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Genetic analysis by means of the parasexual cycle in *Aspergillus niger*

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1. INTRODUCTION

The parasexual cycle is now known to occur in a wide variety of filamentous fungi (see the list in Roper, 1966). At least two processes of segregation and recombination occur during the vegetative multiplication of diploid mycelia: mitotic crossing over, as found in *Drosophila* (Stern, 1936), and mitotic haploidization (Pontecorvo, 1954). Both have been thoroughly analysed in *Aspergillus nidulans* (Pontecorvo, Tarr-Gloor & Forbes, 1954; Pontecorvo & Käfer, 1956, 1958; Käfer, 1958), where because of the presence of a standard sexual cycle it was possible to compare the results of mitotic segregation and recombination with those of the standard meiotic processes. The aim of the work reported here was to extend the first attempt at genetic analysis of the 'imperfect' *A. niger* made by Pontecorvo, Roper & Forbes in 1953, to establish linkage groups and construct chromosome maps, and, finally, to determine the importance, in the genetic system of this species, of the parasexual cycle as a process of genetic recombination.

2. MATERIALS

All strains were derived from the same wild-type strain, isolated by Mr J. L. Yuill in 1951. The list of the markers used in the present work is given in Table 1. Except for *acr3* which occurred spontaneously, all the other mutants were isolated after ultra-violet irradiation (about 5% survival).

Complete medium (CM) and minimal medium (MM) were those used for *A. nidulans* (Pontecorvo, Roper, Hemmons, MacDonald & Bufton, 1953).

3. METHODS

The optimal temperature for growth on solid media was found to be 35°C. and plates were incubated at this temperature throughout.

The techniques of mitotic genetic analysis for *A. nidulans* have been described by Pontecorvo, Tarr-Gloor & Forbes (1954) and Pontecorvo & Käfer (1956, 1958). Most of them were applied unchanged in the investigation of *A. niger*; mention will be made only of those which were especially developed during this work.

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Table 1. *Located mutants of* Aspergillus niger

Phenotype	Symbol	Linkage group and arm		Phenotype	Symbol	Linkage group and arm	
Visible							
olive conidia	*o*	I	right	white conidia epistatic to *a* and *o*	*w1*	III	
fawn conidia epistatic to *o*	*a*	I	left	white conidia epistatic to *a*, partially to *o*	*w4*	IV	
Nutritional							
arginine	*arg*	I	left	methionine	*met4*	IV	
adenine, guanine	*ade2*	III			*met6*	I	right
	ade5	III			*met7*	IV	
	ade6	IV		nicotinic acid	*nic1*	I	left
	ade7	III			*nic2*	IV	
	ade8	IV			*nic3*	I	left
	ade9	III			*nic4*	IV	
histidine	*his*	II		*p*-aminobenzoic acid	*pab1, 5**	I	left
leucine	*leu3*	V					
lysine	*lys2*	III		putrescine	*put*	VI	
	lys3	VI		pyridoxin	*pyr*	VI	
	lys4	III		riboflavine	*rib*	I	right
	lys5	III		'thiazole' aneurin	*thi*	I	right
	lys6	III					
Resistant							
acriflavine	*acr3*	I	left				

* Mutants phenotypically identical have been tested for complementarity in the heterokaryon and diploid. Among those reported here, only these two *pab* mutants were allelic.

(i) *Selection of haploid segregants*

Because of the relatively high frequency of crossing-over in *A. niger* markers could be lost by homozygosis before plating for the selection of haploid segregants; to prevent it, diploids were plated as soon as possible after their synthesis.

(*a*) *On acriflavine*

The acriflavine-resistant marker *acr3* was completely recessive and diploids heterozygous for it produced resistant segregants either by mitotic crossing-over leading to homozygosis or by haploidization. As the marker is very close to the centromere, diploid segregants homozygous for it were very rare; the acriflavine resistant segregants were almost all haploid. The concentration of acriflavine was $8{\cdot}5 \times 10^{-4}$ w/v in CM. This selection was used only for one diploid among those reported.

(*b*) *On* p-*fluoro-phenylalanine*

Diploids were plated on CM containing DL-*p*-fluorophenylalanine (FPA), which induces haploidization in diploid *A. nidulans* (Morpurgo, 1961*a*; McCully & Forbes,

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1965). The optimal concentration for *A. niger* was found to be 1/10,000 w/v in CM. Platings of about fifteen conidia per dish with concentrations ranging from 0·001 to 1 mg./ml. showed no reduction of viable counts up to 0·1 mg. At this concentration

Table 2. *Mean diameter of conidia from haploid, diploid and segregant (on FPA) strains*

