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Correlated meiotic and mitotic maps in *Aspergillus amstelodami**

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1. INTRODUCTION

The demonstration of a parasexual cycle in *Aspergillus nidulans* (Pontecorvo, 1956) provided a system through which extensive genetic analysis of somatic recombination could be described (Pontecorvo & Käfer, 1958; Käfer, 1958). In those two reports a comparative analysis of data from somatic and meiotic recombination in *A. nidulans* showed the existence of eight linkage groups. Numerous other studies have attempted to demonstrate linkage by the analysis of mitotic segregants. These have been described for *Verticullium albo-atrum* (Hastie, 1964), *Penicillium chrysogenum* (Sermonti, 1957), *P. expansum* (Barron, 1962; Garber & Beraha, 1965; Fjeld & Strömnaes, 1966), *P. italicum* (Strömnaes, Garber & Beraha, 1964), *Aspergillus fumigatus* (Strömnaes & Garber, 1963) and *A. niger* (Lhoas, 1967). There are among these, however, some cases of inconsistency in providing information through mitotic recombination. In *A. fumigatus* is was necessary for the authors to rule out the interpretation that each of the 24 of the 26 markers analysed should be assigned to a separate linkage group. Garber & Beraha (1965) accounted for the fact that 13 of the 14 markers studied in *P. expansum* appeared to be on one linkage group by assuming that reciprocal translocation had ocurred during preparation of the multiply marked strains. Barron (1962) working with this organism had previously placed four of the seven markers studied in one linkage group and three in another. Fjeld & Strömnaes (1966) added four markers to Barron's group I and one to his group II; a single discrepancy, however, involving one of Barron's group II markers arose in their work. In *P. italicum* only two of the six markers studied could be tentatively placed in a single 'presumptive' linkage group. Thus it is doubtful whether the principles of parasexuality described for *A. nidulans* can be applied to all filamentous fungi or even to those closely related to this species. More comparative meiotic and mitotic maps are needed, and to this end, 13 markers were mapped meiotically and mitotically in *Aspergillus amstelodami*, an organism in which the parasexual cycle had previously been demonstrated (Lewis & Barron, 1964).

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2. MATERIALS AND METHODS

Strains. Aspergillus amstelodami (University of Guelph collection no. 10163) is a homothallic ascomycete with olive-green conidia. Mutants were produced, isolated and characterized by the methods of Barron & MacNeill (1962). Table 1 shows a list of the strains used in genetic analysis.

Media. The basic minimal medium (bMM) and complete medium (bCM) used have been previously described (Barron & MacNeill, 1962). *A. amstelodami* gave abundant cleistothecia and few conidia on bMM, which was thus used for crosses for meiotic analysis. Abundant conidial production occurred on a modified minimal medium containing 5% NaCl (mMM) and on a modified complete medium with 5% NaCl (mCM). These media were used for mitotic analysis.

Table 1. *Ultraviolet induced mutant strains of* Aspergillus amstelodami *produced by successive irradiation*

No.	Symbols*	Colour and requirement	Strain of origin
1	*wh pab leu*	White conidia, para-amino benzoic acid, leucine	*wh pab*
2	*br* met_2	Brown conidia, methionine	*br*
3	*li* met_1 *lys*	Lime-green conidia, methionine, lysine	*li* met_1
4	*pb ade arg*	Pale-blue conidia, adenine, arginine	*pb ade*

* *wh* = white conidia; *br* = brown; *li* = lime-green; *pb* = pale blue; *pab* = para-amino benzoic acid; *leu* = leucine; *met* = methionine; *lys* = lysine; *ade* = adenine; *arg* = arginine.

Methods of meiotic and mitotic analysis. Heterocaryons and diploids were prepared according to standard procedure (Roper, 1952; Barron & MacNeill, 1962). The techniques of random ascospore analysis (Pontecorvo *et al.* 1953) were used for the analysis of meiotic recombinants. The methods of mitotic analysis used by Pontecorvo & Käfer (1958) were applied to *A. amstelodami*. The techniques of needle-plating (Käfer, 1961), para-fluorophenylalanine (p-FPA) induction of haploids (Morpugo in Lhoas, 1961) and modified replica plating (Mackintosh & Pritchard, 1963) were used in the isolation of haploid segregants.

3. RESULTS

The multiply-marked strains in Table 1 were derived from successive irradiation. Heterocaryons were readily formed between all pairs of strains tested; the results indicated that anastomosis in this species was a regular occurrence. The conidial colour of the heterocaryons was controlled in all cases by autonomous gene action (Pontecorvo, 1946). Heterocaryons exposed to D-camphor vapours for 14–36 h produced diploid strains. Exposure for less than 14 h gave negative results.

