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Non-selfing mutants from selfing (Het^-) strains of *Physarum polycephalum*

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SUMMARY

Plasmodial formation in the Myxomycete *Physarum polycephalum* is under heterothallic control by a mating type (*mt*) locus. In natural isolates only amoebae with different *mt* alleles are able to cross to form diploid plasmodia. A class of mutants isolated from heterothallic amoebae, together with the variant strain *CL*, is able to form plasmodia in pure clones, designated as selfing. Non-selfing mutants have been isolated from *CL* and from other selfing amoebae.

This paper reports the isolation and analysis of 64 non-selfing derivatives (designated Npf^-) from seven selfing (Het^-) strains. The Npf^- mutants could be grouped into eight classes on the basis of their crossing and complementation patterns. The possible significance of these mutants is discussed.

1. INTRODUCTION

The Myxomycete *Physarum polycephalum* is a simple eukaryote that is amenable to genetic analysis (Anderson & Dee, 1977; Wheals, 1970). The life-cycle of *P. polycephalum* has been well reviewed by Gray & Alexopoulos (1968). The differentiation of the microscopic uninucleate amoebae of the organism into macroscopic syncytial plasmodia has aroused considerable interest, and much work has been done on the system.

Plasmodial formation in *P. polycephalum* is controlled by a large number of alleles at the mating-type (*mt*) locus. Heterothallic amoebae are only able to form plasmodia when amoebae with differing *mt* alleles are crossed (Dee, 1973). The ability of heterothallic amoebae to cross is also affected by a mating-compatibility locus, *matB* (or *rac*) (Anderson, 1979; Dee, 1978). Amoebae of different mating types will cross readily if their *matB* alleles differ, less readily if their *matB* alleles are identical. The amoebal strains isolated from Wisconsin or Colonia isolates have the *matB1* allele (Anderson, 1979; Dee, 1978). Heterothallic amoebae normally cannot form plasmodia in pure clones (i.e. 'self'), but they can form spontaneous

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or induced mutants that self rapidly (Adler & Holt, 1977; Gorman, Dove & Shaibe, 1979; Honey, Poulter & Winter, 1981). The natural isolate, Colonia, can form plasmodia in pure clones (Wheals, 1970). The Colonia variant *CL* selfs rapidly in small plaques after 3–4 days' incubation (Cooke & Dee, 1974).

A number of mutants have been isolated from *CL* (and a related strain, *C5–1*) that do not self rapidly in small plaques. Wheals (1973) isolated from *C5–1* four mutants (designated Apt$^-$) by UV mutagenesis. They all crossed with each other, and one was analysed further and the mutation found to be unlinked to the *mt* locus. Anderson (1979) and Anderson & Dee (1977) isolated 32 non-selfing mutants (designated Npf$^-$) from *CL* by NMG mutagenesis. They formed three complementation groups with *npfB* and *npfC* closely linked, and *npfA* unlinked, to the *mt* locus. Honey, Poulter & Teal (1979) isolated 21 mutants (designated Dif$^-$) from *CL* by NMG mutagenesis. They formed two complementation groups, *difA* and *difB*, both closely linked to the *mt* locus. *DifA* and *npfC* are equivalent, as are *difB* and *npfB* (Honey *et al.* 1979). In this paper the two complementation groups will be described as *npfC* and *npfB*. Davidow & Holt (1977) isolated a number of non-selfing mutants from *CL* that formed two complementation groups. These were subsequently shown to be identical to *npfB* and *npfC* (Anderson, 1979). There is some evidence suggesting that the *npfB*$^-$ mutants isolated from *CL* represent a class of revertants to the mt_2 heterothallic state (Anderson & Dee, 1977; Honey *et al.* 1979).

David & Holt (1977) isolated a number of non-selfing derivatives from rapid selfing mutants, and we report the isolation and analysis of an additional series of non-selfing Npf$^-$ mutants. A preliminary report of the isolation of these mutants has been made (Poulter, Honey & Teale, 1977). The Het$^-$ mutations utilized in this work had not been genetically analysed directly because of the difficulty of isolating crosses between the rapid selfing mutants and heterothallic strains (Honey *et al.* 1981). The Het$^-$ mutations were therefore characterized indirectly as part of the analysis of the Npf$^-$ mutations.