Strains	Mean diameter* (μ)
a arg ade7	2·82
	2·90
	2·78
o his met7	3·32
	3·32
	3·36
Diploid $\frac{arg\ a}{+\ +} \cdot \frac{+}{o}\ \frac{+}{his}\ \frac{ade7}{+}\ \frac{+}{met7}$	4·08
	3·96
	4·00
a acr3 thi	2·78
o his	3·12
Diploid $\frac{a\ acr3}{+\ +} \cdot \frac{thi}{+}\ \frac{+}{o}\ \frac{+}{his}$	3·88
Haploid segregants: *a acr3 thi his*	2·74
a acr3 thi	2·80
a acr3 o	2·80
o his	3·16
arg nic3 a	2·72
pab5 o his	3·20
Diploid $\frac{+\ \ arg\ nic3\ a}{pab5\ +\ \ +\ \ +} \cdot \frac{+}{o}\ \frac{+}{his}$	4·08
Haploid segregants: *arg nic3 a his*	2·84
arg nic3 a	2·80
pab5 nic3 a his	2·90
pab5 nic3 a	2·84
arg o	3·20
pab5 o his	3·16
pab5 o	3·20

* Mean diameter of 100 conidia. When several values are given for one strain, the conidia were measured from different colonies.

the growth rate of the colonies derived from diploid conidia is reduced and their sporulation poor. From the diploid colonies, haploid sectors developed.

The ploidy of the segregants produced by diploids was determined by conidial measurement following the method used for *A. nidulans* (Pontecorvo *et al.*, 1953) and for *A. niger* (Pontecorvo, Roper & Forbes, 1953). Conidia were always measured from 5-day colonies on CM. The diameter of conidia from haploid and diploid segregants differed enough to classify them, although olive haploid conidia were

bigger than fawn (Table 2). All segregants, presumed to be haploid after measurement of the conidia, behaved as such when used in the synthesis of another diploid.

A much wider range of diameter was reported by Pontecorvo, Roper & Forbes (1953) for the segregants recovered from diploids of *A. niger*. In their experiment they did not select against aneuploids, as FPA presumably does (see p. 21), so that most of their conidia of intermediate diameter were probably aneuploid.

(ii) *Selection of diploid segregants and determination of map order*

The technique used in *A. nidulans* made use of the fact that a diploid heterozygous for a recessive mutant determining the colour of the conidia or abnormal colony morphology, may give rise to distinguishable homozygous sectors as a result of mitotic crossing-over between the locus concerned and the centromere. This technique proved unrewarding in the present work, because all three available mutants of this kind (*acr3*, *a*, *o*) were found to be linked to each other and close to the centromere.

(iii) *Map order from haploid segregants*

In contrast with *A. nidulans*, where haploidization and mitotic crossing-over occur at such low rates that their coincidence in one nuclear lineage is negligible, haploids of *A. niger* include an appreciable percentage which show recombination between linked markers. By the analysis of the haploids, it is therefore possible not only to assign markers to linkage groups, but also to determine the order of markers and to estimate the distances between them.

4. RESULTS AND DISCUSSION

The genotypes of all diploids referred to in this paper are given in Table 3.

IDENTIFICATION OF SIX LINKAGE GROUPS

An example of the identification of linkage groups by haploidization is given in Table 4. It is clear that segregation of markers among haploids from the heterozygous diploid 1 is not random: *arg*, *a* and *o* appear almost exclusively in parental coupling. We conclude therefore that the few recombinants between them (4 *a* arg^+) are due to crossing-over, as discussed later. That *a* and *o* belong to the same linkage group (designated as linkage group I) had already been shown by Pontecorvo, Roper & Forbes (1953). *His*, *leu3* and *put* segregate independently of one another and of markers of group I and presumably belong to different linkage groups. From this as well as from the study of the other diploids, the following linkage groups were established:

I: *a*, *o*, *acr3*, *pab1,5*, *arg*, *nic1,3*, *thi*, *met6*, *rib*
II: *his*
III: *ade2,5,7,9*, *lys2,4,5,6*, *w1*
IV: *met4,7*, *nic2,4*, *ade6,8*, *w4*
V: *leu3*
VI: *put*, *pyr*, *lys3*

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Table 3. *Genotypes of diploids analysed*

