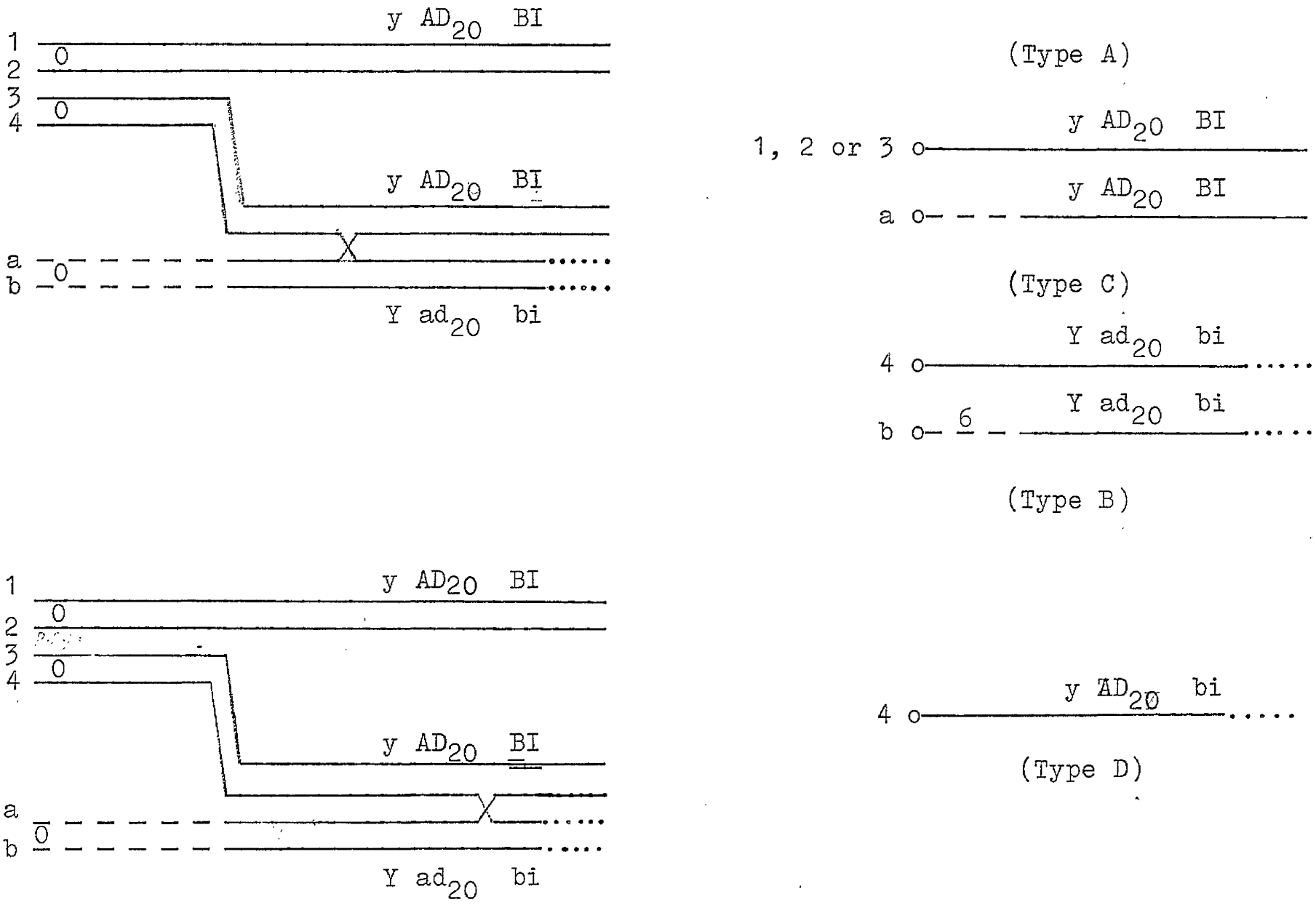


Fig. 3. Proposed mode of origin of recombinants from cross 15. Diplotene in the zygote is shown on the left; the recombinants on the right.

duplication was involved.

The allele ratios with respect to $\underline{ad}_{14}$, $\underline{pro}_1$ and $\underline{paba}_1$ were all approximately 1 : 1 indicating that the duplication does not involve these loci (the strain in which the duplication arose carried the wild type allele at these three loci and the duplication would presumably do so also, if it included these loci, and cause an excess of the wild type allele in crosses in which it was segregating).