2. MATERIALS AND METHODS

(i) *Strains. LU648*: mt_1 $fusA_1$ $fusB_1$; *LU688*: mt_2 $fusA_1$ $fusB_1$ (both partially isogenic with *CL*); *a*: $mt_1 fusA_1 fusB_1$; *i*: $mt_2 fusA_2 fusB_2$ (both from the Wisconsin isolate); *CLd*: mt_h $npfC^-$ $fusA_2$ $fusB_1$ (from the Colonia isolate) (Cooke & Dee, 1975). *OUA9*: $mt_2 fusA_2 fusB_1$; *OUC8*: $mt_1 fusA_2 fusB_1$; *OUD1*: $mt_2 fusA_1 fusB_2$; *OUD3*: $mt_1 fusA_2$ $fusB_1$; *OUD7*: mt_1 $fusA_1$ $fusB_2$; *OUG3*: mt_1 $fusA_1$ $fusB_2$ (all progeny clones from the cross *LU648* × *i*) (Poulter & Honey, 1977). *RP5VI*: mt_h $npfB^-$ $fusA_2 fusB_2$; *RP9VI*: mt_h $npfC^-$ $fusA_2 fusB_2$ (both Npf$^-$ derivatives of *CL*) (Honey, Poulter & Teale, 1979). *NH45*: mt_1 het^- $fusA_1 fusB_1$ (Het$^-$ derivative of *LU648*); *NH01*, *NH34*, *NH35*, *NH48*, *NH49*, *NH51*: all mt_2 het^- $fusA_1 fusB_1$ (Het$^-$ derivatives of *LU688*) (Honey, Poulter & Winter, 1981).

(ii) *Loci. mt*: mating type. Heterothallic alleles mt_1 and mt_2 (Dee, 1966) and selfing allele mt_h (Wheals, 1970). Het$^-$: defect in heterothallic control of plasmodial formation (Honey *et al.* 1981). Het⁻ derivatives of heterothallic amoebae self rapidly. *npfC:* locus required for plasmodial formation (Anderson & Dee, 1977). *npfC* is closely linked to *mt*. *npfB:* locus represented by non-selfing mutants that are suggested to be revertants to the mt_2 heterothallic state (Anderson & Dee, 1977; Honey, Poulter & Teale, 1979). *fusA*, *fusB:* plasmodial fusion type. Identity of *fusA* and *fusB* phenotype is a prerequisite for plasmodial fusion (Dee, 1973; Poulter, 1969). The two alleles $fusA_1$ and $fusA_2$ are codominant, while the allele $fusB_2$ is dominant over $fusB_1$.

(iii) *Experimental methods.* Amoebae and plasmodia were cultured on semi-defined medium (SDM), and the experimental techniques used in this work have been described previously (Honey *et al.* 1979). Npf⁻ mutants were isolated from cultures of Het⁻ strains using a modification of the method employed by Anderson & Dee (1977). Plates of Het⁻ amoebae were mutagenised with *N*-methyl *N*′-nitro *N*′-nitrosoguanidine (NMG) (at a final concentration of 200 ng/ml). Most of the Het⁻ amoebae were permitted to form plasmodia and then suspensions of the remaining amoebae were replated. This procedure was repeated a number of times until non-selfing (Npf⁻) plaques were observed, with no more than one Npf⁻ clone being isolated from any mutagenised plate.

The Npf⁻mutants were analysed by crossing them with the tester strains *OUD3* (mt_1), *i* (mt_2), *RP9VI* (*CL*-derived *npfC*⁻), and *RP5VI* (*CL*-derived *npfB*⁻). The crossing patterns of these testers with *LU648* and *LU688* (both *matB1*) indicate that none of the testers have the *matB1* allele (Table 6 and unpublished observations). The Npf⁻ mutants were all *matB1* (as they were originally derived from *LU648* or *LU688*); therefore, their crossing patterns with the testers are not adversely affected by the *matB* locus.