Diploid No.	Genotype
1.	$\frac{arg\ a}{+\ +} \cdot \frac{+}{o}\ \frac{+}{his}\ \frac{leu3}{+}\ \frac{+}{put}$
2.	$\frac{arg\ a}{+\ +} \cdot \frac{+}{o}\ \frac{+}{his}\ \frac{+}{ade7}\ \frac{+}{met7}\ \frac{leu3}{+}$
3.	$\frac{a}{+} \cdot \frac{+}{o}\ \frac{+}{his}\ \frac{ade7}{+}\ \frac{met7}{+}\ \frac{+}{put}$
4.	$\frac{a\ acr3}{+\ +} \cdot \frac{thi\ +}{+\ o}\ \frac{+}{his}$
5.	$\frac{a\ acr3}{+\ +} \cdot \frac{thi\ +}{+\ o}\ \frac{+}{his}\ \frac{+}{ade7}\ \frac{+}{met7}$
6.	$\frac{+\ arg\ nic3\ a}{pab5\ +\ +\ +} \cdot \frac{+}{o}\ \frac{+}{his}$
7.	$\frac{a}{+} \cdot \frac{+\ +}{o\ met6}\ \frac{+}{his}\ \frac{ade6\ nic2}{+\ +}$
8.	$\frac{pab5\ nic3\ a}{+\ +\ +} \cdot \frac{+\ +}{o\ met6}\ \frac{+}{his}$
9.	$\frac{arg\ a}{+\ +} \cdot \frac{+\ rib}{o\ +}\ \frac{+}{his}\ \frac{+}{met7}$
10.	$\frac{a}{+} \cdot \frac{+\ +}{o\ met6}\ \frac{rib\ +}{+\ his}\ \frac{ade6}{+}$
11.	$\frac{arg\ a}{+\ +} \cdot \frac{+}{o}\ \frac{+}{his}\ \frac{ade7\ +\ +}{+\ w1\ lys2}\ \frac{met7}{+}$
12.	$\frac{a}{+} \cdot \frac{+}{o}\ \frac{+}{his}\ \frac{ade7\ +}{+\ lys4}\ \frac{met7}{+}$
13.	$\frac{a}{+} \cdot \frac{+}{o}\ \frac{+}{his}\ \frac{ade7\ +}{+\ lys5}\ \frac{met7}{+}$
14.	$\frac{a}{+} \cdot \frac{+}{o}\ \frac{+}{his}\ \frac{ade7\ +}{+\ lys6}\ \frac{met7}{+}$
15.	$\frac{arg\ a}{+\ +} \cdot \frac{+}{o}\ \frac{+}{his}\ \frac{+\ ade2}{lys5\ +}\ \frac{+}{met7}$
16.	$\frac{arg\ a}{+\ +} \cdot \frac{+}{o}\ \frac{+}{his}\ \frac{+\ ade5}{lys5\ +}\ \frac{+}{met7}$
17.	$\frac{a}{+} \cdot \frac{+}{o}\ \frac{his}{+}\ \frac{lys5\ +}{+\ ade9}\ \frac{met7}{+}$
18.	$\frac{arg\ a}{+\ +} \cdot \frac{+}{o}\ \frac{+}{his}\ \frac{+\ ade6\ nic2}{met7\ +\ +}$
19.	$\frac{arg\ a}{+\ +} \cdot \frac{+}{o}\ \frac{+}{his}\ \frac{+}{ade7}\ \frac{+\ nic4}{met7\ +}$
20.	$\frac{arg\ a}{+\ +} \cdot \frac{+}{o}\ \frac{+}{his}\ \frac{+}{lys5}\ \frac{+\ ade8}{met7\ +}$
21.	$\frac{arg\ a}{+\ +} \cdot \frac{+}{o}\ \frac{+}{his}\ \frac{+\ met4}{ade6\ +}$
22.	$\frac{arg\ a}{+\ +} \cdot \frac{+}{o}\ \frac{+}{his}\ \frac{+}{ade7}\ \frac{+\ w4}{met7\ +}$
23.	$\frac{arg\ a}{+\ +} \cdot \frac{+}{o}\ \frac{+}{his}\ \frac{+}{ade7}\ \frac{+}{met7}\ \frac{lys3}{+}$
24.	$\frac{arg\ a}{+\ +} \cdot \frac{+}{o}\ \frac{leu3\ +\ put}{+\ lys3\ +}$
25.	$\frac{a}{+} \cdot \frac{+}{o}\ \frac{+}{his}\ \frac{ade7\ put\ +}{+\ +\ pyr}$
26.	$\frac{+\ arg\ nic1\ a}{pab5\ +\ +\ +} \cdot \frac{+}{o}\ \frac{+}{his}$
27.	$\frac{+\ arg\ a}{pab5\ +\ +} \cdot \frac{+}{o}\ \frac{+}{his}\ \frac{leu3}{+}$
28.	$\frac{pab1\ arg\ a}{+\ +\ +} \cdot \frac{+}{o}\ \frac{+}{his}\ \frac{+}{ade7}\ \frac{+}{met7}$
29.	$\frac{pab5\ +\ nic1\ a}{+\ arg\ +\ +} \cdot \frac{+}{o}\ \frac{+}{his}\ \frac{+}{lys5}$

Random segregation of markers of these different linkage groups was unequivocal despite the poor recovery of some mutant alleles among the haploids. This was particularly true for *leu3* among segregants from diploid 1 (Table 4), but the segregation of the wild-type allele left no doubt that this locus was in a linkage group other than I, II and VI.

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Table 4. *Haploid segregants from diploid 1 on FPA*

Constitution of diploid: $\frac{arg\ a}{+\ +} \cdot \frac{+}{o}\ \frac{+}{his}\ \frac{leu3}{+}\ \frac{+}{put}$

		a				*o*				
		arg		*arg*$^+$		*arg*		*arg*$^+$		Totals
		leu3	*leu3*$^+$	*leu3*	*leu3*$^+$	*leu3*	*leu3*$^+$	*leu3*	*leu3*$^+$	
his	*put*	1	6	0	1	0	0	0	8	16
	put$^+$	0	8	1	1	0	0	0	5	15
										31
his$^+$	*put*	1	6	0	1	0	0	0	13	21
	put$^+$	0	8	0	0	0	0	2	13	23
										44
Totals		2	28	1	3	0	0	2	39	75
		30		4		0		41		
		34				41				

One diploid gave a segregation inconsistent with the above assignment of markers to six linkage groups; two markers (*his* and *met7*) segregated from diploid 11 as follows: 16 *his*$^+$ *met7*, 19 *his met7*$^+$, 5 *his*$^+$ *met7*$^+$ and 2 *his met7*. From these results alone, one would have assigned *his* and *met7* to the same linkage group, but, as the segregation of these two markers was independent in other diploids, the most likely explanation is that diploid 11 was heterozygous for a translocation involving *his* and *met7*.