Cross 16 also showed that recombination occurs between the duplication and the homologous segment of the "bi" chromosome. In the absence of recombination of this type, only two classes with respect to y, $\underline{ad}_{20}$ and bi would occur among the progeny; y AD Bi and y AD Bi Dp (Y $\underline{ad}_{20}$ bi). Other types appeared and can only be easily accounted for by recombination.

There were six Y bi colonies without the DP phenotype. Three of these were phenotypically $\underline{ad}_{20}$, not $\underline{ad}_{20}$(R), which was independent evidence for absence of the duplication. The other three carried $\underline{ad}_{14}$ (which does not grow on minimal medium) which masked the $\underline{ad}_{20}$ phenotype, and the absence of the duplication was assumed on morphological grounds alone. In addition there were two Ybi colonies with the Dp phenotype. One was phentoypically $\underline{ad}_{20}$(R) as expected; in the other, $\underline{ad}_{14}$ masked the phenotype with respect to $\underline{ad}_{20}$. The mode of origin proposed for these recombinants is indicated in Fig. 3.

Thirteen colonies were obtained which were yellow with the Dp phenotype. Seven of these were $\underline{ad}_{14}$ but it can be assumed that they were also $\underline{AD}_{20}$ since y and $\underline{ad}_{20}$ are so closely linked as to segregate as a unit. There were also three y bi colonies without the Dp phenotype. They were all $\underline{ad}_{14}$ but can be assumed to have been $\underline{AD}_{20}$. The mode of origin of recombinants of these two types is also given in Fig. 3.

It must be assumed that the duplication includes the whole of the "bi" chromosome distal to bi and essential for viability, otherwise recombinant types A, B and D (Fig. 3) would carry a deficiency and be inviable.

Since recombination, and therefore pairing, occurs between the duplication and the homologous segment of one or other of the "bi" chromosomes, a reduction in pairing and recombination between the two "bi" chromosomes in this region might be expected. Cross 15 provides no information on crossing over in the region covered by the duplication, but in an adjacent region ($\underline{pro}_1$ - $\underline{paba}_1$) the recombination fraction is about half that usually found (Table 22). The recombination fraction between $\underline{ad}_{14}$ and $\underline{pro}_1$ is also reduced although to a lesser extent.

Cross 16 (Table 23) provided the evidence for linkage between $\underline{ad}_1$ and the duplication. When ascospores were plated on medium without adenine (which selects against $\underline{ad}_1$ but not $\underline{ad}_{20}$) a large excess of green colonies, most with the Dp phenotype, was obtained. This

TABLE 23.

Cross showing linkage between ad_1 and duplication.

Cross 16:- $\frac{AD_1 \cdot Dp\ (\text{♀}ad_{20}bi)}{ad_1} \quad \frac{S\,\S}{s\,\S} \quad \frac{yAD_{20}BI}{yAD_{20}BI} \quad \frac{pyro_4}{PYRO_4}$

Selection:- PYRO S §

Classification:-

	yellow		green		Total
	BI	bi	BI	bi	
AD_1 Dp	10	0	43	0	53
AD_1 +	9	2	0	0	11
ad_1 Dp	0	0	4	0	4
ad_1 +	39	1	2	6	48
Total	58	3	49	6	116

Recombination fraction:-

$$ad_1 - Dp = 15/116 = 0.13 \pm 0.031$$

suggested linkage between $\underline{ad}_1$ and the duplication since in the absence of linkage equal numbers of yellow and green colonies were expected. A second plating on minimal medium supplemented with adenine and biotin confirmed this linkage, the data indicating about 13% recombination between the duplication and $\underline{ad}_1$.

It is probable that the duplication is terminally or sub-terminally attached to the "$\underline{ad}_1$" chromosome otherwise colonies of type obtained from cross 15, for example, would be deficient for that part of the "$\underline{ad}_1$" chromosome distal to the duplication and presumably inviable.

3. Discussion

The mode of origin of the duplication cannot at present be determined since it is not known whether or not it is terminally attached to the chromosome carrying $\underline{ad}_1$. The results of genetic analysis show (fig.3) that if the duplication is insertional, then that part of the "ad_1" chromosome distal to it is not lethal when deficient. Similarly, if the duplication originated as a reciprocal translocation, that part of the "ad_1" chromosome transferred to the "bi" chromosome is not lethal when deficient since strains carrying the duplication and a "bi" chromosome different from that

carried by the strain in which the duplication was first found, can be readily obtained.