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Diploid strains were lighter in colour than the olive-green of the haploids and their conidia had a mean diameter of 5·87 μ compared to that of 4·87 μ for the conidia of haploid strains. The heterocaryons used in crosses I, II, III and IV and the results of the analysis of meiotic recombination are shown in Tables 2 and 3. The corresponding diploids A, B, C and D were used in the analysis of mitotic recombination. (Tables 4, 5).

Table 2. *Analysis of meiotic recombinants from crosses I and II*

I: $\frac{br}{BR}\ \frac{met_2}{MET_2}\ \frac{WH}{wh}\ \frac{PAB}{pab}\ \frac{LEU}{leu}$ II: $\frac{br}{BR}\ \frac{met_2}{MET_2}\ \frac{PB}{pb}\ \frac{ADE}{ade}\ \frac{ARG}{arg}$

Hetero-caryon	Expt type	Selected recombinants	Segregation ratios of other loci	Recombination fraction
I	(*a*)	WBR	$\frac{LEU}{leu}$ 63 50	0·442
			$\frac{PAB}{pab}$ 58 55	0·486
			$\frac{MET_2}{met_2}$ 66 47	0·425*
	(*b*)	(i) PAB MET_2 LEU (ii) MET_2 LEU (iii) PAB MET_2	LEU $\frac{WH}{wh}$ 348 298	0·456*
			PAB $\frac{WH}{wh}$ 353 296	0·456*
			MET_2 $\frac{BR}{br}$ 362 226	0·384**
II	(*a*)	PBBR	$\frac{MET_2}{met_2}$ 11 14	—
			$\frac{ADE}{ade}$ 13 12	0·480
	(*b*)	(i) MET_2 ARG (ii) MET ADE	ADE $\frac{PB}{pb}$ 27 23	0·460
			ARG $\frac{PB}{pb}$ 21 20	0·490
			MET_2 $\frac{BR}{br}$ 47 44	0·485

* Significant at the 0·05 level.

** Significant at the 0·01 level. The hypothesis is that free recombination is shown by a 1:1 segregation of alleles.

Three of the selector markers used in the analysis of mitotic segregants, *pb*, *br* and *li*, were not epistatic to each other although a fourth, *wh*, was epistatic to these three. This fact was used in the visual selection of haploid segregants from diploid colonies. From the diploid containing the markers *pb* and *li* all lime-blue segregants (intermediate to the two colours) were haploid. Similarly all light-brown

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segregants were haploid. Haploids of intermediate colour can occur only if the two markers are located on different chromosomes.

A summary of the results of meiotic recombination shows that three definite cases of linkage were determined. They are, *wh–met*$_1$, $6{\cdot}3 \pm 3{\cdot}6$; *pb–met*$_1$, $7{\cdot}7 \pm 1{\cdot}3$;

Table 3. *Analysis of meiotic recombinants from crosses III and IV*

III: $\frac{wh}{WH}\ \frac{pab}{PAB}\ \frac{leu}{LEU}\ \frac{LI}{li}\ \frac{MET_1}{met_1}\ \frac{LYS}{lys}$ IV: $\frac{pb}{PB}\ \frac{ade}{ADE}\ \frac{arg}{ARG}\ \frac{LI}{li}\ \frac{MET_1}{met_1}\ \frac{LYS}{lys}$

Hetero-caryon	Expt type		Selected recombinants	Segregation ratios of other loci	Recombination fraction
III	(*a*)		WLI	$\frac{LEU}{leu}\ \frac{81}{66}$	0·448
				$\frac{PAB}{pab}\ \frac{102}{44}$	0·300**
				$\frac{MET_1}{met_1}\ \frac{10}{137}$	0·068**
				$\frac{LYS}{lys}\ \frac{117}{30}$	0·202**
	(*b*)	(i)	MET_1 LEU	$MET_1\ \frac{wh}{WH}\ \frac{1085}{67}$	0·058**
		(ii)	LEU LYS		
		(iii)	LYS MET_1 LEU	LYS $\frac{LI}{li}\ \frac{327}{101}$	0·290**
		(iv)	†PAB LYS		
		(v)	PAB MET_1		
		(vi)	PAB LEU LYS		
IV	(*a*)		PBLI	$\frac{LYS}{lys}\ \frac{42}{9}$	0·176
				$\frac{met_1}{MET_1}\ \frac{48}{3}$	0·059
				$\frac{ARG}{arg}\ \frac{20}{31}$	—
				$\frac{ADE}{ade}\ \frac{25}{26}$	
	(*b*)	(i)	ARG LYS	LYS $\frac{LI}{li}\ \frac{486}{223}$	0·324**
		(ii)	MET_1 ADE		
		(iii)	LYS ADE		
		(iv)	MET_1 ARG	$MET_1\ \frac{pb}{PB}\ \frac{469}{50}$	0·096**

