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SHORT PAPER

A plasmodial colour mutation in the Myxomycete *Physarum polycephalum*

By R. W. ANDERSON*

Department of Genetics, University of Leicester, Leicester LE1 7RH, England

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SUMMARY

A spontaneous mutation conferring white plasmodial colour on the Myxomycete *Physarum polycephalum* has been analysed. The mutant *whi*-1 allele is recessive to *whi*$^+$ in both heterokaryotic and heterozygous plasmodia. The *whi* locus is unlinked to *mt*, *npfA*, *fusA* and *leu*.

1. INTRODUCTION

The life cycle of the true slime mould (Myxomycete) *Physarum polycephalum* comprises alternating plasmodial and amoebal vegetative phases. Macroscopic, multinucleate, syncytial plasmodia give rise to uninucleate, colourless amoebae by sporulation and germination of spores. Meiosis apparently occurs during sporulation (Aldrich, 1967; Laane & Haugli, 1976; Laffler & Dove, 1977). Amoebae give rise to plasmodia by either sexual or asexual (clonal) mechanisms which are under the control of the mating type locus, *mt*. Techniques for routine genetic analysis are available (Dee, 1973, 1975).

Wild-type plasmodia of *P. polycephalum* are yellow. Since yellow pigment has been implicated in the control of sporulation in this species (Wormington & Weaver, 1976), it is desirable to isolate mutants in which pigmentation is modified or absent. In addition, mutations conferring altered plasmodial colour are of obvious value as genetic markers. This paper reports the genetical analysis of a plasmodial colour mutation obtained fortuitously during the course of other work (Anderson & Dee, 1977). The mutation causes plasmodia to appear white when growing in surface or liquid culture. I believe that this is the first report of a colour mutation in *P. polycephalum*, although two unlinked, recessive mutations affecting plasmodial pigmentation have been isolated in the closely related species *Didymium iridis* (Collins, 1969; Collins & Erlebacher, 1969).

2. MATERIALS AND METHODS

(i) *Loci. mt*, amoebal mating type. The alleles employed were *mt*h (Wheals, 1970, 1973) and the heterothallic alleles *mt*1 and *mt*2 (Dee, 1966). Plasmodia may be formed sexually by cell and nuclear fusion of amoebae carrying different *mt* alleles. Alternatively plasmodia may form from single clones; plasmodium formation occurs in only a small proportion of clonal cultures of heterothallic strains, but all cultures of *mt*h strains are

* Present address: Room 56-720, Department of Biology, M.I.T., Cambridge, Mass. 02139, U.S.A.

normally able to form plasmodia. Crossed plasmodia are not readily obtained from mixtures of *mt*h and heterothallic amoebae because of the asexual formation of many *mt*h plasmodia.

fusA, a locus affecting plasmodial fusion (Poulter & Dee, 1968; Cooke & Dee, 1975). Alleles *fusA*1 and *fusA*2 are co-dominant. Plasmodia fuse on contact if they have the same fusion genotype but fail to fuse if their *fusA* genotypes differ.

leu, a locus affecting plasmodial requirement for leucine (Cooke & Dee, 1975). *leu*-1 is recessive to *leu*$^+$.

npfA, *npfB*, loci affecting the transition from amoeba to plasmodium (Anderson & Dee, 1977). *mt*h *npfA*1 strains fail to form plasmodia within amoebal clones at 28·5 °C but yield a few plasmodia per culture at 26 °C. At 28·5 °C *mt*h *npfA*1 strains readily yield crossed plasmodia when mixed with *mt*1 strains but do not cross with LU688 (*mt*2). *mt*h *npfB*2 strains fail to form plasmodia within amoebal clones and fail to cross with *mt*2 strains.

The above loci are unlinked to one another with the following exceptions: no recombination has been detected between *mt* and *npfB*; and no test of recombination between *npfA* and *leu* had been made prior to the analysis reported in this paper.