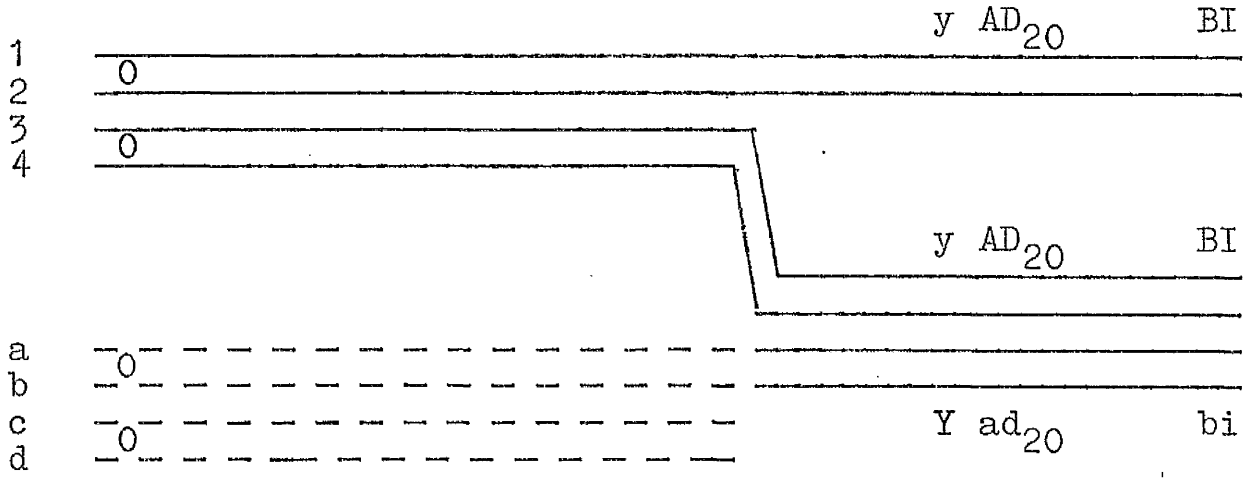
The data from certain crosses can be used to obtain an estimate of the recombination fraction between the duplication and the homologous segment of the "bi" chromosome, and thereby indicate how far the duplication extends proximal to y .

Consider a zygote of the type shown in fig. 4 in which the duplication is paired with the homologous segment of one of the "bi" chromosomes, the other remaining unpaired over a segment as long as the duplication.

Each of the four products of meiosis will receive one of the four chromatids 1, 2, 3, or 4, but it is assumed that the two chromatids a and b will be distributed at random between the four products since the locus ad_1, to which the duplication is closely linked, shows 67% 2nd division segregation (Strickland, unpublished).

Consider first recombination between the proximal end of the duplication and y.

Three types of distribution of the marker pairs $y\ AD_{20}$ and $Y\ ad_{20}$ (the loci y and ad_{20} are so closely linked that they can be assumed to segregate as a unit) between the four strands 3, 4, a and b, are possible. The three types are designated P, F and N.



Ascus type	Genotype of ascospores		Phenotypes produced	Frequency
	"bi"chromosome	Duplication		
P	y AD y AD y AD y AD	Y ad Y ad	y AD Y AD.Dp	1/2 1/2
F	y AD y AD y AD Y ad	Y ad y AD	y AD Y AD.Dp Y ad_{20}(R).Dp Y ad_{20} y AD .Dp	3/8 1/4 1/16 1/8 3/16
N	y AD y AD Y ad Y ad	y AD y AD	y AD Y AD. Dp y AD .Dp Y ad_{20}	1/4 1/4 1/4 1/4

Fig. 4. Phenotypes, with respect to the markers y and ad_{20}, and expected frequencies of colonies produced by the three possible types of ascus that can result from a zygote with pairing of the type shown. The chromosome to which the duplication is attached is indicated with a broken line.

In the P type none of the strands are recombinant between the proximal end of the duplication and y. In the F type there are two recombinant strands and in the N type there are four.