MAP ORDER AND INTERVAL LENGTH

1. *Linkage group I*

(*a*) a–acr3–*centromere*–thi–o

Linkage of these markers appeared from their segregation on FPA: all seventy-eight haploid segregants produced by diploid 4 were, with respect to these markers, of the parental genotypes: forty-four *a acr3 thi* o^+ and thirty-four a^+ *acr3*$^+$ *thi*$^+$ *o*.

Epistasis between the conidial colour markers *a* ('fawn') and *o* ('olive') and the location of the centromere were ascertained from diploid and haploid segregants produced by diploids 4 and 5.

Epistasis of a *to* o. On CM, diploid 4 gave fawn and olive first-order diploid segregants (wild-type conidia are black). No further colour segregant was produced by the fawn, while the olive gave many fawn second-order segregants. Fawn second-order diploid segregants were plated on acriflavine to select haploids. One of the fawn *thi* haploids recovered was combined with strains *a arg* and *o his* to form two diploids. In the diploid with *a arg*, the heads were fawn, in that with *o his* they were olive. In view of the latter result, the haploid recovered from the fawn second-order segregant was *a o*. Its phenotype being fawn, *a* is epistatic to *o*. We note

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therefore that the light olive haploid heads, reported by Pontecorvo, Roper & Forbes (1953) could not have been *a o* double mutants, as they suggested. Light olive heads occurred in these experiments as well, whenever olive heads were produced; but this colour did not persist on subculturing and all the heads turned dark olive. The mechanism of this change of colour has not been investigated.

Location of the centromere. The order *a*–*o*–centromere was excluded by the results just described, since, if this were the correct sequence, the first-order *o* segregants should have been homozygous for a^+ and could not have produced second-order fawn segregants. Two alternative orders in respect of the centromere remained possible *a*–centromere–*o* and centromere–*a*–*o*. The various steps of the formation of the segregants recovered were interpreted as follows: the black diploid

$$\frac{a}{+}\bullet\frac{+}{o} \quad \text{or} \quad \bullet\frac{a \quad +}{+ \quad o}$$

(the dot represents the centromere) produces by single crossing-over *either* fawn first-order segregants

$$\frac{a}{a}\bullet\frac{+}{o} \quad \text{or} \quad \bullet\frac{a \quad o^+}{a \quad o^+},$$

which cannot give further colour segregants, *or* olive first-order segregants

$$\frac{a}{+}\bullet\frac{o}{o} \quad \text{or} \quad \bullet\frac{a \quad o}{+ \quad o} \quad \text{or} \quad \bullet\frac{a^+ \quad o}{a^+ \quad o}.$$

The olive segregants heterozygous for *a* can produce by crossing-over fawn second-order segregants, the genotype of which will be

$$\frac{a}{a}\bullet\frac{o}{o} \quad \text{or} \quad \bullet\frac{a \quad o}{a \quad o}.$$

As for *acr3* and *thi*, the fawn diploid segregants are neither all acriflavine resistant (see also diploid 5), nor homozygous for *thi*. Thus these two markers are located between *a* and *o* or proximally to *a* if *a* and *o* are on the same arm. Since, after plating on acriflavine, one fawn second-order segregant gave fawn haploids, prototrophic and resistant, and another gave fawn haploids, resistant but auxotrophic for thiazole, in respect of the markers of linkage group I, the genotype of the first segregant must have been

$$\frac{a \;\; acr3 \;\; thi^+ \;\; o}{a \;\; + \;\; thi^+ \;\; o},$$

and that of the second

$$\frac{a \;\; acr3 \;\; thi \;\; o}{a \;\; + \;\; + \;\; o};$$

if every recombination is the result of a single crossover, as the following data will show, *thi* must be located between the centromere and *o*.

Diploid 5 plated on CM gave eighty fawn diploid segregants. All were hetero-

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zygous for *thi*, which is consistent with the position just given for this allele; eight were acriflavine sensitive, the remaining seventy-two acriflavine resistant. This locates the centromere between *acr3* and *thi*, with *acr3* proximal to *a*.

These results establish the order: *a*–*acr3*–centromere–*thi*–*o*.