3. RESULTS

(i) *Characterization of Npf⁻ mutants*

Sixty-four Npf⁻ mutants that only selfed occasionally after prolonged culture were isolated from 1 mt_1-derived (*NH45*) and 5 mt_2-derived (*NH01*, *NH34*, *NH48*, *NH49*, *NH51*) Het⁻ strains. The Npf⁻ mutants were characterized by crossing them with the mt_1, mt_2, *npfB*⁻ and *npfC*⁻ tester strains. All plasmodia that formed were fusion tested and confirmed to be of a crossed origin. The Npf⁻ mutants formed eight classes with different patterns of crossing with the four tester strains (Table 1).

The nine Npf⁻ mutants in class 1 crossed readily with mt_1, but not at all with mt_2, *npfC*⁻, or *npfB*⁻. On the basis of their lack of complementation with the *npfC*⁻ tester, these mutants were classified as *npfC*⁻. The mutants did not cross with the mt_2 tester, but the Het⁻ parental strains were all isolated from mt_2 amoebae; and *NH48*, at least, retained a full mt_2 specificity (Honey *et al.* 1981). The mating-type specificity of a mutant was designated by its inability to cross with heterothallic amoebae of a certain mating type. The Npf⁻ mutants in class 1 did not cross with the mt_2 tester, indicating that they all had full mt_2 specificities. If the *npfB*⁻

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mutation, in fact, represents mt_2 heterothallic revertants, the *npfB*⁻ tester would not be expected to cross with the class-1 mutants.

The three mutants in class 2 isolated from *NH34* exactly resembled the *npfC*⁻ mutants isolated from *CL*, and they were therefore classified as *npfC*⁻. When mt_1 and mt_2 strains with identical *matB* alleles were crossed with these *npfC*⁻ mutants,

Table 1. *Characterization of Npf⁻ mutants*

(++, rapid plasmodial formation; +, slow plasmodial formation; +/−, only a proportion of the Npf⁻ mutants crossed; −, no crossed plasmodia formed.)

Class	Parental Het⁻	No. of Npf⁻ derivatives	Crossing behaviour of Npf⁻ derivatives × *D3* mt_1	× *i* mt_2	× *RP9VI* *npfC*⁻	× *RP5VI* *npfB*⁻
1	*NH01*	3	++	−	−	−
	NH35	2	++	−	−	−
	NH48	3	++	−	−	−
	NH49	1	++	−	−	−
2	*NH34*	3	++	+	−	+
3	*NH01*	4	++	−	+	−
	NH35	3	++	−	+	−
	NH48	4	++	−	+	−
	NH49	4	++	−	+	−
	NH51	1	++	−	+	−
	NH34	3	++	−	+	−
4	*NH35*	2	++	+	+	+/−
	NH48	6	++	+	+	+/−
	NH49	2	++	+	+	−
5	*NH35*	3	−	++	++	++
6	*NH35*	1	−	−	−	−
7	*NH45*	15	−	++	++	++
8	*NH45*	4	+	++	++	++

plasmodia formed readily in the mt_1 cross but much less readily in the mt_2 cross. The mutants are therefore described here as having a 'partial mt_2 specificity'. That is, they do not have a full mt_2 specificity (otherwise they would not cross with mt_2 heterothallic amoebae), but have some mt_2 characteristics (as they exhibit reduced crossing with mt_2 heterothallic strains even when the *matB* alleles differ). It should be noted that both *NH34* and *CL* have similar characteristics, described here as a partial mt_2 specificity (Honey *et al.* 1979; Honey *et al.* 1981).

Class 3 comprised 19 Npf⁻ mutants that resembled *npfB*⁻ mutants isolated from *CL*. The mutants crossed readily with mt_1, not at all with mt_2, slowly with *npfC*⁻ tester, and not with *npfB*⁻. These mutants were classified as *npfB*⁻.