The recombination fraction (R) between the proximal end of the duplication and y is therefore given by the expression

$$R = \frac{1}{2}F + N \qquad \text{----------} \qquad (1)$$

Recombination between the proximal end of the duplication and y gives rise to three types of progeny distinguishable from those produced in the absence of recombination. They are $\underline{Y}\ \underline{ad}_{20}(R)$ .Dp, $\underline{Y}\ \underline{ad}_{20}$, and $\underline{y}\ \underline{AD}_{20}$ .Dp (see fig.3), and their expected frequencies are given in fig.4. Thus we can write

$$x = \frac{3}{8}F + \frac{1}{2}N \qquad \text{----------} \qquad (2)$$

where x is the summed frequency of the three types of recombinants.

The frequency of P, F and N types of strand arrangement will depend on the number and arrangment of chiasmata between the proximal end of the duplication and y, and the frequency of 0, 1 and 2, etc. chiasmata will be distributed according to the terms of the Poisson series (Haldane, 1931) in the absence of interference. The frequency of N types can therefore be written in terms of F. The relationship is given approximately by the expression (Papazian, 1952)

$$N = \frac{1}{8}F^2\left(1 + \frac{2}{3}F\right) \qquad \text{----------} \qquad (3)$$

substituting in (1) and (2) we get

$$R = \frac{1}{2}F + \frac{1}{8}F^2\left(1 + \frac{2}{3}F\right) \quad \text{----------} \quad (4)$$

and

$$x = \frac{3}{8}F + \frac{1}{16}F^2\left(1 + \frac{2}{3}F\right) \quad \text{----------} \quad (5)$$

From these two expressions R can be obtained if x is known.

In cross 15 (Table 22) the frequency of $\underline{Y}\ \underline{ad}_{20}$ colonies was $\frac{6}{127}$ (the three $\underline{ad}_{14}\ \underline{Y}$ colonies not carrying the duplication can be assumed to carry $\underline{ad}_{20}$ owing to close linkage between $\underline{y}$ and $\underline{ad}_{20}$). The frequency of $\underline{y}\ \underline{AD}_{20}$ .Dp colonies was $\frac{13}{127}$ (the 7 $\underline{ad}_{14}\ \underline{y}$ .Dp colonies can similarly be assumed not to carry $\underline{ad}_{20}$). There was one $\underline{Y}\ \underline{ad}_{20}(R)$ .Dp colony, but there were also 23 $\underline{ad}_{14}\ \underline{Y}$ .Dp types which may or may not have been $\underline{ad}_{20}(R)$, hence this class cannot be used (green colonies with the duplication may be either $\underline{AD}_{20}$ or $ad_{20}(R)$ in phenotype, the presence of $\underline{ad}_{14}$ masking the phenotype with respect to ad_{20}).

Thus $x = \frac{19}{127} = 0.150$

$$= \frac{5}{16}F + \frac{1}{16}F^2\left(1 + \frac{2}{3}F\right) \quad \text{----------} \quad (6)$$

Equation (6) differs from (5) by $\frac{1}{16}F$, the expected frequency of $\underline{Y}\ \underline{ad}_{20}(R)$ .Dp colonies which are not used.

The value of F obtained from (6) is 0.43 and substituting this in (4) gives a value for R of 0.24.

The recombination fraction can be estimated in the

same way from cross 16 (Table 23) in which

$$x = \frac{18}{116} = 0.155$$

it being again necessary to ignore the class $\underline{Y}\ \underline{ad}_{20}(R)$ .Dp owing to the presence of $\underline{ad}_1$.

From this cross F was found to be 0.445 and R 0.25.

The two estimates of the recombination fraction between the proximal end of the duplication and *y* agree very well but are much higher than expected, since the recombination fraction between *paba* and *y* is about 0.15 and the duplication is known not to extend proximally as far as the locus $\underline{paba}_1$. Moreover the values of 0.24 and 0.25 are both calculated on the assumption that the duplication always pairs with the homologous segment of one of the "bi" chromosomes. If pairing were at random the duplication would remain unpaired in one third of zygotes and the values obtained would need to be increased by $\frac{1}{2}$. If the duplication tended to be excluded from pairing, as might be expected, the values would be even further underestimated.