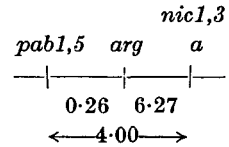
(*b*) pab1,5–arg–nic1,3–a–o

The segregation pattern of these markers (Table 5) shows that they belong to the same linkage group. If it is assumed that the haploid recombinants are due usually to single crossovers, the sequence of the markers must be: *pab1,5*–*arg*–*a*–*o*. The distal position of *pab5* is deduced from the fact that all the fawn arginine-independent haploids from diploids 6, 26 and 27 were *pab*, whilst diploid 29, in which *pab5*

Table 5. *Summary of the data on linkage group I, left arm*

Diploid	Haploids analysed	Recombinants in intervals		
		pab–arg	*pab–a*	*arg–a**
1	75			4
2	61			3
9	74			6
11	27			0
15	47			3
16	66			10
18	67			10
19	40			2
20	61			4
21	52			7
22	71			8
23	62			5
24	95			5
6	310	0		21
26	292	2		18
27	296	2		16
28	302	0		25
29	302	0		9
8	400		16	
Recombination fractions		4/1502	16/400	136/2167

Order of loci and map distances:



* For tests of homogeneity, small classes were pooled as follows: Diploids 1 and 2; 9, 11 and 15; 19 and 20.

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and *a* were in coupling and *arg* in repulsion gave fawn arginine-requiring *pab*$^+$ haploids. No recombination was observed between *a* and *nic1* or *nic3*. This indicates close linkage of these markers.

If for each interval the proportion of recombinants recovered among haploids on FPA is constant, mitotic map distances can be calculated very much as in meiotic recombination analysis; moreover this was the only possible method for estimating interval lengths, as no distally located markers were available in order to select for segregants resulting from mitotic crossing-over and to construct mitotic map by the method of Pontecorvo, Tarr-Gloor & Forbes (1954) and Pontecorvo & Käfer (1956, 1958). The results given by the diploids for the interval *a–arg* are heterogeneous ($\chi^2_{13} = 30{\cdot}61$; $P < 0{\cdot}01$), but most of this heterogeneity is due to diploids 16 and 18, which gave exceptionally high proportions of recombinants. This could be the result of a mitotic crossover having occurred several generations before haploidization, giving rise to a clone of recombinants. If these two diploids are omitted, the results become homogeneous ($\chi^2_{11} = 16{\cdot}55$; $P = 0{\cdot}2$–$0{\cdot}1$). The mean recombination fraction can therefore be taken as the mitotic distance between *a* and *arg*. As *nic1* and *nic3* (diploids 6, 26, 29) always segregated with *a*, the position of the *nic* alleles relative to *a* could not be determined.

The mitotic map derived from these recombination fractions is given in Table 5. Although the distance *pab–a* (4·00) is smaller than the sum of the distances *pab–arg* (0·26) and *arg–a* (6·27), it seems better to assign this discrepancy to the relatively small number of haploids analysed for the first value than to consider *pab* proximal to *arg*; if it were, all four recombinants between the *pab* and *arg* loci (diploids 26 and 27) would have required double crossovers, while no single crossover would have taken place. This emphasizes the difficulty of ordering certain markers by map distances alone.

(*c*) a–o–met6–rib

Linkage between *o* and *met6* is clear from the segregation pattern in the haploids from diploids 7, 8, 10 (Table 6). The order centromere–*o–met6* is suggested by the recovery from diploid 7 of an olive methionine-independent recombinant. If *met6* were located between *a* and *o*, a methionine-independent recombinant should have been phenotypically fawn. The recombinant recovered could not be dismissed as a contaminant, as no olive prototrophic strain was at that time kept in the laboratory.

That *rib* must be located on the same arm, distally to *o*, is shown by the recombinants from diploid 9: the recombinants between *a* and *arg* and those between *o* and *arg* were not recombinants between *a* or *o* and *rib*.

The positions of *rib* and *met6* were checked by the analysis of haploid segregants from diploid 10. All possible segregants for independent markers were recovered, their number varying widely because of the poor viability of *met6* and *his*. One *a met6*$^+$ *rib*$^+$ recombinant was recovered, which places *rib* distally to *met6*. No *met6 rib* haploid was expected as the frequency of *met6* was very low among the haploids.

Table 6. *Summary of the data on linkage groups I, right arm, III and IV*

Linkage group I, right arm

Diploid	Haploids analysed	Recombinants in intervals		
		o–met	*o—rib*	*met–rib*
7	65	1		
8	400	0		
10	147	0		1
9	74		2	
Recombination fractions		1/612	2/74	1/147

Linkage group III

Diploid	Haploids analysed	Recombinants in intervals	
		ade–lys	*lys–w*
11	42	2	0
12	74	0	
13	69	1	
14	73	2	
15	47	0	
16	66	3	
17	59	1	

Linkage group IV

Diploid	Haploids analysed	Recombinants in intervals			
		w–met	*met–nic*	*met–ade*	*nic–ade*
18	67		1		0
19	40		1		
20	61			2	
21	52			0	
22	71	2			

2. *Linkage group III*

(*a*) w1, lys2, ade7

From diploid 11 (Table 6), it is clear that *w1* and *lys2* are linked to *ade7*: all phenotypically white haploids are lysine-requiring and most of them are adenine-independent.

