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Genetic studies on flagellum mutants of *Chlamydomonas reinhardii*

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SUMMARY

Eight newly isolated 9 + 0 mutants each mapped at one of the four previously known loci. Short flagellum mutants were at three loci, two of which (*pf7* and *pf8*) were closely linked; the third, *pf21*, was unlinked to these two and mapped on linkage group II. The long flagellum mutants *lf1* and *lf2* were on linkage groups II and XII respectively. Mutants *pf8A* and *lf1* were both recessive to wild-type. There was no evidence for non-Mendelian flagellum mutants.

1. INTRODUCTION

The first genetic analysis of paralysed mutants in the biflagellate alga, *Chlamydomonas reinhardii*, showed the existence of eleven loci scattered widely over the 11 linkage groups then known (Ebersold *et al.* 1962). A more recent map brought the numbers of known motility loci and of known linkage groups up to 16 (Levine & Goodenough, 1970). Most of these mutants appeared to have structurally normal flagella when examined in the electron microscope (Warr *et al.* 1966); they were presumably impaired in some aspect of flagellar function. Mutations at four of the loci were shown, however, to have '9 + 0' flagella with structurally defective central tubules.

Further flagellum mutants have now been isolated with the aim of identifying genes concerned with the control of flagellar growth and development. They have been partially characterized (see McVittie, 1972) and this paper describes the results of genetic analysis of some of these mutants. Short flagellum mutants defective in flagellar development have been shown to map at three new loci. Two other loci are involved in the control of flagellar length and growth rate. Diploids have been used for dominance tests.

2. METHODS

(i) *Strains, media and culture conditions.* The isolation of the flagellum mutants *NG1, 2, 10, 15* and *37*, *pf7A* and *B*, *pf8A* to *D*, *pf15B*, *pf18E* and *F*, *pf19E*, *pf20B* to *E*, *pf21*, *lf1* and *2* and *AO7* was described by McVittie (1972) and the isolation of *pf18A* to *D*, and *pf20A* by Warr *et al.* (1966). The remaining *pf* mutants and other mutants used for mapping were obtained from Professor R. P. Levine, Harvard

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University. The media and culture conditions were those described by McVittie (1972). Auxotrophs were grown on the following concentrations of supplements: 10 μg/ml L-arginine HCl, 1 μg/ml thiamine, 0·75 μg/ml nicotinamide, 2·0 mg/ml sodium acetate $3H_2O$.

(ii) *Genetic methods.* The crossing techniques of Ebersold & Levine (1959) and Levine & Ebersold (1960) were used. The low efficiency of mating of short flagellum mutants was improved by gently centrifuging (1 min at setting 2 on an MSE bench centrifuge) the mixed suspension of plus and minus gametes into a loose pellet and repeating this about six times every 40–60 min. Zygotes were matured on growth medium solidified with 2% agar (Oxoid Agar No. 3). Supplemented medium was used for zygote maturation only in crosses involving *arg 1*, *arg 2*, *nic 1*, *ac 21* and *ac 157*.

Diploid strains were isolated by the method of Ebersold (1967). The closely linked markers *arg 1* and *arg 2* on linkage group I were used to select against the haploid parental strains and to minimize the recovery of wild-type recombinants. Prototrophic diploid colonies were readily distinguished from those of prototrophic recombinants by their larger size.

3. RESULTS

(i) *First- and second-division segregation in crosses to a centromere marker*

The 2:2 (Mendelian) segregation seen in back-crosses (McVittie, 1972) indicated that the strains tested probably had mutations in chromosomal (nuclear) genes, since mutations in non-chromosomal genes usually give non-Mendelian ratios. By crossing to a centromere marker which, for practical purposes, segregates entirely at the first division of meiosis it was possible to distinguish between first- and second-division segregation of the unknown marker since the former gives ditype (PD and NPD) and the latter tetratype (T) tetrads. In each cross, some tetrads showed first- and some second-division segregation (Table 1). This was interpreted as evidence that these mutations are located on nuclear chromosomes since the occurrence of both first- and second-division segregation is difficult to account for on any other basis (Sager, 1955).