Another possible source of error in the estimation of R is the assumption that the duplication is distributed at random between four products of meiosis. Although this seems the most likely assumption two alternatives exist. When the duplication is paired with one of the "bi" chromosomes the two duplication chromatids and the two chromatids with which it has formed a bivalent

may tend to segregate a) to the same pole or b) to opposite poles at anaphase I. The former alternative would lead to an underestimation and the latter to an overestimation of R.

Crosses 15 and 16 also provide an estimate of the recombination fraction between $\underline{ad}_{20}$ and $\underline{bi}$ in zygotes in which pairing is of the type shown in fig.4. From a zygote of this type recombination between $\underline{ad}_{20}$ and $\underline{bi}$ will result in four distinguishable types: $\underline{y}\ \underline{AD}_{20}\ \underline{bi}$, $\underline{Y}\ \underline{ad}_{20}\ \underline{BI}$, $\underline{Y}\ \underline{AD}_{20}\ \underline{bi}$ .Dp and $\underline{Y}\ \underline{ad}_{20}(R)\left|\underline{BI}\right.$ .Dp . By an argument similar to that previously used it can be shown that the frequency (y) of these four types is given approximately by the expression

$$y = \frac{3}{16} PF_1 + \frac{7}{32} FF_1 + \frac{3}{16} NF_1 \qquad \text{--------} \qquad (7)$$

where P, F and N are used as previously and P_1 and F_1 are the two likely types of strand arrangement with respect to $\underline{ad}_{20}$ and $\underline{bi}$ (N_1 type arrangements are not considered as they will be infrequent owing to close linkage between the two loci). In which case

$$R_1 = \frac{1}{2} F_1 \qquad \text{----------} \qquad (8)$$

In crosses 15 and 16, types $\underline{Y}\ \underline{ad}_{20}(R)\left|\underline{BI}\right.$ .Dp and $\underline{Y}\ \underline{AD}_{20}\ \underline{bi}$ .Dp cannot be used for calculating R_1 owing to the presence of $\underline{ad}_{14}$ in cross 15 and $\underline{ad}_1$ in cross 16 and expression (7) can be shown to become

$$y = \frac{1}{8}\left(PF_1 + FF_1 + NF_1 \right) \qquad \text{----------} \qquad (9)$$

Substituting for P, F and N in (9) the values found in crosses 15 and 16 we get

$$y = 0.125\ F_1 = \frac{3}{127} = 0.024 \quad \text{(cross 15)}$$

and

$$y = 0.125\ F_1 = \frac{5}{116} = 0.043 \quad \text{(cross 16)}$$

Substituting for F_1 in (8) we get

$$R_1 = 0.094 \quad \text{(cross 15)}$$

$$R_1 = 0.17 \quad \text{(cross 16)}$$

These values are again surprisingly high since the map distance between $\underline{ad}_{20}$ and $\underline{bi}$ is about 6 units and the recombination fractions are calculated on the assumption that the duplication always pairs with the homologous segment of one or other "bi" chromosome.

Information of a second type is available which can provide an estimate of the amount of recombination involving the duplication. The strain $\underline{y}\ \underline{pyro}_4$ .Dp ($\underline{Y}\ \underline{ad}_{20}\ \underline{bi}$) is self fertile and plating of ascospores gave the results shown in Table 24.

All colonies had the Dp.phenotype as expected but 9.3% were yellow. 130 green colonies were classified for $\underline{ad}$ and $\underline{bi}$ with the results shown. In the absence of recombination between either duplication and the homologous segment of one of the two "bi" chromosomes all colonies would have been green prototrophs.

TABLE 24.

Recombinant types resulting from self-fertilisation of a strain carrying the duplication.

Strain:- y AD_{20} BI .Dp(Y ad_{20} bi) $pyro_4$

Classification (all colonies have Dp phenotype):-

	green	yellow	Total
	642 (130 tested)	66 (none tested)	708
AD_{20} BI	118		
ad_{20}(R) BI	0		
AD_{20} bi	5		
ad_{20}(R) bi	7		

Frequency:- Yellow = 66/708 = 0.0933

AD_{20} bi = 5/130+13 = 0.0350

ad_{20}(R)bi = 7/130+13 = 0.0489

The new types can therefore provide an estimate of the recombination fraction between duplication and homologue.

Consider a zygote in which the chromosomes are paired as in fig.5. The zygote can be considered as consisting of two identical bivalents but it is assumed that the four chromatids carrying the duplication are distributed at random with respect to the four "bi" chromatids.