The ten mutants in class 4 crossed with mt_1, mt_2, *npfC*⁻, and in four cases with *npfB*⁻. Their ability to cross with mt_2 may indicate a partial loss of mt_2 specificity although all the Het⁻ parents were isolated from mt_2 amoebae; and *NH48*, at least, had a full mt_2 specificity. The mutants probably have not completely lost their mt_2

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specificity; otherwise they would cross readily with mt_2 strains with differing *matB* alleles.

Class 5 comprised three Npf⁻ mutants isolated from *NH35* that resembled mt_1 heterothallic amoebae. The single mutant in class 6, also isolated from *NH35*, failed to cross with any tester strain.

Table 2. *Analysis of amoebal progeny clones from selfed Npf⁻ mutants*

Parental clone	Class	Genotype	Days for selfing	Progeny phenotypes
OUD3	—	mt_1 heterothallic	20	Delayed selfing
CLd	—	mt_h *npfC⁻*	8	Rapid selfing
NH4815I	1	mt_2 Het⁻ *npfC⁻*	10	Rapid selfing
NH3406I	2	mt_2 Het⁻ *npfC⁻*	10	Rapid selfing
NH3402I	2	mt_2 Het⁻ *npfC⁻*	8	Delayed selfing
NH0111I	3	*npfB⁻*	8	Delayed selfing
NH4804I	3	*npfB⁻*	9	Delayed selfing
NH4809I	3	*npfB⁻*	8	Delayed selfing
NH3505I	4		10	Delayed selfing
NH4801I	4		14	Delayed selfing
NH4805I	4		9	Delayed selfing
NH4806I	4		14	Delayed selfing

The 15 Npf⁻ mutants in class 7 were isolated from *NH45* (mt_1-derived Het⁻) and resembled mt_1 heterothallic amoebae. A further four mutants in class 8 were also isolated from *NH45* and crossed with all four tester strains.

A number of the Npf⁻ mutants selfed on a rare basis after prolonged incubation. Some of these were allowed to self, the plasmodia sporulated, and the spores germinated. The selfing phenotypes of the amoebal progeny clones were carefully observed (Table 2). The data for *CLd* (Cooke & Dee, 1974) and the heterothallic strain *OUD3* are included in this analysis (Honey *et al.* 1981). Selfing may occur by reversion of a Npf⁻ mutation to Npf⁺, in which case progeny amoebae derived from selfed plasmodia would be expected to self rapidly (i.e. resemble Het⁻). Alternatively, selfing may occur by a 'leaky' mechanism without any reversion of Npf⁻ to Npf⁺ taking place. Progeny amoebae from such selfed plasmodia would be expected to retain the Npf⁻ phenotype and only self on a rare basis. Only the *npfC⁻* mutants had rapid selfing progeny, suggesting that only they selfed following a reversion of Npf⁻ to Npf⁺. The *npfC⁻* mutant *NH3402I* did not self following reversion to *npfC⁺* and the progeny retained the parental selfing phenotype. This mutation may be leaky, however, as *NH3402I* formed many plasmodia after 8 days' incubation.

(ii) *Isolation of Npf⁻ recombinant clones*

The Npf⁻ mutants were crossed with heterothallic strains, the plasmodia sporulated, and a number of amoebal progeny clones isolated. Each progeny clone

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was crossed with *LU648*, *LU688* and the parental Npf⁻ mutant, and the resulting plasmodia fusion tested (Table 3). In this way the *mt*, *fusA* and *fusB* genotype of each progeny clone was determined. Derivatives of the mutants with new combinations of fusion alleles were thus isolated, which was of value in further analysis. Most of the progeny clones isolated displayed a range of recombinant and

Table 3. *Analysis of the progeny of crosses between Npf⁻ mutants and heterothallic strains*

(A, aneuploid clones detected; P, clones with parental genotypes detected; R, clones with recombinant genotypes detected; S, rapid selfing (Het⁻) clones detected.)

