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Two linkage groups in *Coprinus lagopus*

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INTRODUCTION

During three years' work on *Coprinus lagopus* a variety of mutants have been isolated, characterized and tested for linkage. The chief objective was to find mutants linked with the two independent factors A and B controlling the mating system which could be used in studies of their genetic structure. This paper is a summary of our progress.

In recent years *Coprinus* has been the subject of studies of mutation (Fries, 1948; Mittwoch, 1951; Anderson, 1959), cytoplasmic inheritance (Day, 1959), heterokaryosis (Swiezynski & Day, 1960*a* and *b*), the structure of the A locus (Day, 1960*a*, *b*) and suppressor action (Lewis, 1961).

MATERIAL

C. lagopus belongs to the family Agaricaceae and is a member of a large and complex genus. There is some confusion between it and the related species *C. cinereus* (*C. fimetarius*). The material used by us conforms closely to Buller's (1924) description of *C. lagopus* and so this name will be used. According to Orton (1957), however, the name *C. cinereus* is correct for such forms isolated from dung.

Seven stocks each derived from single basidiospores have been used. Two, 54 (A_1B_2) and 68 (A_2B_1), were isolated by Prof. D. Lewis in 1952 from a fruit-body found growing on an old chaff heap at Barwick Ford, Herts. The third, 1796 (A_3B_3), was isolated by Lewis in 1955 from a dung-heap at Barlaston, Staffs. The remaining four stocks, H1 (A_5B_5), H2 (A_6B_5), H6 (A_5B_6) and H9 (A_6B_6), were isolated in 1957 from one fruit-body collected on a dung-heap at Bayford, Herts. All crosses between stocks from different fruit-bodies are fertile.

METHODS

Media and culture methods have already been described by Day (1959, 1960*a*). For random spore analysis basidiospores were spread on minimal, or complete medium, containing 0·01% furfural (Emerson, 1954). Incubation was at 28° C.

Mutants were produced by irradiating suspensions of oidia with ultra-violet light to kill 95–99%. The method of filtration enrichment (Catcheside, 1954; Woodward *et al.*, 1954) was used to increase the proportion of auxotrophs.

Mutants with similar requirements were tested for complementation by crossing compatible monokaryotic strains and testing the dikaryon for prototrophy.

RESULTS

The numbers and kinds of mutants isolated are shown in Table 1.

Table 1. *Mutants isolated from H9* (A_6B_6) *wild type*

Number of complementation groups shown in brackets

Requirement or phenotype	No. of mutants
Adenine, hypoxanthine	13 (6)
Adenine (not tested further)	54
Methionine	33 (at least 5)
Choline	1
Choline, dimethyl-aminoethanol	3 (1)
Nicotinic acid	4
Nicotinic acid, anthranilic acid	1
Para-aminobenzoic acid	3 (1)
Arginine	1
Uracil	1
Unknown	2
Cycloheximide resistant	1
Penicillin and oligomycin sensitive	2
Morphological mutants	4 (4)

The following mutants were also isolated from 1796 (A_3B_3) wild type: 12 adenine (5), 3 methionine (3), 1 nicotinic acid.

Linkage tests

Sixty-six independently produced mutants were tested for linkage with the *A* or *B* loci. The bulk of the mutants were induced in H9 (A_6B_6) and these were crossed with H1 (A_5B_5). Six loci are within 20 units of the *A* locus and three are within a similar distance of the *B* locus. Table 2 summarizes the segregations and tests for linkage with *A* or *B* for these loci.

A *linked markers*

The three point crosses to determine the order of the markers in the *A* linkage group are summarized in Table 3.

The four independent mutants requiring *p*-aminobenzoic acid which are linked with the *A* locus are non-complementary in all pairwise combinations. Non-complementary dikaryons between the mutants fruited readily when inoculated to dung supplemented with *p*-aminobenzoic acid. Basidiospores from these

Table 2. *Segregation and recombination of markers linked with* A *and* B

All mutants were induced in stock H9 ($A_6 B_6$) except 815 (*me*-2) and 838 (*ad*-5) which were induced in stock 1796 ($A_3 B_3$). Mutants M6 and M2 were isolated by Mr D. Morgan at University College, London.