It can be shown that the frequency (x) of yellow colonies will be given by the expression

$$x = \frac{3}{8} PF + \frac{1}{2} PN + \frac{1}{4} F^2 + \frac{3}{8} FN \qquad \text{---------} \qquad (10)$$

where P, F and N are used as before. In (10), PF is the frequency of zygotes with one bivalent of the P type and one of the F type, and F^2 is the frequency of zygotes in which each bivalent is of the F type.

Expression (10) can be given in terms of F alone since

$$N = \frac{1}{8} F^2 + \frac{1}{12} F^3 \qquad \text{----------} \qquad (3)$$

and

$$P = 1 - (F + N) = 1 - F - \frac{1}{8} F^2 - \frac{1}{12} F^3 \qquad \text{----} \qquad (11)$$

Hence, neglecting terms smaller than F^3, (10) becomes

$$x = \frac{3}{8} F - \frac{1}{16} F^2 - \frac{1}{48} F^3 \qquad \text{----------} \qquad (12)$$

The recombination fraction (R) between the proximal end of the duplication and y for each bivalent will be given

Cross:- $\frac{y\ AD_{20}\ BI}{Y\ AD_{20}\ BI} \cdot Dp\left(\frac{Y\ ad_{20}\ bi}{\cdot}\right) \frac{pyro_4}{pyro_4}$

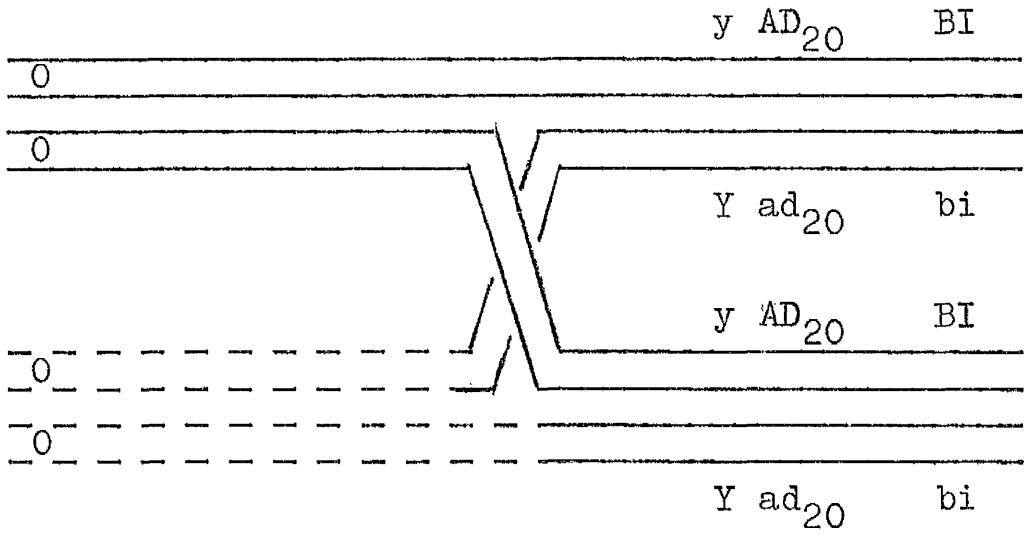


Fig. 5. Type of pairing that can result in the production of recombinant types in a cross in which both parents contribute the duplication.

by

$$R = \frac{1}{2}PF + PN + \frac{1}{2}F^2 + \frac{3}{2}FN + N^2 \quad \text{------} \quad (13)$$

which in terms of F becomes

$$R = \frac{1}{2}F + \frac{1}{8}F^2 + \frac{1}{12}F^3 \quad \text{----------} \quad (14)$$

The frequency of yellow colonies in cross 17 (Table 24) was 0.0933. Solving for F in (12) and substituting the value (0.26) obtained in (14) gives a value for R of 0.14.

The expected frequency of $\underline{Y}\,\underline{ad}_{20}(R)$ colonies will be equal to that of the yellow colonies since the two types are complementary. The observed frequency was in fact 0.0489 (there were 7 $\underline{Y}\,\underline{ad}_{20}(R)$ colonies out of a total of 130 greens, but in calculating the frequency the total was increased by 13 since 9.3% of colonies were yellow). The deficiency of $\underline{Y}\,\underline{ad}_{20}(R)$ colonies seems to be due to lower viability of colonies of this genotype, since a similar deficiency was observed in crosses 13 and 14 (Tables 20 and 21).