(ii) *Mapping of straight (9+0) and curved flagellum mutants*

Each newly isolated straight flagellum mutant except *pf20D* was initially crossed separately to mutants at all four known loci. Crosses were scored for the presence or absence of recombination by allowing mature, chloroform-treated zygotes to germinate in tubes of liquid medium. Motile cells growing vigorously at the top of the tube indicated recombination. The results of these preliminary crosses were mostly unambiguous: each new mutant gave wild-type recombinants with three of the known 9+0 mutants but not with the fourth. The only exception was *pf18F*, which failed to give recombinants in any of the crosses, presumably owing to lack of mating (the mating efficiency was often low when two paralysed mutants were crossed). Crosses which gave no recombination were repeated and tetrads analysed.

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Table 1. *Results of crosses to a centromere marker*

(*lf2* was crossed to *pf2* and the remaining strains were crossed to *ac17*.)

Mutant	Phenotype	Tetrad analysis PD	NPD	T
NG10	Abnormal swimming	4	1	11
NG37	Abnormal swimming	3	2	7
pf7	Short flagellum	3	2	11
pf7A	Short flagellum	3	0	23
pf7B	Short flagellum	1	3	11
pf8A	Short flagellum	1	3	10
pf8B	Short flagellum	1	10	18
pf8C	Short flagellum	0	3	11
pf8D	Short flagellum	1	1	16
pf21	Short flagellum	6	3	4
lf1	Long flagellum	13	8	7
lf2	Long flagellum	1	3	15
A07	Unclassified	2	4	4
NG1	Unclassified	8	6	14
NG2	Unclassified	3	4	9
NG15	Unclassified	5	2	3

Table 2. *Mapping of straight flagellum (9+0) mutants*

(Mutants *pf15A*, *18*, *19A* and *20* are on linkage groups III, II, X and IV respectively.)

Cross	Tetrad analysis PD	NPD	T	Conclusion
pf15B × *pf15A*	24	0	0	Same locus
pf18E × *pf18*	9	0	0	Same locus
pf18F × *pf15A*	2	0	7	Unlinked
pf18F × *pf18D*	27	0	0	Same locus
pf18F × *pf19A*	3	1	4	Unlinked
pf18F × *pf20*	0	2	8	Unlinked
pf19E × *pf19A*	13	0	0	Same locus
pf20B × *pf20*	18	0	0	Same locus
pf20C × *pf20*	19	0	0	Same locus
pf20D × *pf20*	22	0	0	Same locus
pf20E × *pf20*	8	0	0	Same locus

The results showed that each of the eight new mutants was at one of the previously known loci (Table 2).

Germination of mature zygotes in liquid showed that the new curved flagellum mutants *pf1A* and *B* recombined with the known curved flagellum mutant *pf17*, but not with *pf1*. Both strains were again crossed to *pf1* and 30 undissected zygotes were shown to contain mutant cells only. Thus *pf1A* and *B* are at the pf1 locus on linkage group V.

(iii) *Mapping of short flagellum mutants*

Pairs of mutants were first crossed in various combinations to determine the number of loci involved. The efficiency of mating was usually too low to allow the rare zygotes to be seen and manipulated on fresh plates for tetrad analysis.

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Table 3. *Mapping of short flagellum mutants*

	Cross	Recombinant zygotes (T+NPD)	Total zygotes (PD+T+NPD)	Recombination (%)*
1.	*pf7* × *pf7A*	0	17	0
2.	*pf8* × *pf8A*	0	12	0
3.	*pf8A* × *pf8B*	0	218	0
4.	*pf8B* × *pf8D*	0	17	0
5.	*pf8C* × *pf8D*	0	44	0
6.	*pf7* × *pf8A*	7	80	4
7.	*pf7* × *pf8C*	40	430	4·7
8.	*pf7* × *pf8D*	3	24	6
9.	*pf7B* × *pf8A*	10	118	5
10.	*pf7* × *pf21*	4	4	50
11.	*pf8A* × *pf21*	18	20	45
12.	*pf21* × *ac40*	4	33	6
13.	*pf21* × *ac12g*	26	65	20

* Percentage of recombinant zygotes × ½.