Two segregants, recombining *w1 lys2* and *ade7* were recovered, which suggests that, if the three markers are on the same chromosome arm, *ade7* is located outside the segment *w1–lys2*. As for the position of these last two markers in respect of the centromere, the proximity of *w1* to the centromere appears from the very low frequency of white diploid segregant heads compared with that of fawn produced by the same black diploid heterozygous in respect of both. As sixteen white diploids isolated visually from CM were all auxotrophic for lysine, *lys2* must be relatively close to *w1* or else the order is centromere–*w1–lys2*.

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That *w1* is epistatic not only to *o* (the *w1 o* parent strain was phenotypically white) but also to *a*, is suggested by the fact that all fawn haploids, except one, were *ade7*, as were all but one olive; besides, among the white haploids about half were *arg* and thus probably *a*, the other half *arg*$^+$, thus probably *o*. This was checked by synthesizing a heterokaryon between a white segregant, requiring arginine, and a fawn strain, and by plating for diploids. The heterokaryon and diploid heads were fawn, the genotype being therefore

$$\frac{arg\ \ a}{+\ \ a} \bullet \frac{o^+}{o^+}\ \frac{+}{his}\ \frac{lys2\ \ w1}{+\ \ +}\ \frac{met7}{+}.$$

Thus, the white haploid strain carried *a*, and *w1* is epistatic to *a* as well as to *o*.

(*b*) lys4,5,6, ade2,5,9

In diploids 12, 13, 14 (Table 6), the three non-allelic *lys* markers are in repulsion to *ade7*; no double auxotrophic recombinant between any of the three *lys* mutants and *ade7* were recovered, and only three prototrophs were obtained out of 216 haploids. This shows that the *lys* markers belong to the same linkage group as *ade7*.

From the segregation pattern from diploids 15, 16, 17 (Table 6), the *ade2,5,9* markers appear to be linked. Among 172 haploids, only four wild-type recombinants, following a crossover between *lys5* and the *ade* markers, were present. Again, the formation of haploid recombinants carrying the wild alleles, and the absence of the complementary class of double requirers among the few haploids tested can be accounted for by viability effects.

3. *Linkage group IV*

(*a*) nic2,4, ade6,8, met4,7

The segregants from diploids 18, 19, 20, 21 (Table 6) show the linkage of these six markers, but only between *ade6* and *nic2* (diploid 18), and between *ade6* and *met4* (diploid 21) did no crossover take place.

(*b*) w4

There is no doubt from the segregation of diploid 22 (Table 6) that *w4* is linked with *met7*.

A new phenotype appeared in haploids segregating from this diploid

$$\left(\frac{arg\ \ a}{+\ \ +} \bullet \frac{+}{o}\ \frac{+}{his}\ \frac{+}{ade7}\ \frac{+\ \ w4}{met7\ \ +}\right):$$

yellowish heads corresponded to the genotype *w4 o*, while the parent haploid strains were respectively white and olive. The genotype of the yellowish recombinant was confirmed by the synthesis of the following diploids:

(1) $\frac{+\ \ +}{arg\ \ a} \bullet \frac{o}{+}\ \frac{his}{+}\ \frac{w4}{w4}$. This diploid has light brown heads.

(2) $\frac{+\ \ +}{arg\ \ a} \bullet \frac{o}{+}\ \frac{his}{+}\ \frac{w4}{+}$. This diploid is of the usual black colour.

(3) $\frac{+\ \ a^+}{arg\ \ a^+} \bullet \frac{o}{o}\ \frac{his}{+}\ \frac{w4}{+}$. This diploid is olive.

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One of the two recombinants for *w4* and *met7* markers had the genotype *arg* a^+ *his* $w4^+$ *met7* and therefore two crossovers were necessary for its production: one between *arg* and *a*, the other between *w4* and *met7*. This is the only haploid recombinant among more than 3500 haploids analysed that could not be explained by a single crossover.

4. *Linkage group VI*

That *put*, *lys3* and *pyr* belong to linkage group VI was found from diploids 24 and 25. No recombinants between the markers were recovered (among ninety-five haploids produced by diploid 24 and sixty-four haploids from diploid 25) and all classes of expected segregants with respect to other markers were present.

THE PARASEXUAL CYCLE

1. *Haploidization and the action of FPA*

The action of FPA as an inducer of haploidization is confined to a rather narrow range of concentrations; below this range, the growth and sporulation of the colonies are indistinguishable from normal; above it no conidia germinate. Only one concentration (1/10,000 w/v) was used, always in CM, in order to standardize methods: some of the growth factors with which it would have been necessary to supplement MM could have interacted, positively or negatively, with FPA (Morpurgo, 1961*b*).