The recombination fraction between the proximal end of the duplication and $\underline{y}$ calculated from the frequency of $\underline{Y}\,\underline{ad}_{20}(R)$ colonies using (12) and (14) is 0.076.

Of the two values of R it seems probable that the one based on the frequency of yellow colonies is the more reliable owing to reduced viability of $\underline{Y}\,\underline{ad}_{20}(R)$ types. The values of R are calculated on the assumption that each duplication pairs

exclusively with the homologous segment of one of the "bi" chromosomes. If pairing were at random, this type of arrangement would occur in only half the zygotes and the calculated recombination fractions would need to be doubled to obtain the true values.

The data in Table 24 also provide an estimate of the recombination fraction between $\underline{ad}_{20}$ and $\underline{bi}$ when pairing is of the type shown in fig.5 . Recombination between these two loci will result in three distinguishable phenotypes, all with the duplication: $\underline{y}\ \underline{AD}_{20}\ \underline{bi}$, $\underline{Y}\ \underline{ad}_{20}$ (R) $\underline{BI}$ and $\underline{Y}\ \underline{AD}_{20}\ \underline{bi}$. The yellow colonies were not classified for $\underline{bi}$, and the number of colonies of the first type is therefore unknown. There were no colonies of the second type, but 5 of the last.

The frequency of these three colony types is dependent not only on the frequency of recombination between $\underline{ad}_{20}$ and $\underline{bi}$ but also on that between the proximal end of the duplication and $\underline{y}$.

The expected frequency (y_1) of $\underline{Y}\ \underline{AD}_{20}\ \underline{bi}$ types can be shown to be given by the expression

$$y_1 = P^2\left(\frac{3}{8}P_1F_1 + \frac{1}{4}F_1^2\right) + PF\left(\frac{7}{32}P_1F_1 + \frac{5}{32}F_1^2\right) + PN\left(\frac{1}{8}P_1F_1 + \frac{3}{16}F_1^2\right) + F^2\left(\frac{3}{16}P_1F_1 + \frac{1}{8}F_1^2\right) + FN\left(\frac{7}{32}P_1F_1 + \frac{5}{32}F_1^2\right) + N^2\left(\frac{3}{8}P_1F_1 + \frac{1}{4}F_1^2\right) \quad \text{----- (15)}$$

where P, F and N are used as before and P_1 and F_1 are the two likely types of strand arrangement with respect to $\underline{ad}_{20}$ and $\underline{bi}$. N_1 type strand arrangements are not considered as they will occur

with low frequency, in which case

$$P_1 = 1 - F_1 \qquad \text{---------} \qquad (16)$$

and

$$R_1 = \frac{1}{2} F_1 \qquad \text{---------} \qquad (17)$$

Substituting for P, F and N the values obtained from the frequency of yellow colonies (expression 12) we get that

$$y_1 = - 0.1004 F_1^2 + 0.2992 F_1$$

The expected frequency (y_2) of $\underline{Y}\ \underline{ad}_{20}(R)\ \underline{BI}$ types can be shown in a similar way to be given by

$$y_2 = - 0.0151 F_1^2 + 0.0928 F_1$$

and the total frequency

$$y_1 + y_2 = - 0.1155 F_1^2 + 0.3920 F_1 \qquad \text{------} \qquad (18)$$

$$= \frac{5}{143} = 0.0350 \ .$$

This gives a value for F_1 of 0.0918 and for R_1 of 0.0459.

A comparison of the recombination fractions between the duplication and the homologous segment of the "bi" chromosome in the two types of crosses is interesting. Whatever assumptions are made with regard to the frequency of different types of pairing, it seems clear that the amount of recombination in the region of the "bi" chromosome which is involved in the duplication is increased in zygotes in which the duplication is present. The duplication is known not to extend proximally as far as the locus $\underline{paba}$ which is 15 units from $\underline{y}$. In the two crosses in which only one parent

contributed the duplication the recombination fraction between the proximal end of the duplication and y was 0.24 and 0.25. These values are based on the assumption that the duplication is paired in every zygote with one of the "bi" chromosomes. If pairing were random they would need to be increased by half (becoming 0.36 and 0.37) . Moreover recombination would now occur between the two "bi"chromosomes in this region although this could not be observed in either cross since they were both y AD_{20}. If the duplication tended to be excluded from pairing, the values would be even further underestimated.