** Significant at the 0·01 level. The hypothesis is that free recombination is shown by a 1:1 segregation of alleles.

† Recombination values of *pab* and *leu* were not determined because of the bias created by other pairs of markers.

li–lys, $24{\cdot}3 \pm 3{\cdot}0$. Other recombination values were *wh* and *pab*, $41{\cdot}1 \pm 4{\cdot}6$; *wh* and *leu*, $44{\cdot}8 \pm 0{\cdot}3$; *br* and *met*$_2$, $43{\cdot}1 \pm 2{\cdot}4$; *pb* and *ade*, $47{\cdot}0 \pm 0{\cdot}7$; and *pb* and *arg*, $46{\cdot}5 \pm 1{\cdot}8$. The data from these experiments only, indicate that there are two groups of closely linked markers, *wh*, *met*$_1$ and *pb*, and *li* and *lys*. Very little

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Table 4. *Classification of 473 independent segregants from heterozygous diploids A and B in* Aspergillus amstelodami

Diploid A: $\frac{br}{BR}$ $\frac{met_2}{MET_2}$ $\frac{WH}{wh}$ $\frac{PAB}{pab}$ $\frac{LEU}{leu}$

Ploidy	Nutritional requirements	No.	Segregation of unlinked loci
	White segregants		
2n	Wild type	98	wh $\frac{MET_2}{met_2}$ 51/29
n	pab	32	
n	pab met_2	17	$\frac{LEU}{leu}$ 49/31
n	pab leu	19	
n	pab met_2 leu	12	
n	met_2	1	
n	met_2 leu	1	
	Brown segregants		
2n	Wild type	87	br $\frac{MET_2}{met_2}$ 19/15
2n	met_2	3	
n	Wild type	12	$\frac{LEU}{leu}$ 25/9
n	met_2	13	
n	leu	7	
n	met_2 leu	2	
	Wild-type segregants		
n	Not classified	10	

Diploid B: $\frac{br}{BR}$ $\frac{met_2}{MET_2}$ $\frac{PB}{pb}$ $\frac{ADE}{ade}$ $\frac{ARG}{arg}$

Ploidy	Nutritional requirements	No.	Segregation of unlinked loci
	Pale-blue segregants		
2n	Wild type	48	pb $\frac{arg}{ARG}$ 15/31
2n	ade	4	
n	ade	14	
n	ade arg	6	
n	ade met_2	1	
	Brown segregants		
2n	Wild type	41	br $\frac{met_2}{MET_2}$ 18/12
2n	met_2	1	
n	Wild type	3	$\frac{ARG}{arg}$ 17/13
n	met_2	7	
n	met_2 arg	2	
n	arg	2	
	Light-brown segregants		
n	ade	9	
n	ade met^2	7	
n	ade arg	7	
n	met_2 ade arg	2	
	Wild-type green segregants		
n	arg	2	
n	met_2	3	

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Table 5. *Classification of 591 independent segregants from heterozygous diploids C and D in* Aspergillus amstelodami

Diploid C: $\frac{wh}{WH}$ $\frac{pab}{PAB}$ $\frac{leu}{LEU}$ $\frac{LI}{li}$ $\frac{MET_1}{met_1}$ $\frac{LYS}{lys}$

Diploid D: $\frac{pb}{PB}$ $\frac{ade}{ADE}$ $\frac{arg}{ARG}$ $\frac{LI}{li}$ $\frac{MET_1}{met_1}$ $\frac{LYS}{lys}$

Ploidy	Nutritional requirements	No.	Segregation of unlinked loci
	White segregants		
2n	Wild type	120	wh $\frac{leu}{LEU}$ 56/47; $\frac{LYS}{lys}$ 51/52
n	pab	28	
n	pab leu	23	
n	pab lys	19	
n	pab leu lys	33	
n	lys	2	
	Lime-green segregants		
2n	Wild type	1	li $\frac{LEU}{leu}$ 25/34
2n	lys	35	
n	met_1 lys	23	
n	met_1 lys leu	34	
n	lys	2	
	Wild-type green segregants		
2n	met_1	4	WHLI $\frac{LEU}{leu}$ 23/20
n	met_1	23	
n	met_1 leu	20	

Ploidy	Nutritional requirements	No.	Segregation of unlinked loci
	Pale-blue segregants		
2n	Wild type	39	pb $\frac{arg}{ARG}$ 67/39
n	ade	17	
n	ade arg	38	
	Lime-green segregants		
2n	Wild type	1	li $\frac{ARG}{arg}$ 27/9
2n	lys	7	
n	met_1 lys	27	
n	met_1 lys arg	9	
	Lime-blue segregants		
n	lys ade	22	
n	lys ade arg	29	
	Wild-type green segregants		
n	met_1	18	PBLI $\frac{arg}{ARG}$ 17/18
n	met_1 arg	17	

information about the number of linkage groups in this species is provided from meiotic analysis in these experiments.