Mutant	Isolate number	Total progeny	No. of mutants	% recombination with *A*		s.e.
ad-8	1904	401	254†	1·98		0·74
	2242	320	128†	1·27		0·63
	3231	64	35	0·0	(2·43)*	—
	M6	128	75	0·0	(3·22)*	—
me-2	815	877	176†	6·8		2·5
me-5	1905	118	60	34·7		4·4
me-6	2214	96	41	12·6		3·4
paba-1	2519	1184	529†	0·54		0·21
	3445	62	42†	1·2		0·88
	3463	64	38	0·0	(4·60)*	—
	M2	101	54	0·0	(0·45)*	—
arg-1	2246	95	39	6·4		2·5
				with *B*		
ad-5	838	364	236†	7·1		1·6
chol-1	2212	240	135	9·9		2·3
	3245	153	81	15·1		2·9
	3409	64	29	2·2		1·5
chol-2	2222	224	98	11·4		2·7

* 5% upper fiducial limit.
† Differs from a 1:1 ratio at 0·01 probability level.

Table 3. *Estimates of recombination between* A *linked markers based on prototroph frequencies*

The cross *ad*-8 × *arg*-1 was analysed as tetrads, all the progeny of the cross *ad*-8 × *me*-5 were characterized.

Cross a		Cross b	Progeny	Prototrophs	Prototroph *A* mating types a	Prototroph *A* mating types b	% recombination
paba-1 (2519)	×	*ad*-8 (2242)	ca. $1·9 \times 10^5$	2685	721	1774	1·35
ad-8 (2242)	×	*arg*-1	183 (tetrads)	8	0	8	2·18
	×	*me*-2	320	84	2	76	52·5*
	×	*me*-6	256	14	1	13	10·9
	×	*me*-5	96	11	0	11	27·0
arg-1	×	*me*-2	430	37	7	30	17·2
	×	*me*-6	384	43	4	38	22·4
	×	*me*-5	1248	257	13	81	41·18
me-2	×	*me*-5	512	56	4	52	21·8
me-6	×	*me*-5	128	11	6	5	17·2

* Frequency of prototrophs approaches 25% due to segregation of *me*-2 suppressor from *me*-2$^+$ parent.

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fruit-bodies gave rise to low frequencies of prototrophs. The prototroph frequencies are shown in Table 4 together with the mating types of randomly selected prototrophs. Approximately 10^6 viable spores were analysed in each cross.

Table 4. *Prototroph frequencies and mating types from crosses between mutants at the* paba-1 *locus*

Cross			Prototrophs per 10^5 viable spores	No. tested	Prototroph mating types		
A_5		A_6			A_5	A_6	$A_?$
2519	×	M2	4·3 ± 0·8	49	4	27	18
	×	3463	9·9 ± 1·4	32	7	12	13
	×	3445	24·4 ± 2·5	32	7	23	2
	×	2519	0·0	—	—	—	—
3445	×	2519	35·9 ± 3·8	22	20	2	0
	×	M2	31·8 ± 6·4	50	48	2	0
	×	3463	29·1 ± 9·8	15	15	0	0
	×	3445	0·0	—	—	—	—
M2	×	2519	0·0	—	—	—	—
	×	3463	0·0	—	—	—	—
	×	3445	18·6 ± 4·6	19	1	15	3
	×	M2	0·0	—	—	—	—

The A mating types of the prototrophs are chiefly those which would be expected if the mutant order were:

—3445——3463——*M*2——2519——*A*—

The same order is indicated by the prototroph frequencies (see Fig. 1). The prototrophs listed as $A_?$ are compatible with both A_5 and A_6 testers. They are the subject of further investigation.

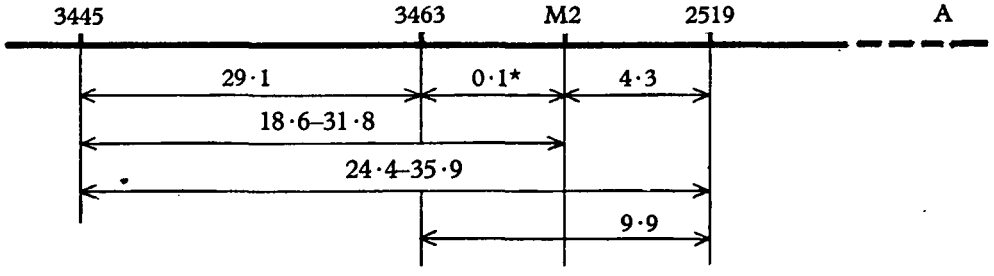

Fig. 1. Map of the *paba*-1 locus showing prototroph frequencies per 10^5 viable basidiospores.
* 5% upper fiducial limit.

