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A new type of plasmodium formation in *Physarum polycephalum*

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SUMMARY

Haploid amoebae of *Physarum polycephalum* may form plasmodia sexually by 'crossing', which involves cellular and nuclear fusion, or asexually by 'selfing', which occurs without nuclear fusion. In most amoebal strains, selfing is seen in clonal cultures only at very low frequency. In the present study, we have shown that selfing occurs at a similarly low frequency in mixtures of crossing-incompatible amoebae, but is stimulated in crossing-compatible mixtures. In certain compatible mixtures involving mutant strains, where crossing is temperature-sensitive, selfing may be stimulated even at a temperature that largely or wholly abolishes crossing. The extent to which selfing is stimulated appears to be influenced by *matB*, a locus which is known to affect the frequency of amoebal fusion. We have failed to detect any filter-transmissible factor that might be responsible for the effects we have observed. We suggest a sequence of events that might bring about 'stimulated selfing' as a consequence of abortive crossing.

1. INTRODUCTION

The life cycle of *Physarum polycephalum*, a Myxomycete or true slime mould, comprises two alternating, vegetative forms: uninucleate amoebae which grow and divide by binary fission, and multinucleate syncytia called plasmodia, which grow without regular cell divisions and may attain diameters of many centimetres. The conversion of amoebae into plasmodia has attracted interest as an example of eukaryotic cell differentiation that is amenable to the combined application of genetic and biochemical analyses. For recent reviews, see Gorman & Wilkins (1980); Haugli, Cooke & Sudbery (1980) and Dee (1982).

Plasmodium formation in *P. polycephalum* may occur sexually or asexually. In sexual development ('crossing'), pairs of haploid amoebae undergo cellular and nuclear fusion to generate diploid zygotes, which differentiate into diploid plasmodia. Crossing is under the control of a mating-type system, such that plasmodia

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form in this way only when compatible amoebal strains are grown together in mixed culture. There are two unlinked mating-type loci, both of which are multiallelic (Dee, 1966, 1978; Youngman *et al.* 1979). Heteroallelism for the *matA* locus (also known as *mt*) is normally essential to permit zygotes to differentiate into plasmodia (Youngman, Anderson & Holt, 1981), while the second mating-type locus, *matB*, influences the efficiency with which amoebae fuse to form zygotes. Mixtures homoallelic for *matB* yield 10^2–10^3 times fewer zygotes than are formed in heteroallelic mixtures (Youngman *et al.* 1979).

Although the mating-type system does not allow crossing to occur in clonal cultures, amoebal strains grown alone may generate plasmodia. Such plasmodia arise by an asexual pathway ('selfing') which apparently occurs without nuclear fusion (Cooke & Dee, 1974; Adler & Holt, 1975). In some strains, such as the Colonia strain (Wheals, 1970), selfing may involve more than 10% of the amoebae in a culture (Youngman *et al.* 1977), but in most strains selfing is a very rare event. Strains that self only rarely are described as heterothallic. They typically yield plasmodia in clonal cultures at a frequency of less than one in 10^8 amoebae (Adler & Holt, 1975, 1977). In contrast, about one in four of the amoebae in a mixed culture of heterothallic strains may participate in crossing if the mixture is heteroallelic for both *matA* and *matB* (Youngman *et al.* 1981).

During the course of experiments designed to detect crossing between certain mutant, heterothallic strains in weakly compatible mixtures (Anderson, 1976), the amoebae were marked genetically in such a way that plasmodia formed by selfing within the mixtures would be distinguishable from those formed by crossing. Unexpectedly, selfing seemed to occur far more frequently in the mixed cultures than in corresponding clonal controls. We have now characterized this 'stimulated selfing' and report preliminary studies which suggest that the phenomenon may occur as a result of abortive crossing events.

2. MATERIALS AND METHODS

(i) *Amoebal strains.* All amoebae were of inbred 'Colonia background' strains (Cooke & Dee, 1975; Adler & Holt, 1974) or were derived from such strains. Four of the fourteen known *matA* alleles (Collins & Tang, 1977) were represented, in combination with three of the thirteen known *matB* alleles (Kirouac-Brunet, Masson & Pallotta, 1981).