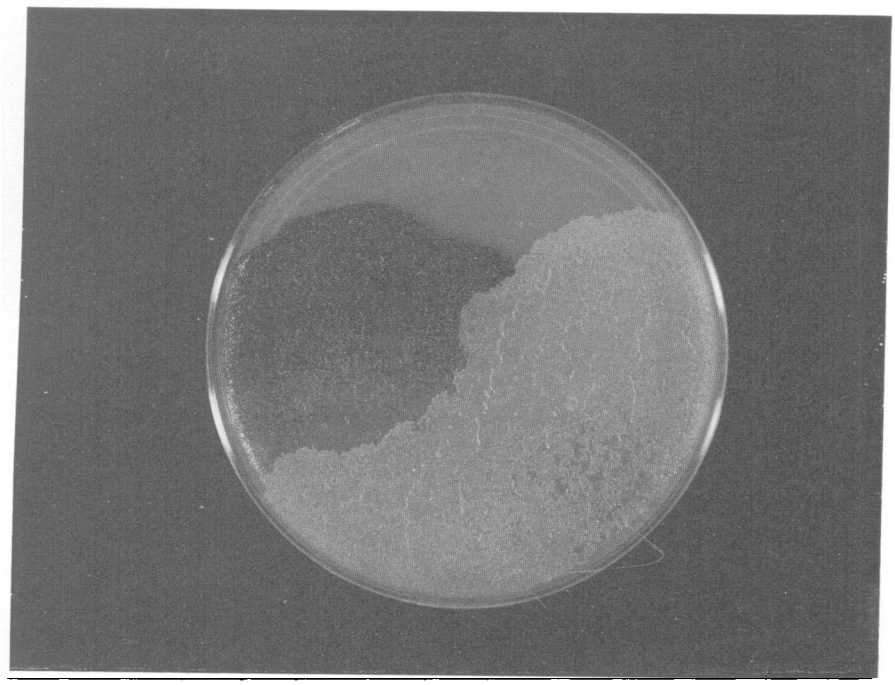
(ii) *Strains*. CL6082, *mt*h *npfB*2 *fusA*2 (Anderson & Dee, 1977). LU688, *mt*2 *fusA*1 (Cooke & Dee, 1975). LU853, *mt*1 *fusA*2 *leu*-1 (Cooke, 1974). LU858, *mt*2 *fusA*1 *leu*-1 (Cooke, 1974). LU867, *mt*h *npfA*1 *fusA*1 (Anderson & Dee, 1977).

(iii) *Methods*. Amoebal culture, plasmodium formation tests and plasmodial fusion tests were carried out as described by Anderson & Dee (1977). Amoebae were routinely cultured at 26 °C with *Escherichia coli* on liver infusion agar. Plasmodium formation tests were carried out with *E. coli* on dilute semi-defined medium (DSDM) agar. Plasmodia were cultured at 26 °C on semi-defined medium (SDM) agar or in liquid SDM (Dee & Poulter, 1970). Leucine requirement was tested as described by Cooke & Dee (1975). Spore formation, spore plating and clone isolation were as described by Wheals (1970).

3. RESULTS

During a previous investigation (Anderson & Dee, 1977) it was found that attempted crosses between amoebal strains of the genotypes *mt*h *npfB*$^-$ and *mt*2 did not yield hybrid plasmodia. One of the crosses attempted was between CL6082 (*mt*h *npfB*2) and LU688 (*mt*2). Amoebae of these two strains were co-inoculated into ten drops of bacterial suspension on DSDM agar plates and incubated for six weeks at 26 °C. A single plasmodium arose and was transferred to SDM agar. It was noticed that this plasmodium was white instead of yellow (Plate 1) and was thus possibly mutant. The plasmodium grew vigorously and retained its white colour over successive passages in surface culture and also in liquid culture. Overgrown cultures were sometimes pale yellow but always clearly distinguishable from wild-type cultures. Sclerotia (plasmodial resting structures which are yellow in wild-type strains) were pale yellow or cream coloured.

CL6082 and LU688 carry different plasmodial fusion alleles (*fusA*2 and *fusA*1, respectively). Thus the fusion behaviour of the white plasmodium was tested in order to determine its origin. It failed to fuse with a heterozygous *fusA*1/*fusA*2 plasmodium and with a *fusA*2 plasmodium but fused with a yellow *fusA*1 plasmodium. Since the white plasmodium expressed only the *fusA*1 allele, it was apparently formed asexually from LU688. Cytoplasmic mixing following fusion of the white and yellow plasmodia initially yielded a pale yellow plasmodium which darkened over a few hours to a colour similar to that of the yellow tester plasmodium and retained this colour during further growth. White colour was therefore recessive to yellow in a heterokaryotic plasmodium.