The failure of the four *paba* mutants to complement each other and the short length of chromosome which they occupy (*ca.* 0·056 map unit) suggest that they are all mutants at different sites within the same functional unit or cistron (Benzer, 1955) *paba*-1.

All attempts to fruit crosses between the four non-complementary adenine-requiring mutants at the *ad*-8 locus failed.

B *linked markers*

Three point crosses among the *B* linked markers are summarized in Table 5.

Table 5. *Estimates of recombination between* B *linked markers based on prototroph frequencies*

Cross a	Cross b	Progeny	Prototrophs	Prototroph *B* mating types a	Prototroph *B* mating types b	% recombination
chol-1 (2212) ×	*chol*-2 (2222)	256	8	8	0	6·2
,, ,,	,,	52 tetrads	10	10	0	9·6
ad-5 ×	*chol*-1 (2212)	454	67	19	48	18·9
			19*	17*	2*	
,, ,,	,,	81 tetrads	2	0	2	1·1
ad-5 ×	*chol*-2	$2{\cdot}7 \times 10^6$	377	190	170	0·028

* Double mutants.

The first two crosses in Table 5 have established the gene orders:

—*B*——*chol*-2——*chol*-1— and —*B*——*ad*-5——*chol*-1—

In the cross *ad*-5 × *chol*-1 the difference between random spores and tetrads is most likely due to the fact that the parent stocks were different in each case. The random spores came from a cross between the original mutants, the tetrads came from a cross between stocks derived from the mutants.

The cross between *ad*-5 and *chol*-2 was made three times and the data from these crosses has been bulked in Table 5. The frequency of prototrophs varied between $2{\cdot}9 \times 10^{-4}$ and 15×10^{-4} in different fruit-bodies from the same dikaryon fruited at different times and from $1{\cdot}7 \times 10^{-4}$ to $15{\cdot}0 \times 10^{-4}$ in different crosses. The *B* mating types of the prototrophs were determined to see if they could be used to place the two markers *ad*-5 and *chol*-2 in a consistent order. Both parental *B* tester stocks were used so that prototrophic dikaryons, common *A* heterokaryons and disomics heterozygous for *B*, which would all be compatible with both testers, could be rejected. Three prototrophs were also crossed to wild type to see if they were disomic or diploid, but homozygous or hemizygous for *B* and thus not detectable by the mating reaction. No auxotrophs were recovered among the progenies of these crosses in tests of at least 100 spores.

The mating types of the prototrophs did not reveal a consistent order for the three markers *B*, *ad*-5 and *chol*-2. We are indebted to our colleague Dr J. R. S. Fincham for suggesting the following explanation. The two mutants were induced in different wild-type stocks; *ad*-5 in 1796 (A_3B_3) and *chol*-2 in H9 (A_5B_5). If the chromosome region carrying the markers *ad*-5 and *chol*-2, but not the centromere, were inverted in one of the mutants, it would follow that, if the two segments paired, a single crossover between the markers would yield duplication-deficiency chromatids which would almost certainly be inviable. A viable prototrophic combination would only result from a two-strand double exchange within

the inversion where one of the crossovers was between the markers. The position of the other crossover, to the right or left, would then determine the *B* mating type of the prototroph. Thus the proportion of one *B* mating type to the other among the prototrophs would depend on the distribution of crossovers within the inversion. This would vary from one cross to another as a result of differences in chiasma distribution consequent on differences in pairing in the mutually inverted segments. The explanation also accounts for the apparent very close linkage between *ad*-5 and *chol*-2.

The three independent mutants at the *chol*-1 locus are non-complementary but dikaryons between different *chol*-1 mutants readily produce fruit-bodies when inoculated to dung. The frequencies of the prototrophs produced and their *B*

Table 6. *Prototroph frequencies and mating types from crosses between mutants at the* chol-1 *locus*

Cross			Prototrophs per 10^5 viable spores	No. tested	Prototroph mating types	
B_5		B_6			B_5	B_6
2212	×	3409	$34{\cdot}5 \pm 5{\cdot}0$	61	41	20
2212	×	3245	$13{\cdot}8 \pm 2{\cdot}1$	56	36	20
3245	×	3409	$41{\cdot}6 \pm 4{\cdot}1$	123	76	47

mating types are shown in Table 6. In each progeny there is an excess of B_5 prototrophs suggesting the order

—*B*——3409——3245——2212—

However the recombination frequencies suggest the order

—*B*——3409——2212——3245—

The most likely reason for this discrepancy is that the recombination frequencies varied in the different crosses because they were subject to differences in genetic background between the crosses.