In the cross in which both parents contributed the duplication the recombination fraction between the proximal end of the duplication and y was 0.14. This value is based on the assumption that each duplication pairs exclusively with one of the "bi"chromosomes. It seems much more likely however, that the two "bi" chromosomes, and the two duplications, tend to pair with each other in which case the recombination fraction would be greatly underestimated. Even if it were assumed that pairing was random the estimated recombination fraction would need to be doubled, becoming 0.28.

It may be significant that the recombination fractions from crosses in which the duplication is represented only once agree more closely with that from the cross in which the duplication is represented twice if it is assumed that pairing is at random

rather than that the duplication is always paired with one of the "bi" chromosomes.

The amount of recombination between the "bi" chromosome and the duplication in the segment $\underline{ad}_{20} - \underline{bi}$ also appears to be greater than the normal value of 6.0% . The fractions from the three crosses were 0.094, 0.17 and 0.046 on the assumption that the duplication always pairs with one of the "bi" chromosomes. These values become 0.14, 0.25 and 0.092 respectively on the assumption of random pairing.

This is not the first case of the presence of a duplication resulting in an increase in crossing over. Rhoades (1936) showed that a duplication in Maize of one arm of a chromosome resulted in an increase in crossing over, not only in the duplicated arm but also in the other arm of the chromosome. In our case there is some evidence of a reduction of crossing over in the "bi" chromosome outside the duplicated region (Table 22).

A clue to the possible cause of the increase in crossing over in zygotes with the duplication compared with normal diploid zygotes may be found in the observation (see Review of Schultz and Redfield , 1951) that in triploid Drosophila regional changes in the amount of crossing over are found when a comparison with diploid flies is made. The maps are increased in length near the centromere and at the chromosome ends and reduced in the mid-regions. These changes result in better agreement between the

genetic and cytological maps. The nature of the regional changes in the map distances between loci are however difficult to interpret in triploids since it is not easy to separate changes in the frequency and distribution of crossing over from apparent changes due to inviability of unbalanced gametes produced by irregular segregation. Duplications of the type discovered in Aspergillus might be more satisfactory material for studying changes in crossing over induced by rearrangements since disjunction is probably normal.

4. Summary

1. A duplication of the terminal part of one arm of the "bi" chromosome has been found. It is attached to a second chromosome. Its origin is unknown.

2. Crossing over occurs between the duplication and the homologous segment of the "bi" chromosome and a method has been devised to estimate the recombination fraction.

3. The results suggest that the amount of crossing over between the duplication and the "bi" chromosome is greater than expected from the map distances between the loci which are duplicated.

VI GENERAL SUMMARY.

1. Tests for allelism between adenine-requiring mutants showed that they could be divided into five groups. Mutants of different groups were complementary; those of the same group were physiologically allelic.

2. The mutants of each group were found to be located at approximately the same position on the chromosome map. Different groups occupied different map positions, but two of the groups, each represented by a single mutant, were extremely closely linked.

3. Two mutants, both X - ray induced, were found to be associated with a rearrangement.

4. Four physiologically allelic mutants of one group were tested against each other and shown to occupy different but extremely closely linked loci. Three others of the same group were shown to occupy loci different from two of the first four, but their location with respect to the other two and to each other has not yet been worked out.

5. Two different suppressors of one mutant were found, one is specific for this mutant; even mutants which are physiologically allelic with the suppressed mutant are not suppressed.

6. It was discovered that about 0.1 per cent of ascospores of A.nidulans are diploid. In crosses involving strains carrying

rearrangements the frequency may be as high as 1.0 per cent of the viable ascospores. An attempt has been made to ascertain the origin of diploid ascospores. The results, although not conclusive, suggest that two mechanisms are largely responsible (a) absence of a meiotic division of the zygote nucleus and (b) non-disjunction at anaphase II of meiosis in the zygote.

7. A duplication of the terminal part of one chromosome, about 20 units in length and attached to a second chromosome, has been found. Crossing over occurs between the duplication and the homologous segment of the duplicated chromosome. The recombination fraction is greater than expected from the known distance between the loci which are duplicated. It therefore appears that the presence of the duplication results in an increase in the amount of crossing over in the duplicated region of the chromosome.

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