Parental Het⁻	No. of Npf⁻ derivatives	Npf⁻ phenotypes	Crossed with heterothallic	Total no. of progeny from crosses	Analysis of progeny from crosses
NH35	2	Class 1 *npfC⁻*	*OUD3*	36	P+R
NH48	3			60	P+R
NH49	1			34	P+R
NH01	2			37	P+R
NH01	1			16	Only Npf⁻ P
NH34	2	Class 2 *NpfC⁻*	*OUD3*	40	P+R
NH49	2	Class 3 *npfB⁻*	*OUD3*	37	P+R
NH51	1			12	P+R
NH01	3			57	P+R
NH01	1			15	9A+P+R
NH34	2			37	P+R
NH34	1			19	12A+Npf⁻
NH35	1			19	P+R
NH35	1			16	4A+P+R
NH48	2			38	P+R
NH48	1			20	3A+P+R
NH35	1	Class 4	*OUD3*	19	P+R
NH49	2			42	P+R
NH48	5			113	P+R
NH48	1			17	Only Npf⁻ (P+R)
NH35	3	Class 5	*i*	32	18A+P+R
NH45	4	Class 7	*i*	76	73 Npf⁻ P+ 3 mt_2 P
NH45	1	Class 8	*i*	20	Only Npf⁻ P
NH45	2	Class 8	*i*	25	17A+P
NH45	1	Class 8	*OUD7*	64	17S+P+R

parental genotypes, indicating that the plasmodia had passed through diploidy. Sixty-three of the progeny clones, however, had aberrant characteristics, such as abnormal plaque morphologies, abnormal selfing characteristics, poor amoebal or plasmodial viabilities, and heterozygosity at the *fusA* locus.

The progeny analyses allowed the possible linkage of the Het⁻ and Npf⁻ mutations to *mt* to be investigated. The *npfC* locus has previously been found to be closely linked to the *mt* locus (Anderson & Dee, 1977) and the present data are consistent with this conclusion. If a Het$^-$ mutation was unlinked to *mt npfC*$^-$, then a cross with a heterothallic strain might be expected to result in 25% progeny that selfed rapidly. This was not observed for any Het$^-$ *npfC*$^-$ mutant analysed, suggesting that these Het$^-$ mutations (present in the clones *NH01*, *NH34*, *NH35*, *NH48*, and *NH49*) are probably all linked to *npfC*, and thus also to *mt*. The class-3

Table 4. *Summary of complementation tests between different classes of Npf*$^-$ *mutants*

(+, complementation, crossed plasmodia detected; −, no complementation; +/−, only a proportion of the mutants complemented; NT, complementation test not performed.)

Class	Class 1 *npfC*$^-$	Class 2 *npfC*$^-$	Class 3 *npfB*$^-$	Class 4	Class 7	Class 8
1	−	−	−	+	NT	NT
2	−	−	+	+	+	+
3	−	+	−	+	+	+
4	+	+	+	+/−	NT	NT
7	NT	+	+	NT	−	−
8	NT	+	+	NT	−	−

mutant isolated from *NH51* may be a mt_2 revertant of the Het$^-$ mutation, and therefore the possible linkage of this Het$^-$ mutation and *mt* cannot be determined. Only the mutant *NH4511* had 25% progeny that selfed rapidly, indicating that either the mt_1-derived Het$^-$ or the Npf$^-$ mutation is unlinked to the *mt* locus.

(iii) *Analysis of Npf*$^-$ *mutants*

The Npf$^-$ mutants were initially characterized on the basis of their crossing patterns with four tester strains, then subsequently examined in greater detail. The complementation data are summarized in Table 4. All of the *npfC*$^-$ mutants isolated in this work and all of the 11 NpfC$^-$ (difA$^-$) mutants isolated from *CL* by Honey *et al.* (1979) were combined in different pair-wise combinations. No crossed plasmodia were detected. Thirteen of the *npfB*$^-$ mutants isolated in this work and six of the *npfB*$^-$ (*difB*$^-$) mutants isolated from *CL* by Honey *et al.* (1979) were combined in different combinations. No crossed plasmodia were observed.