Although the three *chol*-1 mutants appear to be mutant sites within the same functional unit or cistron their recombination values with the *B* locus vary from 2·2 (3409) to 15·1 (3245) in crosses of the original mutants, induced in H9, with the wild-type stock H1 (Table 2). These crosses and the cross between *chol*-2 (2222) and H1 were repeated using the same stocks as before. At the same time the four mutants were crossed to an A_5B_5 stock which had been produced by backcrossing H1 to H9 six times. We are indebted to Mr D. Morgan for giving us a culture of this backcrossed stock. The results are shown in Table 7. After backcrossing, the recombination frequencies were increased from 2 to 6 times.

Similar effects of backcrossing have been noted by Stadler (1956) and Towe (1958) on the second division segregation frequency of the marker *asco* in *Neurospora crassa*.

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Table 7. *The effect of backcrossing on linkage estimation*

	Total progeny	Auxotrophs	% recombination with *B*	S.E.
chol-1				
2212 × H1	145	77	10·0	3·3
× BC*	108	57	21·3	3·9
3245 × H1	87	53	10·1	3·6
× BC	177	92	27·4	3·8
3409 × H1	106	54	5·1	2·2
× BC	183	105	36·5	4·3
chol-2				
2222 × H1	128	60	11·4	3·6
× BC	130	67	20·0	3·6

* BC = H1 (A_5B_5) backcrossed to H9 (A_6B_6) six times.

Mapping the centromeres

Three crosses were analysed as tetrads with the object of mapping the centromeres of the *A* and *B* chromosomes using the method suggested by Whitehouse (1957) for unordered tetrads. This method makes use of the fact that the frequency of tetratype tetrads for two unlinked loci depends on the frequency of second division segregation of the two loci.

If the data include three loci, two of which are linked, then, provided one of the linked loci is close to the centromere, second division segregation frequencies can be calculated. In fact each cross included three linked markers and one unlinked marker. Thus the second division segregation frequencies could be calculated in three ways according to which pair of linked loci were selected. The data on which these calculations are based are given in Table 8.

Table 8. *Numbers of tetratype tetrads for all possible marker pairs obtained in three different crosses*

	Markers a	b	c	d	No. of tetrads	Marker pairs ab	ac	ad	bc	bd	cd
i.	$\frac{A_5}{A_6}$	+	*arg*-1	$\frac{B_6}{B_5}$	180	9	14	72	5	68	66
ii.	$\frac{A_6}{A_2}$	$\frac{B_5}{B_6}$	+	*chol*-1 / +	81	11	6	8	7	9	2
iii.	$\frac{A_6}{A_5}$	$\frac{B_5}{B_6}$	*chol*-2	*chol*-1 / +	52	13	13	22	6	16	10

If one of the linked markers is close to the centromere, then it is valid to assume that the tetratype frequency for the pair of linked markers is equal to the sum or difference of their second division segregation frequencies. The relative positions of the linked markers and the centromere are determined by trial and error by finding which of three equations gives a real solution.

Among 183 tetrads from cross (i) there were 3 which resulted from two-strand double exchanges between *A* and *arg*-1. These were the only double exchanges and they have been excluded from the calculations. Real solutions to the equations given by Whitehouse were only obtained for the following order of markers on the *A* chromosome:

—*A*——*ad*-8——centromere——*arg*-1—

The tetratype frequencies from cross (ii) did not give a consistent position for the centromere in relation to the *ad*-5 locus. Real solutions to the equations involving the pair of linked markers *B*-*ad*-5 are only obtained if the centromere is assumed to be between them. The same is also true of the equations for the pair *ad*-5–*chol*-1. It was earlier suggested that the *ad*-5 locus may be present in an inversion to explain the low frequency of recombination between it and *chol*-2 and the anomalous recombination with the adjacent *B* marker. Accordingly the data from cross (ii) have been treated assuming that no recombination between *ad*-5 and the centromere took place.

The tetratype frequencies from cross (iii) indicate the marker order

—*B*——centromere——*chol*-2——*chol*-1—

The frequency of recombination between *B* and *chol*-1 in cross (iii) is approximately three times that in cross (ii) (15·4 % compared with 5·5 %), showing that crossing-over is reduced in this region of the *B* chromosome when the *ad*-5 marker is present, which would be expected if it were present in an inversion.

The second division segregation frequencies for *A*, *B* and the linked markers are given in Table 9.

