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Four new linkage groups in *Coprinus lagopus*

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1. INTRODUCTION

Coprinus lagopus has in recent years been the chosen material in studies of a diversity of genetic problems. Moreover, as one of the few Basidiomycetes which is the subject of wide-ranging research, its importance is likely to increase.

Day & Anderson (1961) established the only linkage maps available up to now, but restricted their analysis to mutants showing linkage with the independent incompatibility factors A and B. The present paper contains data which considerably extend our knowledge of linkage maps in this fungus.

2. MATERIALS AND METHODS

The taxonomic position of *C. lagopus* is briefly considered by Day & Anderson (1961). The origins of the various wild strains presently in use are also detailed in that paper. Most of the mutants used in the linkage studies now to be reported were originally isolated by D. H. Morgan. For the most part they were found to be allelic with mutants previously isolated by P. R. Day and G. E. Anderson, whose stocks were also used in the course of this work. Day's H-series wild-types were also used, though more extensive use was made of a series of backcrossed strains prepared by D. H. Morgan. These had been backcrossed six times to the H9 wild-type strain, and have the stock numbers; BC6/66, BC6/65, BC6/56, and BC6/55 (mating types A_6B_6, A_6B_5, A_5B_6, and A_5B_5 respectively).

Media and culture methods followed closely those described by Day (1959) and Lewis (1961). Glaxo 'Crystapen' benzylpenicillin was routinely added to all media at a final concentration of 200 μg./ml. Basidiospores were germinated on complete medium to which dung extract had been added (*ca.* 20% v/v). The extract was prepared by boiling or autoclaving 200 gm. of dried horse dung in about 1 l. of water. The resultant mess was filtered through cheesecloth and then concentrated to about 400 ml. by boiling. Sporelings were individually transferred from the germination medium to normal complete medium after 18 to 24 hours' growth at 37°C. They

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were grown up to about 2 cm. diameter and then tested for mating type and/or nutritional requirements. Selective methods of progeny classification were avoided; the progeny of all crosses were completely characterized whenever possible.

The vast majority of tetrads were isolated from dry preparations of gill tissue on glass slides as described previously (Moore, 1966*a*).

3, RESULTS

(i) *Tetrad analysis*

Seven crosses were analysed entirely by tetrads (a total of 729 tetrads) with two aims in view: firstly the establishment of new centromere markers to aid in linkage screening, and secondly to map the *B* linkage group. Because of the suggested occurrence of chromosomal aberrations in certain stocks carrying members of this group (Day & Anderson, 1961) it was decided to use tetrads to map it so that any aberration which became apparent could be the more easily traced. Also, the use of strains quoted by Day and Anderson as possible carriers of the supposed paracentric inversion was avoided. No evidence was found of chromosomal abnormalities in the strains used.

The tetrad distributions which were obtained are summarized in Table 1. Some of the distributions displayed there are summed from two or more individual

Table 1. *Summarized tetrad distributions*

	B	*ad*-5	*chol*-1	*nic*-4	*paba*-2	*pdx*
A	$\frac{239:237}{253}$	$\frac{39:35}{38}$	$\frac{46:63}{97}$	$\frac{92:99}{145}$	$\frac{25:24}{26}$	$\frac{20:30}{50}$
B	—	$\frac{77:2}{33}$	$\frac{102:7}{97}$	$\frac{136:132}{68}$	$\frac{29:21}{25}$	$\frac{25:29}{46}$
chol-1	—	—	—	—	—	$\frac{45:34}{21}$

Individual distributions are presented as $\frac{\text{PD:NPD}}{\text{T}}$.

Terminology follows Perkins (1953); PD = parental ditype, NPD = non-parental ditype, and T = tetratype.

With the exception of the known linked gene pairs (*B*/*ad*-5 and *B*/*chol*-1) there is no significant deviation from 1:1 in any PD:NPD ratio.

crosses. This was done only after the individual results were determined to be homogeneous with one another.