Zygotes were therefore allowed to germinate on the maturation plates after chloroform treatment and were then classified as non-recombinant, when they gave rise to no motile progeny (PD tetrads), or recombinant when motile progeny were produced (T and NPD tetrads). The results are given in Table 3. The combined results of crosses 2–5 indicated that *pf8* and *pf8A* to *D* are at the same locus; cross 1 showed that *pf7A* was at the *pf7* locus. From crosses 6 to 8 it was evident that the *pf7* and *pf8* loci were closely linked at a distance of about 5 units. The evidence that *pf7B* was allelic with *pf7* came from cross 9, in which the percentage recombination between *pf7B* and *pf8A* was shown to be the same as that between *pf7* and the *pf8* locus; the alternative explanation that *pf7B* and *pf7* represented two loci equidistant from *pf8* and on opposite sides of it was considered unlikely.

Crosses 10 and 11 showed that *pf21* represented a third locus unlinked to the other two. Further crosses to markers on various linkage groups showed it to be on linkage group II. The data (Table 3, crosses 12 and 13) were consistent with a location for *pf21* on the left arm of linkage group II between *ac40* and the centromere (Fig. 1), although the actual map distances (equivalent to percentage recombination) were subject to some error for the following reasons: the *ac40*–*pf21* distance (cross 12) was based on analysis of only 33 tetrads; the *pf21*–centromere distance was based on 13 tetrads (Table 1) and was therefore only a rough estimate; the *pf21*–*ac12* distance (cross 13), although based on a larger number of tetrads, was probably an underestimate since a few of the recombinant tetrads may have been NPD (double cross-over) rather than T (single cross-over) tetrads.

The map locations of *pf7* and *pf8* were not established. The percentage recombination between *pf7* and *pf8* mutants and their centromere was obtained from the results of crosses to the centromere marker, *ac17* (Table 1). The average values (equal to one half of the percentage of T tetrads) of 38 for the *pf7* mutants and 42 for the *pf8* mutants indicated that these loci were too far from the centromere for accurate determination of the gene–centromere distance.

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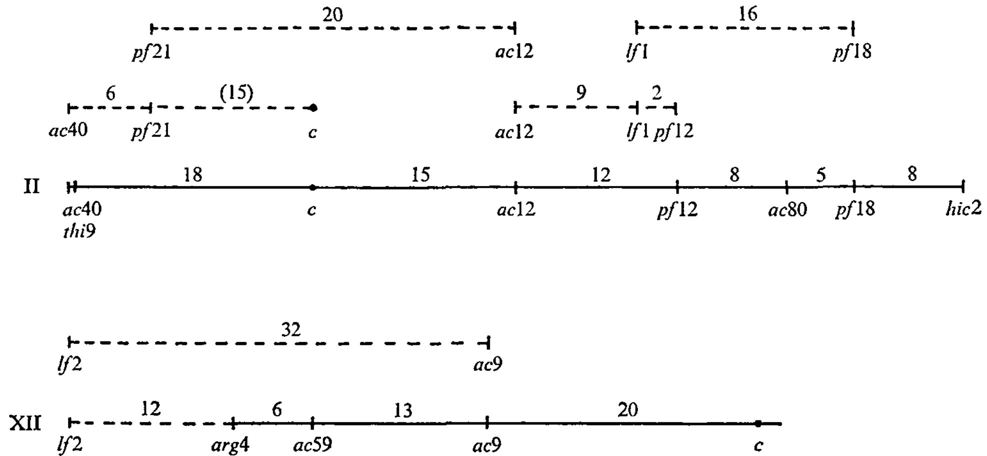

Fig. 1. Genetic maps of linkage groups II and XII. Solid lines represent data from Levine & Goodenough (1970). Dotted lines represent new data showing map positions of *pf21* and *lf1* on linkage group II and of *lf2* on linkage group XII. *c* = centomere.