The *npfC*$^-$ mutants *NH0106VI*, *NH4811VI* and *NH4814VI* were combined with each of 23 different *npfB*$^-$ mutants isolated from six Het$^-$ strains and *CL*, but no crossed plasmodia were observed. Seven Npf$^-$ mutants isolated from *NH48* (*npfC*$^-$, *npfB*$^-$, and class-4 mutants) were combined with each other in all possible pair-wise combinations. Crossed plasmodia were only observed in the combinations of the class 4 mutants with the *npfC*$^-$ and *npfB*$^-$ clones.

Four Npf$^-$ derivatives of *NH34* were combined with eight Npf$^-$ derivatives of *NH48*. Crossed plasmodia were observed in the combination *npfC*$^-$ (*NH34*

Table 5. *Complementation tests between class 4 mutants*

(Numbers of plasmodia formed: —, no plasmodia formed; ×, cross not performed; *, number of plasmodia formed in pure clones.)

	NH4805VI	*NH4717VI*	*NH3505VI*	*NH4810VI*	*NH4806VI*	*NH4801VI*	*NH4807VI*	*NH4909VI*	*OUD3*	*i*
NH4805I	*82	100	89	3	44	125	3	55	10^3	10^3
NH4817I	×	*49	×	×	×	×	×	×	×	×
NH3505I	11	21	*6	—	2	1	1	39	10^3	10^3
NH4810I	3	13	1	*2	2	—	—	76	10^3	10^2–10^3
NH4806I	3	20	3	—	—	—	—	25	10^3	10^3
NH4801I	18	39	4	—	—	—	—	22	10^3	10^3
NH4807I	—	4	2	—	—	—	—	60	10^3	10^3
NH4909I	10^2–10^3	29	25	10^3	7	10^2–10^3	31	—	10^3	77
LU648	10^3	60	92	10^3	51	10^3	79	27	×	10^3
LU688	11	—	1	4	—	1	—	—	10^3	×

origin) × *npfB*⁻ (*NH48* origin), but not in the reversed combination *npfC*⁻ (*NH48* origin) × *npfB*⁻ (*NH34* origin).

Eight class-7 mutants (isolated from *NH45*) were combined with ten *npfB*⁻ (mt_2 revertants) isolated from five Het⁻ strains and *CL*. Crossed plasmodia were readily formed in each combination. Nine class-7 and class-8 mutants isolated from *NH45* were combined with each other in all possible combinations. No rapidly forming plasmodia, indicating the occurrence of complementation, were observed in any combination.

Table 6. *Relative rates of crossing between different amoebal strains*

(+, slow crossing [a few plasmodia at ⩾ 4–5 days]; + +, moderate crossing [a moderate number of plasmodia at 2–3 days]; + + +, fast crossing [many plasmodia at ⩽ 2 days].)

	mt_1 *LU648*	*OUG3*	*a*	*OUC8*	*OUD3*	*OUD7*
mt_h *CLd*	+	+	+ +	+ +	+ +	+ +
mt_2 *LU688*	+	+	+ +	+ +	+ +	+ +
OUD1	+	+	+ + +	+ + +	+	+ +
OUA9	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
i	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +

(iv) *Class-4 mutants*

The class-4 mutants were examined in greater detail by combining them in pair-wise combinations and observing the formation of plasmodia. The pairs of mutants had suitable fusion genotypes such that any crossed plasmodia would be fusion class IV. The mutants were also crossed with mt_1 and mt_2 heterothallic strains. Each pair of mutants was combined by making suspensions of the clones in distilled water (at 10^6 amoebae per ml) and spreading 0·05 ml of each suspension onto a 5 % SDM plate. The formation of plasmodia was quantitated by carefully counting the total number of plasmodia formed on each cross-plate (Table 5). Each plasmodium was destroyed with a Gallenkamp electrode immediately after it was counted. A number of crosses formed so many plasmodia ($> 10^3$) that they could not be counted accurately and an approximation of 10^3 was estimated in these cases. The pairs of mutants were allowed to self and the numbers of plasmodia formed counted. The average of each pair of numbers was calculated and is indicated on the diagonal of Table 5. These figures thus indicate the approximate numbers of selfed plasmodia formed on the different cross-plates. A range of plasmodia from the cross plates were confirmed crossed by fusion tests.