(ii) *Culture methods and media.* Amoebal culture (Anderson, 1979), plasmodial culture (Adler & Holt, 1974), sporulation (Wheals, 1970), plasmodial fusion tests (Anderson, 1977) and preparation of dilute plasmodial rich medium (dPRM) agar (Adler & Holt, 1974) were as described elsewhere. Dilute simplified medium (dSM) agar, which was essentially equivalent to dPRM agar, was a 20-fold dilution of simplified medium (Brewer & Prior, 1976) in 1·5% agar. Sometimes dSM was supplemented with 10 mM-$MgSO_4$ (dSM+S) to enhance crossing (Shinnick *et al.* 1978).

(iii) *Selfing assay.* Selfing was studied in 'spot cultures' (Youngman *et al.* 1979) on dPRM, dSM or dSM+S agar in 10 cm Petri dishes. These media were employed because they gave good growth of plasmodia and allowed easy scoring of

plasmodial colours. Tests of *gad*$^-$ *npf*$^-$ strains (see Results) were usually performed on dSM + S agar, since crosses of such strains often yielded few plasmodia. Other tests, with the exception of some *matB*-homoallelic combinations, were performed on dPRM or dSM agar. Each culture was inoculated with $1–2 \times 10^4$ amoebae as well as $10^8–10^9$ live *Escherichia coli* as food, and was incubated for up to 14 days, usually at 26 °C. Stimulation of selfing was detected by comparing the frequencies of selfing in mixed and clonal cultures involving the same pair of strains. Since the strains in most mixtures were crossing-compatible, it was necessary to distinguish plasmodia formed by selfing from those formed by crossing. To achieve this end, the members of each pair were marked with different alleles of at least two loci. The mixtures were nearly always heteroallelic for the *fusA* locus (Poulter & Dee, 1968; Adler & Holt, 1974), which controls plasmodial fusion. Plasmodia with identical fusion phenotypes fuse freely on contact, but non-identical plasmodia do not fuse. Since the *fusA* alleles are codominant, plasmodia formed by selfing of either amoebal strain in the mixture would not fuse with hybrid plasmodia resulting from crossing. One strain of each pair carried the recessive mutant allele *whi-1* (Anderson, 1977) which confers white colouring upon plasmodia in place of the yellow colour of *whi*$^+$ strains. Inspection of the cultures allowed selfing of the *whi-1* strain to be detected, since the white plasmodia were easily distinguishable from yellow plasmodia formed either by crossing or by selfing of the *whi*$^+$ strain. Neither *fusA* nor *whi* appears to have any effect upon crossing or clonal selfing. It also seems likely that stimulated selfing is unaffected by these loci: both *fusA1* and *fusA2* strains proved capable of being stimulated by strains carrying the alternative allele, and stimulated selfing was demonstrated in two *whi*$^+ \times$ *whi*$^+$ mixtures by testing the individual fusion phenotypes of 299 plasmodia picked at random from the mixtures.

3. RESULTS

(i) *Detection of stimulated selfing*

Haploid, heterothallic amoebal strains were mixed in pairs in order to ascertain whether selfing would occur more readily in the mixed cultures than in clonal control cultures. One member of each pair carried the recessive mutation *whi-1*, which confers on plasmodia a white coloration readily distinguishable from the wild-type yellow (Anderson, 1977). The other member carried the dominant *whi*$^+$ allele. Crossing between *whi-1* and *whi*$^+$ amoebae, or selfing of *whi*$^+$ strains, results in the formation of yellow plasmodia. Thus, selfing of *whi-1* amoebae was specifically detectable by the generation of white plasmodia. By determining the frequencies with which white plasmodia arose in control cultures of each *whi-1* strain alone, and in mixtures of the same *whi-1* strain with various *whi*$^+$ strains, it was possible to compare the clonal selfing frequency of the *whi-1* strain with its selfing frequencies in the various mixtures.