Table 4. *Results of pf8A crosses*

Linkage group	Marker	Gene-centromere distance of marker*	Tetrad analysis PD	NPD	T	Recombinant zygotes/total
I r†	*arg1*	10	1	0	7	17/19
II l†	*ac40*	18	1	0	6	—
II r	*pf18*	40	—	—	—	17/20
III l	*pf15A*	27	—	—	—	16/20
III c†	*ac17*	0·1	1	3	10	—
IV r	*pf20*	37	1	2	3	15/20
V l	*ac31a*	14	1	0	3	—
			0	0	4	—
V r	*pf1*	20	0	2	8	15/20
VI l	*mt*	24	0	0	4	—
VI r	*pf14*	10–15	—	—	—	18/19
VIII l	*thi1*	16	3	0	13	—
IX l	*pf16*	12	—	—	—	17/20
IX r	*pf13*	30	—	—	—	15/20
X l	*pf19*	10	—	—	—	19/20
X l (or c)	*nic13*	4	—	—	—	14/16
XI r	*ac21*	23	1	1	8	—
XII l	*ac9*	20	0	2	7	—
XII l	*arg4*	39	—	—	—	11/16
XIII l	*pf9*	50	2	4	9	—
XV l	*nic1*	11	0	0	7	—
XVI c	*ac46*	1	2	0	8	—

* Data from Levine & Goodenough (1970).
† r = right, l = left arm, c = centromere marker.

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Table 5. *Mapping of long flagellum mutants*

Cross	Tetrad analysis PD	NPD	T	Recombination (%)*
lf1 × *ac12g*	39	0	8	9
lf1 × *pf12*	287	0	13	2
lf1 × *pf18*	35	0	16	16
lf2 × *arg4*	83	0	26	12
lf2 × *ac9*	11	0	19	39

* % T tetrads × ½.

The results of crossing *pf8A* to markers representing the left and right arms of most of the 16 linkage groups are given in Table 4. The gene–centromere distances of these markers are included to give some idea of the percentage recombination expected in the event of linkage between *pf8A* and any of the markers. Tetrads were analysed in most crosses and in a few of these additional, undissected zygotes were scored for presence or absence of recombination. In some crosses only undissected zygotes were examined. The data failed to show any linkage and were provisionally interpreted as excluding a location for *pf8A* (and hence for the other *pf8* and *pf7* mutants) on linkage groups II, III, V, VI, IX and XVI, on the left arms of VIII, X, XII, XIII and XV and on the right arms of I, IV and XI. The data probably also excluded the right arm of X (*nic13* cross). No markers are known on the right arms of XII, XIV and XV and the left arm of XI, although in the latter case *pf2* is probably sufficiently close to the centromere to enable linkage to be detected on the opposite arm. A few crosses additional to those shown in Table 4 were unsuccessful, either because the markers used were not scorable (*ac209* on I left and *ac177* on XIV left) or because the majority of tetrads were incomplete (*nic11* on IV left and *pf17* on VII right).

(iv) *Mapping of long flagellum mutants*

Mutant *lf1* was found to be on the right arm of linkage group II between *ac12* and *pf12*. The relevant crosses are given in Table 5 and the map location in Fig. 1. In the cross to *pf12*, 47 dissected zygotes gave 45 PD:0 NPD:2 T. The remaining 253 undissected zygotes gave 242 non-recombinant:11 recombinant. Since the expected frequency of NPD tetrads was negligible it was assumed that these 11 recombinants were all T tetrads and the data were combined with those of the dissected zygotes (Table 5).

A map location for *lf2* was established on the left arm of linkage group XII (Table 5; Fig. 1).

(v) *Dominance tests with* pf8A *and* lf1

Two diploid strains were isolated, each containing one of the above mutations together with its wild-type allele. To obtain the heterozygous *pf8A* diploid, a *pf8A arg1* double mutant was crossed to *arg2* and the zygotes were plated on non-supplemented medium. The strain isolated was tested for diploidy by back-crossing and examining the segregants for arginine requirement. Both *arg1* (growth on

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arginine or citrulline) and *arg2* (growth on arginine only) segregants were obtained. This could be taken as evidence for diploidy since in all cases tested by Ebersold (1967), heterozygosity for the markers used to select prototrophs was accompanied by heterozygosity for other non-selected markers. More than half the tetrads resulting from the back-cross were incomplete, in agreement with the findings of Ebersold (1967) and Starling (1969).