From the growth rate of haploid sectors on diploid colonies, it became clear that the action of FPA differed according to the ploidy of the mycelium. Haploid sectors sporulated normally, while diploid colonies usually gave only a few conidial heads and the growth rate of a haploid sector was higher than that of the diploid from which it segregated. Growth rates of haploid and diploid mycelia on CM with and without FPA were compared. To reduce possible selective effects of certain markers, a diploid homozygous for two nutritional markers and heterozygous for two others was plated on both media, together with haploid strains corresponding to those from which the diploid was synthesized, but differing in colour. Diploid

$$\begin{array}{cccccc} a & his & ade7 & + & met7 \\ \overline{a} & \overline{his} & + & lys5 & \overline{met7} \end{array}$$

was inoculated on the same dish as either *o his ade7 met7* or *o his lys5 met7*, and

$$\begin{array}{cccccc} o & his & ade7 & + & met7 \\ \overline{o} & \overline{his} & + & lys5 & \overline{met7} \end{array}$$

was inoculated with either *a his ade7 met7* or *a his lys5 met7*. All experiments gave the same results. On FPA, haploid colonies grew evenly, more quickly than the diploids, and sporulated normally; the diploids grew very slowly, did not sporulate well, and gave vigorous sectors with normal sporulation, which turned out to be haploid. The markers used in this test did not influence growth rates. If we compare the growth rates with and without FPA (Table 7), it appears that on CM with

Table 7. *Diameter (mm.) of haploid and diploid colonies on CM with and without FPA*

Strains	Hours after inoculation: On CM + FPA 48	72	96	120	144	On CM 24	48	72	96	120
a his ade7 met7	5	11	15	21	25	8	23	43	61	79
	7	12	17	23	28	9	24	44	63	80
a his lys5 met7	8	13	18	23	28	9	24	44	63	81
	8	12	17	22	27	11	27	46	64	80
o his ade7 met7	8	13	18	23	28	9	25	45	63	82
	8	12	17	23	28	10	25	44	61	79
o his lys5 met7	8	13	18	22	27	10	26	44	60	79
	7	12	17	23	28	9	25	43	61	79
Mean (haploids)	7·4	12·2	17·1	22·5	27·4	9·4	23·6	44·1	62·0	79·9
$\frac{a\ his\ ade7\ +\ met7}{o\ his\ +\ lys5\ met7}$	2	4	7	9	11	10	30	49	68	86
	2	4	6	8	10	10	29	49	69	86
	2	4	6	8	10	11	29	49	68	85
	2	4	6	8	10	10	28	48	66	85
$\frac{o\ his\ ade7\ +\ met7}{a\ his\ +\ lys5\ met7}$	2	5	7	9	11	10	32	50	69	86
	3	6	8	10	12	10	33	50	70	87
	2	5	7	9	11	11	33	51	71	89
	3	5	8	10	12	10	33	50	71	87
Mean (diploids)	2·2	4·6	6·9	8·9	10·9	10·2	30·9	49·5	69·0	86·4

Of each haploid strain two, and of each diploid strain four colonies were measured.

1/10,000 FPA a haploid colony grows at about 30% of the rate on normal CM (mean increase of diameter per day: 5 and 17·6 mm.), while a diploid grows only at about 10% of its normal rate (mean increase of diameter per day: 2·25 and 19·2 mm.).

The hypothesis was made (Lhoas, 1961) that the difference between these two rates was the result of a disturbance of mitosis induced by FPA, leading to chromosome losses. After the loss of a chromosome, the diploid nuclei could go on dividing, though at the lower rate of aneuploids, until the haploid stage was reached by further losses of chromosomes. Chromosome loss in a haploid nucleus, on the other hand, would stop it dividing further, so that it would not disturb the growth rate of the rest of the colony: in the haploid colony, the elimination of nuclei lacking one or more chromosomes would be responsible for the reduction by 30% of the growth rate, while in a diploid colony the much slower growth would result from the presence of numerous aneuploid nuclei.

This hypothesis was tested by the following experiment. Diploid

$$\frac{arg\ a}{+\ +} \bullet \frac{+}{o}\ \frac{ade7\ +}{+\ lys5}\ \frac{nic4\ +}{+\ met7}$$

was inoculated at one point on MM + FPA (1/20,000 w/v), supplemented either with adenine and nicotinic acid (on which monosomics requiring arginine, lysine or methionine could not grow), or with lysine and methionine (on which monosomics requiring arginine, adenine or nicotinic acid could not grow). As a control the same

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diploid was inoculated on MM + FPA supplemented with all five growth factors. If the hypothesis was correct, the growth rate on MM supplemented only with two growth factors, on which only three types of monosomics can mutiply, should be greater than that on the control medium supplemented with all five growth factors, on which all types of monosomics can multiply. The growth rates on MM + FPA with either pair of growth factors was about twice that on MM + FPA with all five growth factors (Table 8). A direct effect of the growth factors added to the medium is unlikely as an explanation, since in that case better growth should have occurred on the more fully supplemented medium, as in the absence of FPA. The results obtained therefore support the hypothesis.