Eighty-five pairs of strains were tested. In each test, about twenty cultures contained the mixture of *whi-1* and *whi*$^+$ amoebae, and a similar number of control cultures contained only amoebae of the *whi-1* strain. The clonal controls of most *whi-1* strains formed no plasmodia at all. Out of a total of 852 clonal cultures scored

in this study, only six (0·7 %) contained any plasmodia, and no more than two plasmodia were observed in any individual culture. The maximum proportion of clonal cultures yielding plasmodia for any strain was two out of twenty, and this high frequency was shown by only one strain. Thus, in interpreting the results obtained with mixed cultures, we define stimulated selfing as the formation of white

Table 1. *Tests of stimulated selfing by* CH929 *amoebae*

	Numbers of cultures		
	With white plasmodia	With yellow plasmodia	Total
Clonal control:			
CH929 (*matA1 matB3 whi-1*)	0	0	19
Mixtures:			
CH929 × CH928 (*matA1 matB3 whi*$^+$)	0	0	20
CH929 × CH934 (*matA2 matB3 whi*$^+$)	8	20	20
CH929 × CH815 (*matA3 matB3 whi*$^+$)	4	20	20
CH929 × CH941 (*matA4 matB3 whi*$^+$)	18	20	20

plasmodia in more than two out of a set of twenty replicate cultures. Typical results from a series of mixtures involving one *whi-1* strain and several *whi*$^+$ strains are shown in Table 1, which demonstrates stimulated selfing in three of the four mixtures tested.

Among the mixtures that exhibited stimulated selfing, the extent of the stimulation was variable, both in the numbers of cultures yielding white plasmodia, and in the numbers of white plasmodia that formed in each culture. At one extreme of behaviour were a few mixtures which formed up to twenty white plasmodia in every culture; at the other extreme were several mixtures that formed one or a few plasmodia in only three cultures out of twenty. Some of the variation between different mixtures was clearly not significant, since large differences were sometimes observed between repeated tests on the same mixture, even to the extent that a mixture might not always show stimulation in every test. Because of this inconsistency in our results, failure to detect stimulation in one test was not, by itself, taken to indicate that a mixture was necessarily unable to exhibit stimulated selfing.

(ii) *Effect of crossing compatibility on stimulated selfing*

An obvious difference between mixed and clonal cultures of amoebae is that crossing occurs frequently in mixed cultures, but never in clonal ones. In order to determine whether stimulation of selfing might be associated only with cultures in which crossing occurred, we compared selfing in crossing-compatible and crossing-incompatible mixtures. The major determinant of crossing is, ordinarily, the *matA* locus. The effect of *matA* upon selfing stimulation was examined by testing mixtures which represented all possible combinations of the four available *matA* alleles (see Table 2). This analysis included tests homoallelic and heteroallelic for *matB*. Four or five *matA*-homoallelic mixtures were tested for each *matA* allele,

and in no test was either crossing or stimulated selfing detected. Moreover, the overall frequency of selfing associated with each *matA* allele in these homoallelic mixtures was essentially the same as in clonal cultures. Between four and seven mixtures were tested for each heteroallelic combination of *matA* alleles. Crossing was detected in every test, and was shown to be associated with stimulated selfing

Table 2. *Fraction of cultures showing white plasmodia for each combination of* matA *alleles*

	matA1 whi$^+$	*matA2 whi*$^+$	*matA3 whi*$^+$	*matA4 whi*$^+$	Clonal controls
matA1 whi-1	0·7 % (1/136)	20·6 % (35/170)	30·2 % (42/139)	57·8 % (78/135)	0·6 % (1/166)
matA2 whi-1	44·3 % (51/115)	0·0 % (0/92)	39·0 % (30/77)	30·0 % (27/90)	3·0 % (4/134)
matA3 whi-1	17·6 % (15/85)	31·5 % (29/92)	1·0 % (1/97)	32·3 % (29/90)	0·0 % (0/99)
matA4 whi-1	26·7 % (31/116)	29·9 % (81/271)	21·0 % (25/119)	0·0 % (0/120)	0·0 % (0/210)

in at least two mixtures for each combination of alleles. Overall, the frequency of cultures showing selfing for each *matA*-heteroallelic combination was at least ten-fold higher than the corresponding frequency in clonal controls of the *whi-1* strains, or in *matA*-homoallelic mixtures. Thus, stimulation of selfing was detected only in pairs of strains that crossed with one another.