White and yellow plasmodia growing on the surface of SDM agar in a 9 cm Petri dish. The white plasmodium appears pale in comparison with the smaller, yellow plasmodium. The plasmodia carry different *fusA* alleles and have therefore not fused with one another.

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The white plasmodium was caused to sporulate. The spores were dark brown or black as in wild-type strains. The progeny of some plasmodia formed asexually from heterothallic strains are found to carry mutations conferring the ability to form plasmodia in all clonal cultures (Adler, 1975; Anderson, 1976). Thus ten amoebal progeny clones were derived from the spores of the white plasmodium and tested for their ability to form plasmodia within clonal cultures. The strains were plated on DSDM agar to give well-separated colonies (plaques). Amoebae proliferated and covered the plates but did not form plasmodia within 14 days.

Table 1. *Analysis of progeny of the cross LU853 × LU887*

Parental genotypes: LU853 *mt*1 *fusA*2 *whi*$^+$ *leu*-1
LU887 *mt*2 *fusA*1 *whi*-1 *leu*$^+$

*mt*1 *fusA*2	*whi*$^+$	*leu*$^+$	7	22	96
		leu-1	1		
	whi-1	*leu*$^+$	8		
		leu-1	6		
*mt*1 *fusA*1				74	
*mt*2 *fusA*2					
*mt*2 *fusA*1					

The preferred method for the analysis of a recessive plasmodial character carried by a heterothallic strain is to cross the heterothallic strain with *mt*h *npfA*1 amoebae. Since clones carrying the *mt*h allele are able to form plasmodia in all cultures, the character can be scored in the *mt*h progeny without carrying out test crosses (Dee, Wheals & Holt, 1973; Anderson & Dee, 1977). Unfortunately, crosses between *mt*2 strains and *mt*h strains could not readily be carried out; the white character was isolated in a *mt*2 strain and it was therefore necessary to modify the above procedure by first making a cross with a *mt*1 strain. A single amoebal progeny clone obtained from the white plasmodium was selected and designated LU887. Amoebae of strain LU853 (*mt*1 *fusA*2 *leu*-1) were crossed with LU887 amoebae (*mt*2 *fusA*1) and a yellow plasmodium which fused with a *fusA*1/*fusA*2 tester plasmodium was obtained. The plasmodium LU853 × LU887 was caused to sporulate and 96 progeny clones were derived from the spores. When co-inoculated with LU887 amoebae (*fusA*1), 22 progeny clones gave rise to plasmodia of *fusA*1/*fusA*2 fusion behaviour, and these clones were thus classified as *mt*1 *fusA*2 recombinants. The fraction of progeny in the *mt*1 *fusA*2 class did not differ significantly at the 5% level from the expected one-quarter. Fourteen of the *fusA*1/*fusA*2 plasmodia obtained from the backcrosses of *mt*1 *fusA*2 progeny with LU887 were white and 8 were yellow (Table 1). The expected segregation if white plasmodial colour was conferred by a recessive allele (*whi*-1) at a locus unlinked to *mt* or *fusA* was 1:1, while linkage to either *mt* or *fusA* would have created a shortage of white plasmodia. The observed ratio was not significantly different from 1:1 ($P > 0{\cdot}05$).

Clones in the *mt*1 *fusA*2 class were crossed with LU858 amoebae (*mt*2 *fusA*1 *leu*-1) and the resulting *fusA*1/*fusA*2 plasmodia were tested for leucine requirement. The segregation of *leu*$^+$ and *leu*-1 (Table 1) was not significantly different from 1:1 ($P > 0{\cdot}05$) and there was no evidence of linkage of *whi* and *leu*.