Wide variations in the numbers of plasmodia formed in the crosses between the mutants and the heterothallic strains were observed. In order to study this phenomenon further, a number of heterothallic strains were crossed in pairs and the appearance of plasmodia noted (Table 6). The pattern observed was easily distinguished and quite reproducible.

4. DISCUSSION

In this paper we describe the isolation and analysis of 64 Npf⁻ mutants derived from six independently isolated Het⁻ clones. Eight classes of mutants displaying different patterns of crosses with the four tester strains were observed. The ability of different mutations to cross is affected by both their respective mating type specificities and their Npf⁻ mutations. Thus Npf⁻ mutants will not cross with heterothallic clones of the same *mt*. This factor complicates the interpretations of the natures of the Npf⁻ mutations. Twelve of the Npf⁻ mutants did not cross with the $npfC^-$ tester and were classified as $npfC^-$. These mutants did not display a homogeneous behaviour when crossed with the mt_2 or $npfB^-$ testers. This is likely to be a reflexion of the full mt_2 specificities of *NH01*, *NH35*, *NH48*, and *NH49* and the partial mt_2 specificity of *NH34* (with a reduced ability to cross mt_2 heterothallic amoebae, similar to *CL*). In general, the $npfC^-$ mutants only selfed following reversion of $npfC^-$ to $npfC^+$ and thus the progeny of the selfed plasmodia resembled the original Het⁻ strains. Further analysis of the $npfC^-$ mutants showed that they were all due to single mutations closely linked to the *mt* locus. The analysis showed that the Het⁻ mutations were probably also closely linked to *mt*.

Nineteen Npf⁻ mutants resembled the *npfB* complementation group isolated from *CL*. The $npfB^-$ mutants derived from *NH34* and *CL* did not cross with mt_2 strains, even though the parental selfing clones had only a partial mt_2 specificity (that is, could cross occasionally with mt_2 testers, Adler & Holt, 1978; Honey *et al*. 1981). It is not clear how the mutagenesis of a *npfB* gene would result in this change in *mt* specificity. A number of the mutants selfed on an occasional basis, and the progeny of all of these selfed plasmodia retained the Npf⁻ rare selfing phenotype. Selfing therefore did not occur following the reversion of a Npf⁻ gene to Npf⁺, but probably occurred by a leaky mechanism. The 19 $npfB^-$ mutants crossed with $npfC^-$ mutants with a partial mt_2 specificity, but failed to cross with any $npfC^-$ mutant with a full mt_2 specificity. This behaviour would be expected of revertants to the mt_2 heterothallic state, but not of mutants in a Npf gene. This group of mutants may therefore be better described as mt_2 revertants.

The ten Npf⁻ mutants in class 4 displayed an unexpected phenotype. They crossed with the mt_1, mt_2 and $npfC^-$ testers, and in some cases with the $npfB^-$ tester. Although these mutants crossed with the $npfC^-$ mutants with both partial and full mt_2 specificities, they did not seem to represent a separate *npf* gene. The mutants did not self by the reversion of Npf⁻ to Npf⁺, but apparently by a leaky mechanism. The mutations are closely linked to the *mt* locus as shown by progeny analysis. They were all isolated from Het⁻ strains with a full mt_2 specificity but now had only a partial mt_2 specificity and crossed occasionally with mt_2 testers.

Eight of the ten class-4 mutants were crossed with each other and with heterothallic strains, but did not show a homogeneous response (Table 5). The mutants *NH4805I*, *NH4817I*, *NH3505I*, *NH4810I*, *NH4806I*, *NH4801I*, *NH4807I* (all $fusA_1$ $fusB_1$) and their $fusA_2$ $fusB_2$ derivatives did not cross with each other. They crossed readily with *i* but not at all, or only very rarely, with *LU688*.