Although heteroallelism for *matA* is normally essential to permit zygotes to differentiate into plasmodia, crossing occurs readily in certain mixtures that appear to be homoallelic for *matA* (Shinnick, Anderson & Holt, 1983). One of the participating strains in each mixture is a double mutant of a particular kind, designated *gad*$^-$ *npf*$^-$. The precise nature of the mutations is unclear, and one possibility is that some of them may represent changes in *matA* itself. For a full discussion of this idea, see Anderson & Holt (1981). To determine whether stimulated selfing could be associated with crossing that was dependent upon *gad*$^-$ *npf*$^-$ double mutants, we tested mixtures that included such strains. Three out of six double-mutant strains stimulated selfing of heterothallic *whi-1* strains with which they were mixed (see Table 3). Since the three strains that did not stimulate selfing in these crosses were tested only once each, it is possible that they would have caused stimulation in further tests. We conclude that stimulated selfing is not restricted to crosses of the 'ordinary' type, but can also occur in at least some of these mutant crosses.

As shown in Table 3, the mutant mixture OX115 × CH839 was tested at three temperatures. These strains were incompatible under the crossing conditions used in a previous study (Shinnick *et al.* 1983) but we found that crossing in the mixture was temperature-dependent, occurring in all cultures at 21 °C, but in none at 30 °C. At 26 °C, half the cultures formed no yellow plasmodia, and half formed one or two, of which five were tested and found to have been formed by crossing. Stimulated selfing was detected at both 21 °C and 26 °C, but not at 30 °C. Although only half the 26 °C cultures yielded any yellow plasmodia, every one yielded

between ten and twenty white plasmodia. Thus, in some cultures, selfing of the *whi-1* strain was stimulated in the absence both of crossing and of selfing by the *whi*$^+$ double mutant. A similar result (not shown) was obtained when a second temperature-sensitive cross (CH932 *matA2 gad*$^+$ *npf*$^+$ *whi-1* × RA245 *matA2 gad-14 npfA1 whi*$^+$) was performed at its non-permissive temperature of 26 °C. Selfing of

Table 3. *Stimulated selfing in crosses involving double mutants*

	Temp. (°C)	Number of cultures: With white plasmodia	With yellow plasmodia	Total
LU910 (*matA3 matB3 whi-1*)	26	0	0	19
LU910 × CH840 (*matA3 matB1 whi*$^+$ *gad-11 npfF1**)	26	17	17	17
CH942 (*matA4 matB3 whi-1*)	26	0	0	20
CH942 × OX133 (*matA4 matB1 whi*$^+$ *gad-13 npfF1*)	26	1	19	19
CH932 (*matA2 matB2 whi-1*)	26	0	0	20
CH932 × CH946 (*matA2 matB1 whi*$^+$ *gad-14 npfF1*)	26	16	16	16
CH931 (*matA2 matB2 whi*-1)	26	0	0	19
CH931 × APT1 (*matA2 matB1 whi*$^+$ *gad-h npfF1*)	26	0	20	20
CH931 × CL6143 (*matA2 matB1 whi*$^+$ gad-h npfC*3*)	26	0	20	20
OX115 (*matA3 matB1 whi-1*)	21	0	0	15
OX115 (*matA3 matB1 whi-1*)	26	0	0	20
OX115 (*matA3 matB1 whi-1*)	30	0	0	20
OX115 × CH839 (*matA3 matB3 whi*$^+$ *gad-11 npfA1*)	21	11	15	15
OX115 × CH839 (*matA3 matB3 whi*$^+$ *gad-11 npfA1*)	26	20	10	20
OX115 × CH839 (*matA3 matB3 whi*$^+$ *gad-11 npfA1*)	30	0	0	20

* Formerly designated *aptA1*. Information on the *gad* and *npf* mutations is given elsewhere (Anderson & Holt, 1981; Shinnick *et al.* 1983).

the *whi-1* strain did not occur in clonal controls, but was stimulated in all 17 mixed cultures examined, where it occurred in the complete absence of yellow plasmodium formation. It should be noted that, although these mixtures demonstrate stimulated selfing in the absence of crossing, they are mixtures in which crossing occurs readily under other conditions.