Additional information concerning linkage and the order of genes was obtained from mitotic analysis (Tables 4 and 5). The *wh–pab* linkage relationship was indicated from the 183 white, *pab*-requiring haploids from diploids A and C. The absence of *pab*-requiring white diploid segregants indicated that the *pab* locus was either on the other arm of the chromosome or closely linked to the centromere on the same arm. The absence of *pab*-requiring brown segregants from diploid A reflected the epistasis of *wh* over *br*. Proof that *wh* and *br* were not in the same linkage group was shown by the isolation of ten olive-green haploids. The *pb–ade* linkage group was indicated from the *pb*, light-brown and lime-blue segregants of diploids B and D. Four of the 91 *pb* diploid segregants were *ade*-requiring, indicating that *pb* was linked distally to *ade*. The *wh–pb–met*$_1$ linkage group first detected in meiotic analysis was readily confirmed on analysis of the segregants of diploids C and D. Four diploid and 78 haploid wild-type green segregants were all *met*$_1$-requiring. Analysis of the *li* segregants indicated that *lys* was distally linked to *li*.

Classification of the mitotic segregants showed several cases of unusual segregation. Four *br*, *met*$_2$-requiring diploid segregants from diploids A and B were either non-disjunctional diploids or resulted from simultaneous crossovers on different chromosomes. Two prototrophic diploid *li* segregants from diploids C and D appear to represent coincident crossovers on the same arm. Six cases of crossing over followed by haploidization in the same nucleus were detected. Four *wh*, *pab*-independent haploid segregants from diploids A and C and 2 *li*, *met*$_1$-independent haploids from diploid C probably arose in this way.

The combined results of meiotic and mitotic analyses show that *wh*, *pb* and *met*$_1$ are closely linked in a cluster which is distal to and 50 or more units from two other markers, *pab* and *ade*. These five markers comprise linkage group I. *Li*, and *lys* 24·3 units distal to it, comprise linkage group II.

4. DISCUSSION

The results obtained in mapping experiments with *Aspergillus amstelodami*, first through meiotic analysis and then confirmed and extended through mitotic analysis, agree well with the principles established by Pontecorvo and his co-workers with *A. nidulans*.

The analysis of mitotic segregants has been shown to be a highly efficient means of establishing linkage groups (Pontecorvo & Käfer, 1958; Käfer, 1958; McCully & Forbes, 1965; Roper, 1966). Crossing over in a chromosome heterozygous for given markers will result in homozygosity for all markers distal to the point of crossing over, if they are crossed in repulsion to the selector marker. By the proceess of mitotic haploidization whole chromosomes reassort at random during mitosis, producing nuclei with recombined complements. Thus markers which are located on the same chromosome, when crossed in repulsion, will rarely appear simultaneously in a haploid segregant and should always appear simultaneously when

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crossed in coupling. These observations and their exceptions were confirmed here. The instances of coincident crossovers on the same or different chromosomes or crossing over preceding haploidization, 1·2 % and 1·0 % respectively, were similar in frequency to those reported by Pontecorvo & Käfer (1958), 1·0 % and 0·62 %. The data are quite unlike those reported by Hastie (1964) in which he reported frequencies of crossing over preceding haploidization as high as 16·7 % for *Verticillium albo-atrum*.

The results of meiotic recombination show that for the *wh–pab*, *wh–leu* and *br–met*$_2$ pairs of markers factors other than chance were acting in some experiments to produce a deviation from 1:1 among ratios of wild type to mutant alleles. In other experiments the recombination frequencies were in agreement with ratios expected from free recombinaion. The observed deviation could be due to factors which affect the survival of the mutant allele, as has been demonstrated by Coy & Tuveson (1964).

The lack of epistatasis among the *pb*, *br* and *li* markers of *A. amstelodami* differs from the interaction of colour markers in *A. fumigatus*, where Strömnaes & Garber (1964) working with 15 colour markers noted that segregants with both colour markers were not detected. They suggested that epistasis might be responsible for this observation. In *A. nidulans* Käfer (1958) reported that *Bw*, brown was dominant and epistatic to *w*, white, and *y*, yellow.

SUMMARY

Thirteen markers in *Aspergillus amstelodami* were mapped through meiotic and mitotic recombination, resulting in good correlation of the two linkage groups established by each method. Mapping via mitotic analysis proved the more efficient of the methods because of the long distances between some of the markers established in this organism.

Visual selection of haploid segregants from mitotic recombination was aided by the ability to recover conidia which differed in colour from the haploid parents. These contained pairs of conidial colour markers which were not epistatic to each other.

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