The heterozygous *pf8A* diploid had a wild-type phenotype. The cells had two flagella of normal length and showed normal motility, thus indicating that *pf8A* was recessive to wild-type. An attempt to carry out a complementation analysis of the five mutants at the *pf8* locus was unsuccessful owing to difficulties in mating of arginine requiring, short flagellum double mutants.

The *lf1* heterozygous diploid was isolated from the cross *lf1arg2* × *arg1*. Diploidy was established as described above; segregants from the back-cross were also examined for motility and found to include both mutant and wild-type strains. The diploid showed normal flagellum length and motility, the mutant allele again being recessive to wild-type.

4. DISCUSSION

Mutations have been described above as being at the same locus where recombination has not been detected in crosses between them. It should be borne in mind that the numbers of tetrads analysed in these crosses do not exclude the possibility of low levels of recombination. Thus the term 'locus' as used here may represent either a single cistron or two or more closely linked cistrons. These alternatives could be distinguished by using diploid strains for complementation analysis, but this has so far been carried out only for the *pf18* locus (Starling, 1969).

No new 9 + 0 loci were found, and since 22 mutations of this phenotype have now been isolated it seems likely that the four known loci are the only ones. Two new curved flagellum mutants were located at an existing locus.

Modification of the mating procedure improved the efficiency of mating of short flagellum mutants sufficiently to carry out some genetic analysis. The shorter gametogenesis time gave more flagellated cells and spinning into a loose pellet increased the possibilities for cell contact. The *pf7* and *pf8* loci were found to be closely linked. The map distance of 4·7 (Table 3, cross 7) is likely to be accurate since it is based on analysis of 430 zygotes. For such a short distance the expected frequency of NPD tetrads is negligible and inability to distinguish them from T tetrads when undissected zygotes are analysed should not affect the accuracy of the calculated distance. The *pf7* and *pf8* loci are likely to be on one of the following linkage groups: VII, XIV, the left arm of I, IV, XI or the right arm of XII, XIII or XV.

It is desirable to determine map distances from analysis of 100 or more tetrads (Ebersold *et al.* 1962). Thus the *ac40–pf21* distance can only be an approximate one. The location of *pf21* to the right rather than the left of *ac40* is, however, firmly established by the cross to *ac12* (Fig. 1).

The *lf1–pf12* map distance of 2 is accurate, and although the *lf1–ac12* and *lf1–*

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pf18 distances are less accurate because fewer tetrads were analysed, additivity of distances is good. It is clear that *lf1* is to the left of *pf12* since only this arrangement gives good additivity (Fig. 1). The *lf2* map position is accurate since the *lf2–arg4* distance of 12 is based on analysis of more than 100 tetrads and the cross to *ac9* clearly places *lf2* to the left rather than to the right of *arg4* (Fig. 1). The additivity is again good.

The recessivity of *pf8A* and *lf1* in heterozygous diploids is in agreement with the findings of Ebersold (1967) and Starling (1969) that all mutations so far tested in *Chlamydomonas reinhardii* are recessive to their wild-type alleles. The recessivity of *lf1* was also shown by Starling & Randall (1971) using an alternative method based on the examination of temporary dikaryons.

Chlamydomonas reinhardii is known to have a non-Mendelian genetic system (reviewed in Gillham, 1969; see also Sager & Ramanis, 1970), but there is so far no evidence for the existence of non-Mendelian genes controlling any aspect of flagellar structure or function. It was shown previously that mutants defective in the formation of central tubules, as well as those lacking flagellar function but having no apparent structural abnormality, were due to mutation in chromosomal genes (Warr *et al.* 1966). It has now been shown that mutations in chromosomal genes can also result in defective development of the organelle, as in short flagellum mutants, or in defective regulation of flagellar length and elongation characteristics, as in long flagellum mutants.

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