Coprinus tetrads are unordered; the tetrad distributions were therefore considered in trios (i.e. cycles of three genes) and second division segregation frequencies calculated using the method detailed by Whitehouse (1957). Where appropriate, standard sampling errors were determined using the method given by Haldane in Whitehouse (1957). The second division segregation frequencies of the

Table 2. *Second division segregation frequencies calculated from the data of Table 1*

Trio	*A*	*B*	*ad*-5	*chol*-1	*nic*-4	*paba*-2	*pdx*
**A/B/ad*-5	25·2	15·4	14·1	—	—	—	—
**A/B/chol*-1	27·3	12·5	—	36·2†	—	—	—
A/B/nic-4	33·8	1·8	—	—	18·9	—	—
A/B/paba-2	21·4	19·5	—	—	—	19·5	—
A/B/pdx	25·2	15·3	—	—	—	—	39·8
A/chol-1/*pdx*	44·8	—	—	6·9‡	—	—	15·8
**B/chol*-1/*pdx*	—	39·8	—	7·3‡	—	—	15·4

* These trios involve a linked gene pair.

† Values of 33·8% and 40·0% obtained from two different crosses.

‡ These frequencies obtained from the same cross and have a standard sampling error of approximately ± 12%. The second division segregation frequencies of *pdx* and *A* calculated from the same trios have sampling errors of 2·8% and 8·1% respectively.

seven genes investigated in these tests are shown in Table 2. Comparatively large variations in the estimates of second division segregation frequencies are evident in this table. Such estimates seem, in general, to be imprecise (see, for example, the different values quoted for the mating type gene in *Saccharomyces* by Hawthorne & Mortimer, 1960) unless very large numbers of tetrads are analysed, and this requires a very considerable technical effort. Nevertheless sufficient data are available to map the *B* group and to establish three new centromeres each satisfactorily marked with a vitamin deficiency mutation thus: Group III, characterized by the centromere marker *pdx* (pyridoxal-less) strain stock number M47; Group IV, with *nic*-4 (nicotinic acid-less) as centromere marker, stock number M48; and Group VI, centromere marked with *paba*-2 (*p*-aminobenzoic acid-less), strain M4. Random spore analysis showed that these three genes were independent of one another, tetrad analysis (Table 1) having already established their independence of the two known linkage groups.

(ii) *Linkage tests*

Mutants existing in stock were tested for linkage by random spore analysis with the centromere markers previously established. The results are presented in Table 3. The groups will now be considered in turn; the conclusions are summarized in Fig. 1, below.

(*a*) *Group I* (*the* A *linkage group*)

No new markers were found to be associated with this group. However, the linkage of *me*-5 with the *A* mating type factor was confirmed by the discovery of two new alleles of the *me*-5 locus. The figure of 29·6% recombination (from 359 progeny) between *me*-5 and *A* obtained in the present investigation compares well with the 34·7% (from 118 progeny) recorded by Day & Anderson (1961). An

Table 3. *Summarized recombination data*

Group	Interval	Total progeny	Recombinants	% Recombination	S.E.
I	*A—me*-5	359*	106	29·6	2·4
II	*B—ad*-5	225	41	18·2	2·4
II	*B—chol*-1	418*	126	30·2	2·2
III	*pdx—ad*-2	1443*	219	15·2	0·94
III	*pdx—me*-1	1164*	50	4·3	0·59
III	*pdx—nic*-1	797*	102	12·8	1·2
III	*ad*-2—*me*-1	410	87	21·2	2·02
III	*ad*-2—*nic*-1	421	10	2·4†	0·76
III	*me*-1—*su*-4–*me*-1	966	14	1·5	0·37
III	*me*-1—*su*-3–*me*-1	315	100	31·7	2·6
IV	*ad*-1—*nic*-4	640*	111	17·4	1·5
IV	*ad*-1—*me*-9	448	144	32·2	2·2
IV	*me*-9—*nic*-4	571*	90	15·7	1·5
V	*me*-8—*ad*-6	377*	128	34·0	2·4

* These results summed from two or more crosses.

† This value is derived from five prototrophs recovered from the 421 progeny analysed of the cross *nic*-1 × *ad*-2. A value of 7·5% recombination for this interval, however, can be derived from the three-point cross detailed in Table 5 (Cross 2). Because of the uncertainty attached to this three-point cross however (see text), the figure given in the present table is used in the construction of the Group III map.