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These mutants therefore retained a certain degree of mt_2 specificity. *NH4909I* and its derivative *NH4909 VI* crossed moderately readily with the other mutants, but only very rarely with *i*. The mutant therefore had a marked degree of mt_2 specificity (and resembled normal mt_2 strains) but did not resemble the other mutants at all. The nature of the mutations is not clear, but seems to involve the *mt* locus in some way.

Fifteen of the Npf⁻ mutants isolated from *NH45* (mt_1-derived Het⁻) did not cross with the mt_1 test strain, but did cross with all test strains of mt_2 origin. These mutants resembled revertants to the mt_1 heterothallic state, analogous to the mt_2 revertant (class 3) clones. The four mutants in class 8 did cross with mt_1 on an occasional basis. The nature of the defects is uncertain, but may be analogous to the class-4 mutants of mt_2 origin. The analysis of the class-8 mutant *NH4511I*, however, suggested the presence of a mt_1-derived Het⁻ or Npf⁻ mutation unlinked to the *mt* locus. Only a single mt_1 Het⁻ strain had been available for mutagenesis, and the isolation of more Npf⁻ mutants from other Het⁻ clones is desirable. A range of possible Npf⁻ mutations could then be looked for and compared to those of mt_2 origin.

The data summarized in Table 4 indicate the presence of modifying factors affecting the rates of crossing separate from the effects of the Npf⁻ mutations, these modifying factors being similar or identical to the *matB* (or *rac*) locus (Anderson, 1979; Dee, 1978).

The mutants *NH4801I* → *NH4807I* (all isolated from *LU688*) displayed a homogeneous behaviour when crossed with *OUD3*, *i*, and *NH4909 VI*. Their $fusA_2$ $fusB_2$ derivatives (isolated from crosses with *OUD3*) showed a variation in the number of plasmodia formed (by a factor of 10 or more) when crossed with *LU648* and *NH4909I*. This suggests the segregation of two modifying alleles at a single locus.

A number of different heterothallic strains were crossed together in pairs and the formation of plasmodia observed (Table 6). There is a good correlation between the total number of plasmodia formed and the time of appearance of plasmodia. The data support the suggestion of the segregation of alleles modifying the rates of crossing. However, the segregation appears more complex than that of two alleles at a single gene. The Wisconsin strains used in this work plus the strains of *CL* origin have the *matB1* alleles (Anderson, 1979; Dee, 1978). The heterothallic amoebal strains described here are all derived from *CL* and the Wisconsin isolate and should be *matB1*, suggesting that the modifying alleles detected here may be different from *matB1* or *matB2*.

The genetic control of plasmodial formation in *P. polycephalum* is complex, with the *mt* region playing a crucial role in this process. In addition to the *mt* gene, most of the selfing (Het⁻) and almost all of the non-selfing (Npf⁻) mutations studied are closely linked to the *mt* locus (Adler & Holt, 1977; Anderson & Dee, 1977; Gorman *et al.* 1979; Poulter & Honey, 1979; Shinnick & Holt, 1977). The *matB* gene is unlinked to *mt* and affects plasmodial development by modifying the rate of crossing of amoebae. The *mt* locus has many alleles and the combination of any two alleles will permit plasmodial formation, suggesting that this process is unlikely to require a specific interaction of different *mt* alleles, analogous to the yeast mating type. The single *mt* allele present in an amoeba prevents plasmodial formation, while the combination of two different *mt* alleles in a cross relieves this block. Youngman, Anderson & Holt (1979) suggest that *matB* controls cell fusion and *mt* controls zygote formation. The control over plasmodial development may be negative, with a single *mt* allele in an amoeba inhibiting differentiation (Honey *et al.* 1979). Alternatively, control may be positive, with the presence of two different *mt* alleles being necessary to initiate differentiation (Anderson & Dee, 1977).

In conclusion, the Npf$^-$ mutants provided an analysis of the amoebal differentiation system of *P. polycephalum*. A *npf* gene closely linked to the *mt* locus was observed; a group of revertants to the heterothallic state were identified; and a group of mutants of a novel type were isolated.

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