A clone of the genotype *mt*1 *fusA*2 *leu*-1 *whi*-1 (LU896) was crossed with LU867 (*mt*h *fusA*1 *npfA*1). One hundred and nineteen progeny clones were derived from the

plasmodium LU867 × LU896 and amoebae were plated on DSDM agar and incubated at 26 °C for 14 days. The progeny were classified for *mt* and *npfA* alleles (Table 2). Clones which formed many plasmodia within each plaque after 3–4 days were designated *mt*h *npfA*+. Clones which gave a few isolated plasmodia per plate in 7–10 days were classified as *mt*h *npfA*1. Clones which grew as amoebae and did not form plasmodia were designated *mt*1. Plasmodia derived from *mt*h clones were classified for *fusA*, *whi* and *leu* alleles (Table 2). Allele ratios for *fusA*, *leu*, *mt*, *npfA*, and *whi* did not differ significantly from 1:1 ($P > 0{\cdot}05$) and there was no significant deviation from free recombination of alleles at all 5 loci ($P > 0{\cdot}05$).

Table 2. *Analysis of progeny of the cross LU867 × LU896*

Parental genotypes: LU867 *mt*h *npfA*1 *fusA*1 *whi*+ *leu*+
LU896 *mt*1 *npfA*+ *fusA*2 *whi*-1 *leu*-1

Assigned genotypes					Number
mt	*npfA*	*fusA*	*whi*	*leu*	in class
h	+	1	+	+	3
h	+	1	+	1	2
h	+	1	1	+	3
h	+	1	1	1	3
h	+	2	+	+	5
h	+	2	+	1	4
h	+	2	1	+	2
h	+	2	1	1	2
h	1	1	+	+	4
h	1	1	+	1	2
h	1	1	1	+	5
h	1	1	1	1	4
h	1	2	+	+	4
h	1	2	+	1	3
h	1	2	1	+	4
h	1	2	1	1	3
h	1	*	+	+	1
h	1	*	*	*	3
1	*	*	*	*	62
				Total	119

* = Not scored.

4. DISCUSSION

The results presented in this paper show that *whi* is an easily scored genetic marker which is unlinked to four other loci and that *whi*-1 is recessive to *whi*+ in both heterokaryotic and heterozygous plasmodia. This is also the first demonstration that *npfA* and *leu* are unlinked. The failure to detect linkage of *whi* and any of the other loci used in the analysis is not surprising in view of the fact that the haploid chromosome number of *P. polycephalum* is about 40 (Mohberg, 1977). Since progeny of the original asexually formed white plasmodium did not acquire the ability to form plasmodia efficiently within clones, formation of the plasmodium presumably did not occur as an effect of the *whi*-1 mutation. The dominance of *whi*+ over *whi*-1 suggests that the white plasmodium arose from a *whi*-1 amoeba, rather than by mutation within a syncytial plasmodium. Other plasmodia clonally derived from LU688 amoebae have been yellow (Anderson, 1976), so that *whi*-1 apparently arose as a spontaneous mutation in LU688.

It is found that white plasmodia are able to sporulate as efficiently as yellow plasmodia and sometimes more rapidly. This is surprising, since it has been claimed that sporulation is induced by a yellow photoreceptor pigment (Wormington & Weaver, 1976). Only one of several pigment fractions has been shown to have sporulation-inducing capacity, however, and it has been noted here that *whi*-1 plasmodia may sometimes show a pale yellow colour in overgrown cultures. Detailed comparisons of sporulation abilities and pigment extracts from mutant and wild-type plasmodia may lead to an improved understanding of the role of plasmodial pigments in *P. polycephalum*.

Plasmodial fusion tests involving pairs of plasmodia differing in colour are scored much more easily than tests involving plasmodia of the same colour. Thus fusion tests in our laboratories are now routinely carried out using pairs of plasmodia which differ in colour. In addition, the white character can eliminate the need to carry out plasmodial fusion tests when identifying selfed and crossed plasmodia from crosses in which one strain forms plasmodia asexually. The selfing strain must carry the *whi*-1 allele; the non-selfing strain must carry the *whi*$^+$ allele. The strains must also differ in their plasmodial fusion genotypes, in such a way that selfed and crossed plasmodia do not fuse with one another (e.g. *fusA*1 and *fusA*2). When a cross of two such strains is carried out, plasmodia derived asexually from the selfing strain are white and thus easily distinguishable from the yellow crossed plasmodia.

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