Strains used (original mutant stock numbers): *ad*-1, G1912; *ad*-2, M39 and M58; *ad*-6, P809; *chol*-1, G2212 and G3245; *me*-1, P827; *me*-5, M23 and M37; *me*-8, G2301; *me*-9, M61; *nic*-1 C758 (as strain PR701); *nic*-4, M48; *pdx*, M47; *su*-3–*me*-1, Ms29; *su*-4–*me*-1, Ms25.

inconsistency between the two investigations is evident though in the case of the second division segregation frequency of the *A* locus itself. Calculations from seven crosses analysed by tetrads in the present investigation regularly indicate this frequency to be in excess of 20% (average *ca.* 28%), while Day and Anderson give about 7% (two crosses) and 22% (one cross). If the larger value is introduced into the published map, additivity of recombination percentages is greatly improved. It is thus likely that the *A* locus is, at least in many crosses, about four times as distant from its centromere as hitherto supposed.

The three alleles of *me*-5 were found to give fertile intercrosses, and the sites of the mutations can thus be ordered within the locus. The two new alleles, M23 and M37, were each crossed with two strains, DR1 and DR2, derived from the original *me*-5 allele G1905. DR1 was isolated from a cross of *me*-5 × *ad*-8; DR2 from a further cross of DR1 × *paba*-1. The four dikaryons were all auxotrophic. They fruited, producing both aborted and fertile sporophores; sporophore tissue was also auxotrophic. Prototrophs were scored after 60 hours' incubation of spread spores on minimal medium, and a sample was isolated for mating type testing. Full details are shown in Table 4. The prototroph frequencies together with the *A* mating type specificities of the prototrophs indicate the order:

M37—(11·2)—G1905—(8·0)—M23——*A*—

Table 4. *Results of crosses between alleles of the* me–5 *locus*

Cross	%V*	Viable spores plated $\times 10^{-6}$	Prototrophs	Prototrophs as fraction of viable spores $\times 10^{5}$	*A* mating type A_5	A_6
MR37.03 (A_5) × DR1 (A_6)	89	10·6	1442	13·0	21	7
MR37.03 (A_5) × DR2 (A_6)	8	2·4	12	0·5	10	2
MR23.03 (A_5) × DR1 (A_6)	94	7·8	626	8·0	8	20
MR23.03 (A_5) × DR2 (A_6)	34	2·6	208	8·0	6	20

* %V = % viability.

the figures in parentheses showing the number of prototrophs per 10^5 viable spores. This is the first demonstration of intragenic recombination at a methionine locus in *C. lagopus.*

(*b*) *Group II* (*the* B *linkage group*)

In the calculation of second division segregation frequencies of Group II members (Tables 1 and 2) real solutions to the Whitehouse equations were only obtained on the basis of the orders *B—centromere—ad*-5 and *B—centromere—chol*-1. The second division segregation frequencies obtained are quite consistent with these orders, as are the data from random spore analyses shown in Table 3. There is one other known member of Group II, namely *chol*-2. According to Day & Anderson (1961) this locus shows only 0·03% recombination with *ad*-5, though they suggest this low value could be due to the effects of an inversion. Six attempts to fruit dikaryons made between *ad*-5 (the M36 allele which is not suspected of carrying an inversion) and various strains carrying *chol*-2 were unsuccessful so this point could not be examined.

(*c*) *Group III*

Small samples of tetrads were analysed from two crosses to order members of the group with respect to the centromere. The *B* mating type specificity of the progeny was determined in each case so that this unlinked marker could be used in the analysis.

Sixteen tetrads were analysed from the cross MR58.01 (B_5; *ad*-2) × M47 (B_6; *pdx*). Tetrad types obtained for *pdx* and *ad*-2 were 10PD : 0 NPD : 6 T; for *pdx* and *B*, 9 PD : 4 NPD : 3 T; and for *ad*-2 and *B*, 5 PD : 3 NPD : 8 T. Real solutions to the

Whitehouse equations could only be obtained on the basis of the order *centromere —pdx—ad*-2.