Table 9. *Percentages of second division segregation calculated from tetratype data in Table 8*

Cross	Trio of markers			Percentage second division segregation						
				A	*ad*-8	*arg*-1	*B*	*chol*-2	*chol*-1	*ad*-5
i.	*A*	*ad*-8	*B*	6·6	1·6	—	37·0	—	—	—
	A	*arg*-1	*B*	7·6	—	0·2	36·6	—	—	—
	ad-8	*arg*-1	*B*	—	2·6	0·16	36·6	—	—	—
ii.	*B*	*chol*-1	*A*	6·7	—	—	7·6	—	3·5	—
	B	*ad*-5	*A*	7·4	—	—	8·6	—	—	0*
	ad-5	*chol*-1	*A*	7·4	—	—	—	—	2·5	0*
iii.	*B*	*chol*-1	*A*	23·7	—	—	1·9	—	28·8	—
	B	*chol*-2	*A*	21·0	—	—	5·8	5·8	—	—
	chol-2	*chol*-1	*A*	6·6	—	—	—	20·4	39·6	—

* See text for explanation.

DISCUSSION

The groups of markers linked with the *A* and *B* loci have been mapped. The only inconsistencies in the present data are the relative positions of *ad*-5, *chol*-2 and the centromere and the order of two of the sites within the *chol*-1 locus on the *B* chromosome. Maps of the two linkage groups are shown in Fig. 2.

The difficulty in determining the relative positions of *ad*-5 and *chol*-2 is most likely due to the fact that they were mutants induced in different wild-type stocks. Frost (1961) has analysed the consequences of using different stocks of *Neurospora crassa* and has shown how different genetic backgrounds may have profound effects on recombination frequencies within a given interval. We have now adopted H9 as a standard stock for mutation experiments. When mutants derived from H9 are intercrossed with compatible stocks produced by backcrossing to H9 difficulties due to stock heterogeneity are minimized.

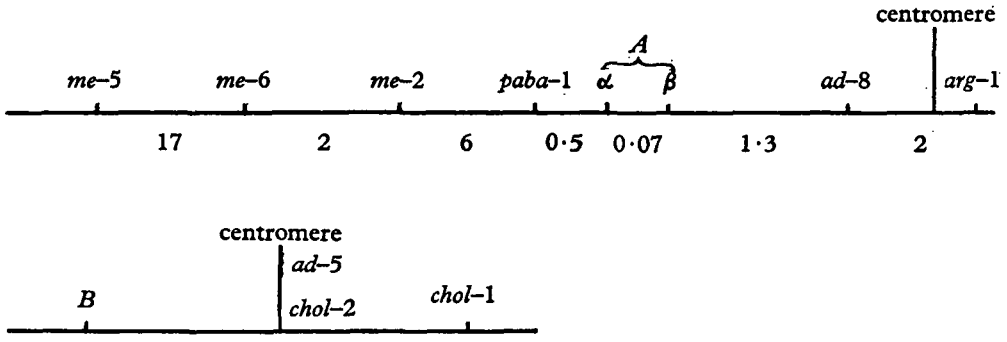

Fig. 2. Maps to show the linear order of the *A* and *B* linked markers.

Some of the variation in recombination between repeats of the same cross (compare Tables 2 and 7) could be attributed to our use of sterilized manure instead of a synthetic fruiting medium. While our stocks of *Coprinus* will fruit on synthetic fruiting medium (Madelin, 1956) they do so more slowly and far less readily than on manure.

The spectrum of mutants obtained in *Coprinus* is broadly similar to those found in other fungi. The two mutants described which were sensitive to penicillin and oligomycin, both of which have no effect on the wild type, are of some interest. They may show a method for studying the mode of action of antibiotics from physiological comparisons between resistant and derived sensitive forms.

The pseudoallelic nature of the *paba*-1 and *chol*-1 mutants is a characteristic feature of most organisms in which the fine structure of individual loci has been studied. All the more interest therefore centres on the basic problem of the fine structure of the *A* and *B* loci for which this work has been a necessary preliminary study.

SUMMARY

Sixty-six independently produced markers have been tested for linkage with the *A* and *B* mating-type factors. Six loci linked with *A* and three linked with *B* were found. Maps of the two linkage groups which include the centromeres were constructed.

Non-identical alleles at two loci, *paba*-1 and *chol*-1, were found and crosses between different mutants were used to map the positions of the mutant sites within each locus.

It is a pleasure to acknowledge the helpful discussions we have had with our colleagues Dr Fincham, Dr Holliday and Mr Gilbert and the valuable technical assistance of Mrs Margaret Lamdin.

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