Table 8. *Diameter (mm.) of diploid colonies*

$$\frac{\text{arg}\ \ a\ +}{+\ \ +\ o} \quad \frac{\text{ade7}\ \ +}{+\ \ \text{lys5}} \quad \frac{\text{nic4}\ \ +}{+\ \ \text{met7}}$$

on MM, MM with growth factors and MM with FPA and growth factors

	Hours after inoculation				
	72	96	120	144	168
MM	25	33	41	49	56
	24	32	41	49	57
MM + adenine, nicotinic acid	30	42	55		
	29	41	53		
MM + lysine, methionine	27	40	54		
	28	41	56		
MM + adenine, nicotinic acid arginine, lysine, methionine	32	46	60		
	33	47	61		
MM + FPA, adenine, nicotinic acid			4	7	10
			5	8	10
MM + FPA, lysine, methionine			4	7	10
			5	7	10
MM + FPA, adenine, nicotinic acid, arginine, lysine, methionine			2	4	5
			2	3	4

2. *Parameters of the parasexual cycle*

The data already available on the proportions of diploid nuclei in a heterokaryotic colony, of haploid nuclei in a diploid colony, and on the incidence of crossing-over in a diploid nucleus give an idea of the part played in this 'imperfect' fungus by the parasexual cycle. It must, however, be emphasized that the figures given here are very crude; the results obtained did not allow, for example, efficient correction for clonal effects by Luria & Delbrück's (1943) method.

The proportion of diploid nuclei in a freshly synthesized heterokaryon is given by the number of diploid colonies over the total number of colonies from platings of conidia from a heterokaryotic colony. $0{\cdot}8 \times 10^{-4}$ (Table 9) is the lowest estimate of the proportion of diploid nuclei in a young heterokaryotic colony.

The rate of haploidization was found by plating diploid 4 on acriflavine and taking

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Table 9. *Proportion of diploid colonies in platings of conidia from balanced heterokaryons*

Heterokaryon	No. of conidia plated	No. of diploid colonies	Proportion of diploid colonies $\times 10^4$
2	6×10^5	424	7·06
4	2×10^6	162	0·81
8	1×10^6	530	5·30
9	1×10^6	356	3·56

advantage of the close linkage between *acr* and the centromere. Several platings were made from different single conidium colonies (Table 10). $0{\cdot}4 \times 10^{-2}$ is the lowest estimate of the proportion of haploid *acr* nuclei in a diploid colony; the minimum proportion of all haploids produced (*acr* and *acr*$^+$) should then be $0{\cdot}8 \times 10^{-2}$.

Table 10. *Proportion of haploid colonies selected from platings of diploid*

$$\frac{\text{a acr3}}{+\ +} \bullet \frac{\text{thi} + +}{+\ \text{o his}}$$

on CM + acriflavine

No. of conidia plated	No. of haploids selected	Proportion of *acr3* haploids $\times 10^2$
1000	14	1·4
1000	11	1·1
1000	6	0·6
2000	8	0·4
600	33	5·5
600	18	3·0
600	36	6·0

These proportions of diploid nuclei in a heterokaryon and of haploid nuclei in a diploid colony give an estimate of the ratio of diploid to haploid nuclei in a population at equilibrium, in the absence of selection: $0{\cdot}8 \times 10^{-4}/0{\cdot}8 \times 10^{-2} = 10^{-2}$.

The number of exchanges in a diploid nucleus may be estimated from the proportion of recombinants between *arg* and *a* (6·27%). Considering that this segment is only a part (but the largest found so far) of the first chromosome and that there are at least six chromosomes in *A. niger*, 20% is a reasonable minimum estimate of the incidence of mitotic crossing-over in a population of nuclei.

Darlington's (1937) formula for calculating the 'meiotic recombination index' was corrected and adapted as follows to mitotic recombination by Pontecorvo (1958):

$$I_{mi} = [E + (n-1)h]d$$

where I_{mi} = mitotic recombination index
E = the number of exchanges per diploid nucleus
n = the number of chromosome pairs
h = the proportion of diploid nuclei which undergo haploidization
d = the proportion of diploid nuclei per colony.

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Using the estimates given above:

$$I_{mi} = [2 \times 10^{-1} + (6-1) \times 0{\cdot}8 \times 10^{-2}] \times 10^{-2} = 2 \times 10^{-3}.$$

This value is 100 times the mitotic recombination index in *A. nidulans* (Pontecorvo, 1958).

Even though the estimates given are based on very limited data, they show nevertheless the importance of the parasexual cycle in *A. niger* for storing and recombining genetic variation.

SUMMARY

An investigation of mitotic segregation and recombination in *A. niger* gave the following results:

1. Thirty-one non-allelic markers have been assigned to six linkage groups (containing 11, 9, 6, 3, 1 and 1 markers respectively) by the analysis of haploid mitotic segregants from synthesized diploids.

2. The sequence of nine markers in one linkage group was determined and some of the map intervals were estimated by the analysis of haploids, recombinants for linked markers.

3. Almost all the haploid segregants were obtained on medium supplemented with the aminoacid analogue, *p*-fluoro-phenylalanine, the action of which is interpreted as an induction of chromosome losses.

4. The rates of mitotic crossing-over and haploidization are much higher than in the sexual species *A. nidulans* and the data support Pontecorvo's (1958) suggestion that the parasexual cycle can be a substantial alternative to the sexual cycle.

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