Twenty-three tetrads were analysed from the cross PR701 (B_2; *nic*-1) × PR2304 (B_1; *me*-1). Tetrad distributions obtained were: for *me*-1 and *nic*-1, 14 PD: 2 NPD: 7 T; for *nic*-1 and *B*, 6 PD: 5 NPD: 12 T; and for *me*-1 and *B*, 11 PD: 2 NPD: 7 T. Calculation again indicates that both genes are on the same arm, *me*-1 being proximal to the centromere.

Two simple three-point crosses analysed as random spores are detailed in Table 5. Cross No. 1 clearly shows that the order is; *me*-1—*pdx*—*ad*-2. In the case of cross 2, however, the two double crossover classes cannot be reliably identified, since the

Table 5. *Data from three-point crosses between members of Group III*

Cross	Genotypes			Frequency
1. PR 2301 (*me*-1)	*pdx*	*ad*-2	+	150
×	+	+	*me*-1	175
DR2256 (*pdx, ad*-2)	+	*ad*-2	*me*-1	28
viability = 87%	*pdx*	+	+	38
	pdx	*ad*-2	*me*-1	7
	+	+	+	10
	pdx	+	*me*-1	0
	+	*ad*-2	+	2
2. PR701 (*nic*-1)	*pdx*	*ad*-2	+	195
×	+	+	*nic*-1	164
DR2256	*pdx*	+	*nic*-1	29
viability = 87%	+	*ad*-2	+	19
	pdx	+	+	5
	+	*ad*-2	*nic*-1	8
	pdx	*ad*-2	*nic*-1	9
	+	+	+	11

four rarest classes are all about equally frequent. Spores from the same fruit were therefore plated on to minimal medium + pyridoxal hydrochloride to select for nic^+ ad^+ recombinants. From approximately 5×10^4 viable spores plated, 151 nic^+ ad^+ recombinants were recovered, and only eight of these were found to be fully prototrophic. This 143 : 8 ratio of pdx^{++} to $^{+++}$ is obviously quite different from the 5 : 11 ratio of the same two genotypes originally found (Table 5, cross 2), even though both samples of spores were from the same fruit. The reason for the discrepancy is not clear but the data of Table 5 seem likely to have been in error in some way. In any case, the indicated order for these three markers is; *pdx*—*nic*-1—*ad*-2.

Lewis (1961) showed that the two suppressors of *me*-1, *su-3–me*-1 and *su-4–me*-1, were both linked to that gene; it was therefore considered desirable to include them in this investigation of Group III. Two crosses were analysed by random spores; cross 3 between Ms25 (*me*-1, *su-4–me*-1) and DR22.56 (*pdx, ad*-2), and cross 4

Table 6. *Data from four-point crosses between members of Group III: mapping suppressors of* me–*1*

Genotypes	Phenotypes	Frequencies: Cross 3 ($\frac{su\text{-}4\text{–}me\text{-}1 \quad me\text{-}1 \quad + \quad +}{+ \quad + \quad pdx \quad ad\text{-}2}$)	Frequencies: Cross 4 ($\frac{su\text{-}3\text{–}me\text{-}1 \quad me\text{-}1 \quad + \quad +}{+ \quad + \quad pdx \quad ad\text{-}2}$)
su me + + *su* + + + + + + +	prototroph	354	72
su me pdx ad *su* + *pdx ad* + + *pdx ad*	*pdx, ad*	444	128
su me + *ad* *su* + + *ad* + + + *ad*	*ad*	83	22
su me pdx + *su* + *pdx* + + + *pdx* +	*pdx*	78	33
+ *me pdx ad*	*me, pdx, ad*	0	1
+ *me* + +	*me*	7	50
+ *me* + *ad*	*me, ad*	0	9
+ *me pdx* +	*me, pdx*	0	0

between Ms29 (*me*-1, *su-3–me*-1) and DR22.56. The frequencies of all phenotypes which could be recognized are shown in Table 6. The phenotype distributions obtained from cross 3 can only be explained if the order

su-4–me-1—*me*-1—(*pdx*—*ad*-2)

is assumed; likewise the results of cross 4 can be most readily explained by the order

su-3–me-1—*me*-1—*pdx*—*ad*-2

The observed 31·7% recombination between *su-3–me*-1 and *me*-1, coupled with the known order, and the closeness of the linkage between *me*-1 and the centromere places this suppressor locus on the other arm of the chromosome to that occupied by *me*-1.

The genes *me*-1, *me*-3, and *me*-7 form a tightly clustered group of apparently

Table 7. *Recombination between members of the Group III methionine cluster*

Cross	Progeny	Prototrophs	% recombination
me-3 × *me*-1 (M60) (PR2301)	$1{\cdot}82 \times 10^5$	135	0·148
me-7 × *me*-1 (MR1.01) (PR2301)	$1{\cdot}0 \times 10^5$	28	0·056
me-3 × *me*-7 (M60) (MR1.01)	$1{\cdot}74 \times 10^5$	240	0·276

related function, although they are quite easily distinguished physiologically (Moore, 1966*b*). The results of tests for recombination between them are summarized in Table 7. The recombination frequencies suggest the order;

—*me*-7—*me*-1—*me*-3—

This order has not been confirmed by other tests, and the markers cannot at present be ordered with respect to other members of the group.

(d) Group IV

A single three-point cross of M61 (*me*-9) × DR20.55 (*nic*-4, *ad*-1) was analysed both by random spores and by tetrads (see Table 8).

Table 8. *Recombination between members of Group IV: random spore and tetrad data*

Cross 5. Random spores from the cross M61 × DR20.55

Genotypes			Frequency
+	*nic*-4	*ad*-1	164
me-9	+	+	160
me-9	*nic*-4	*ad*-1	30
+	+	+	24
+	*nic*-4	+	27
me-9	+	*ad*-1	23
+	+	*ad*-1	8
me-9	*nic*-4	+	12

Cross 6. Tetrads from the cross M61 × DR20.55

	Tetrad distribution		
Marker pair	PD	NPD	T
B & *me*-9	4	5	9
B & *ad*-1	2	4	12
B & *nic*-4	4	5	9
me-9 & *ad*-1	13	0	5
me-9 & *nic*-4	17	0	1
ad-1 & *nic*-4	14	0	4

The data from random spores clearly indicate the order:

—*me*-9—*nic*-4—*ad*-1—

The results from the eighteen tetrads analysed are not entirely satisfactory, nevertheless the purpose of the cross—positioning of the centromere—is served by the *nic*-4/*ad*-1/*B* trio. For this trio real solutions for the Whitehouse equations are only obtained if it is assumed that the order is *centromere—nic*-4—*ad*-1. The centromere is thus placed almost midway between *nic*-4 and *me*-9.

(*e*) *Group V*

This was distinguished originally as carrying the methionine auxotroph *me*-8 which showed no linkage with any of the centromere markers of the test strains. The second division segregation frequency of *me*-8 was estimated to be 27·2% from a cross of G2301 (*me*-8; B_6) × MR48.01 (*nic*-4; B_5) which gave the following tetrad distributions; for *nic*-4 and *me*-8, 5 PD: 9 NPD: 7 T; for *nic*-4 and *B*, 10 PD: 7 NPD: 4 T; and for *me*-8 and *B*, 8 PD: 6 NPD: 7 T.

The gene *ad*-6, which shows 34% recombination with *me*-8, was estimated to have a second division segregation frequency of approximately 9·3% from the cross P809 (*ad*-6; B_3) × MR48.01 (*nic*-4; B_5). A total of twenty-three tetrads were analysed, the frequencies of the various types being: for *ad*-6 and *nic*-4, 12 PD: 7 NPD: 4 T; for *ad*-6 and *B*, 9 PD: 6 NPD: 8 T; and for *nic*-4 and *B*, 7 PD: 8 NPD: 8 T. A standard sampling error of ± 21% can be attached to the value of 9·3% for the second division segregation frequency of *ad*-6. In view of this very large error one can only draw the tentative conclusion that *ad*-6 does show centromere linkage. Since it would not be possible to demonstrate this if *ad*-6 were distal to *me*-8 on the same arm (as this would place *ad*-6 about 48 units from its centromere) the most likely order is *ad-6—centromere—me-8*. At present, though, this can only be considered a provisional representation.

(*f*) *Group VI*

This is represented by a single gene, *paba*-2, which was one of the centromere markers established at the start of this investigation. Its close centromere linkage (*ca.* 9·5 units) together with the demonstration of its independence of all members of Groups I to V qualify it for rank as a separate group.

A second division segregation frequency of 58% was recorded for the gene *ad*-3 (strain M8), which also segregated independently of all centromere markers and many other members of the major groups. As this is not a convincing demonstration of an independent group, the designation Group G (instead of VII) has been adopted for this marker to indicate that rank as a separate chromosome is not satisfactorily established.

No centromere linkage at all could be demonstrated for the gene *nic*-3 (strain M33).

4. DISCUSSION

Four, and possibly five, entirely new linkage groups have been established and mapped, and corrections made to the existing maps of Groups I and II. Maps of all linkage groups are shown in Fig. 1.

In addition to the genes shown in that figure, Lewis has mapped the gene controlling the methionine-activating enzyme (*MAE* or *ethr*-1, strain E2) some 4 units distal to *me*-6 in linkage group I, and *ad*-9 *ca.* 7·5 units distal to *me*-5 in the same linkage group. The morphological mutant 'dendroid' (*den*), which forms a tight cushion of growth, shows about 6 to 8% recombination with *me*-1, although its precise

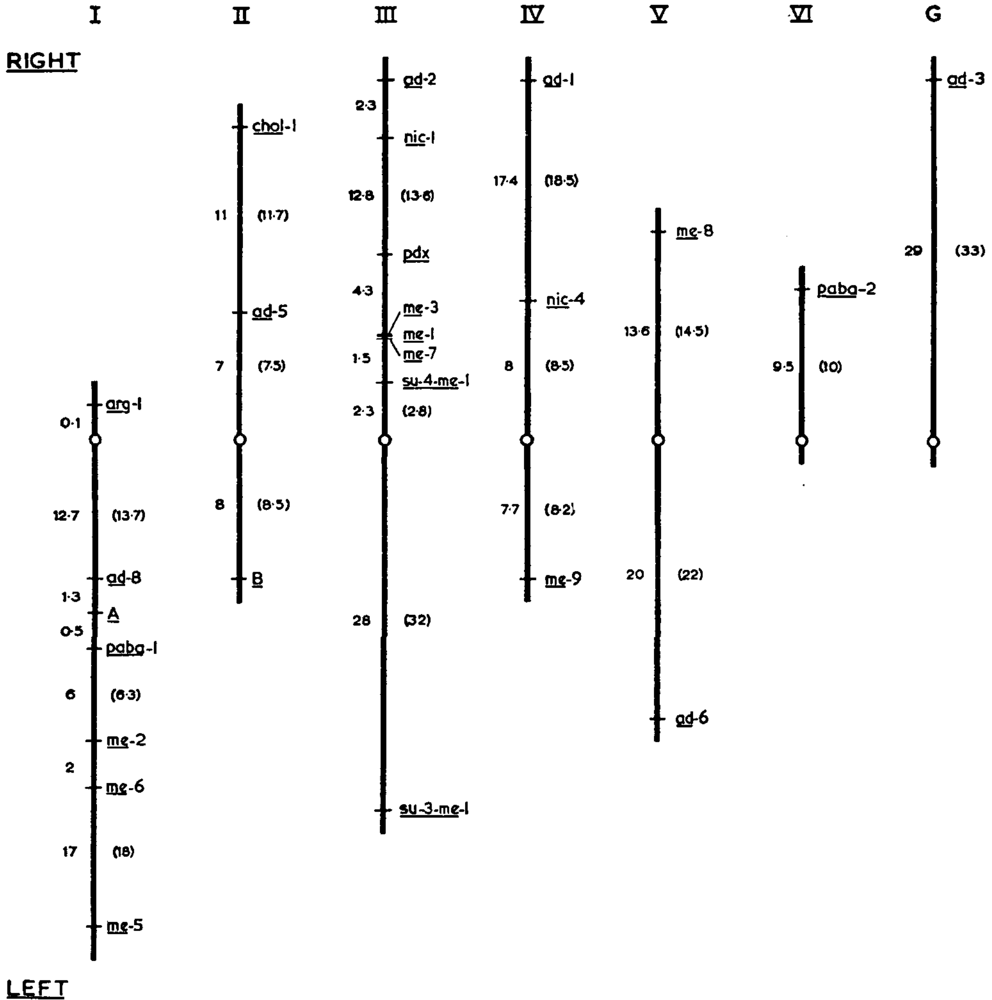

Fig. 1. Linkage groups of *Coprinus lagopus*. Centromeres are shown as open circles and corrected values of the larger distances appear in parentheses. The map of linkage group I distal to *ad*-8 is reproduced, with permission, from Day & Anderson, 1961.

position within Group III is not yet known (J. W. Cowan, personal communication). Another morphological mutant called 'oidial' (*oid*), which has abnormally abundant oidia, has been shown to be linked (*ca.* 20% recombination) with the *A* mating type factor (P. R. Day, *Notes on Fungal Incompatibility* for April, 1966).

The data to hand from the present investigation allow the definite conclusion that the six linkage groups described (omitting Group G from consideration) are

actually situated on different chromosomes. Correlation between genetically and cytologically observed chromosomes is not yet possible to any great extent. However, it is interesting to note that the genetic studies reveal two linkage groups which are much more extensive than the rest (Groups I and III), while the cytological observations of B. C. Lu (personal communication) have established a haploid chromosomal complement composed of eight small and two relatively much larger chromosomes.

A total of 4092 interval tetrads have been fully characterized in this work, and in this number only one possible case of gene conversion was observed. This was seen in a tetrad derived from a cross of the form M48 (A_6; B_6; *nic*-4) × BC6/55 (A_5; B_5; +). The spores of the tetrad concerned had the genotypes;

(*a*) A_6; B_5; +
(*b*) A_5; B_6; *nic*-4
(*c*) A_5; B_5; +
(*d*) A_6; B_6; +

suggesting conversion of the mutant allele of *nic*-4 to its wild-type allele. Though miss-scoring was ruled out by repeated tests, this tetrad was one of the few which were isolated directly from fresh gill tissue and the possibility of contamination due to technical error cannot be completely excluded.

SUMMARY

1. By means of tetrad analysis three vitamin-requiring mutants have been established as markers of centromeres distinct from those of the two known chromosomes.

2. Mutants existing in stock were tested for linkage with these centromere markers, and four previously unknown linkage groups with independent centromeres thereby established and accurately mapped.

3. Corrections were made to the maps of existing linkage groups. The *A* locus (Group I) was shown to be approximately four times as distant from its centromere as hitherto supposed (at least in most crosses); and several markers of Group II were mapped for the first time.

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REFERENCES

Day, P. R. (1959). A cytoplasmically controlled abnormality of the tetrads of *Coprinus lagopus*. *Heredity, Lond.* **13**, 81–87.

Day, P. R. & Anderson, G. E. (1961). Two linkage groups in *Coprinus lagopus*. *Genet. Res.* **2**, 414–423.

Hawthorne, D. C. & Mortimer, R. K. (1960). Chromosome mapping in *Saccharomyces*: centromere linked genes. *Genetics*, **45**, 1085–1110.

LEWIS, D. (1961). Genetical analysis of methionine suppressors in *Coprinus*. *Genet. Res.* **2**, 141–155.
MOORE, D. (1966*a*). New method of isolating the tetrads of Agarics. *Nature, Lond.* **209**, 1157–1158.
MOORE, D. (1966*b*). The formal genetics of *Coprinus lagopus* (*sensu* Buller). Ph.D. Thesis, University of Hull.
PERKINS, D. D. (1953). The detection of linkage in tetrad analysis. *Genetics*, **38**, 187–197.
WHITEHOUSE, H. L. K. (1957). Mapping chromosome centromeres from tetratype frequencies. *J. Genet.*, **55**, 348–360.