(iii) *Effect of* matB *on stimulated selfing*

The *matA*-heteroallelic crosses described in (ii) included all possible homoallelic and heteroallelic combinations of the three *matB* alleles used, and analysis of the results indicated that strains representing each of the three *matB* genotypes were stimulated in combinations both homoallelic and heteroallelic for *matB*. Selfing was detected in *matB*-homoallelic mixtures at about twice the frequency found in

heteroallelic ones: 39 % versus 18 % of cultures, respectively. Nevertheless, this was not necessarily a true comparison of the efficiencies with which white plasmodia were formed, since the 'background' levels of yellow (crossed) plasmodium formation were usually very different in homoallelic and heteroallelic mixtures. Crosses homoallelic for *matB* normally generated less than ten visible, yellow plasmodia per culture, while heteroallelic crosses generated so many yellow plas-

Table 4. *Stimulated selfing of* matA3 whi-1 *progeny of the cross OX110 × OX118**

	Numbers of progeny forming white plasmodia in mixtures with				
matB allele of progeny clone	CH839 (*matB3*) only	CH951 (*matB1*) only	CH839 > CH951	CH951 > CH839	Neither
matB1	5	0	2	0	1
matB3	0	7	0	0	1

* *matA2 matB1 whi-1 × matA3 matB3 whi-1.*

modia that they were not individually countable. It therefore seemed very likely that the efficiency of detection of white plasmodia in *matB*-heteroallelic mixtures was much lower than in *matB*-homoallelic ones, and we suspected that white plasmodia might, in fact, be generated more readily in the heteroallelic combinations.

In order to minimise the difference between background yellow plasmodium formation in *matB*-homoallelic and heteroallelic mixtures, we tested temperature-sensitive, double-mutant mixtures at a temperature that inhibited the formation of plasmodia by crossing. Two experiments were performed, and in both cases the results suggested that selfing was stimulated more strongly in *matB*-heteroallelic mixtures than in homoallelic ones. In the first experiment, CH839 (*whi*$^+$ *gad-11 npfA1 matA3 matB3*) was paired with OX115 (*whi-1 gad*$^+$ *npf*$^+$ *matA*3 *matB1*) and with a sibling strain, OX118 (*whi-1 gad*$^+$ *npf*$^+$ *matA3 matB3*). Twenty cultures of each mixture were tested at 26 °C. All cultures of the *matB*-heteroallelic mixture yielded ten to twenty white plasmodia, while only one or two white plasmodia were formed in each of the nineteen homoallelic, mixed cultures that yielded plasmodia. This difference in the extent of selfing by OX115 and OX118 was maintained in two subsequent tests. In the second experiment, sixteen *whi-1 matA3* progeny were obtained from a single cross, eight progeny carrying the *matB1* allele, and eight *matB3* (see Table 4). All sixteen sibling strains were tested both against CH839, which carries the *matB3* allele, and against a similar strain that carries *matB1*. Of fourteen progeny that gave rise to white plasmodia in these mixtures, twelve formed white plasmodia only in the *matB*-heteroallelic combination, and two gave white plasmodia in both combinations, but to a greater extent in the heteroallelic one. We infer from these two experiments that *matB* influences selfing stimulation. We cannot, of course, entirely exclude the possibility that some gene closely linked to *matB* might be responsible for the effects we observed.

(iv) *Tests for an extracellular stimulator of selfing*

One explanation for stimulated selfing of heterothallic strains might be that unfused, haploid amoebae are stimulated to self by an extracellular factor that acts in mating-compatible mixtures, but not in incompatible mixtures or in clonal cultures. The production by haploid amoebae of one or more extracellular factors has already been demonstrated (Youngman *et al.* 1977; Pallotta *et al.* 1979). These factors are probably necessary for crossing of heterothallic strains, and for clonal

Table 5. *Tests for a diffusible stimulator of selfing*

	Upper cultures	Lower cultures	Cultures with white plasmodia	Cultures with yellow plasmodia	Total tests
(*a*)	CH926 (*matA1 matB2*)	LU863 (*matA4 matB2*)	0 (0)*	0 (0)	20
	CH926	CH926 × LU863	0 (17)	0 (18)	18
	CH926	CH926	0 (0)	0 (0)	18
(*b*)	LU910 (*matA3 matB3*)	CH840 (*matA3 matB1 gad-11 npfF1*)	0 (0)	0 (0)	19
	LU910	LU910 × CH840	0 (19)	0 (19)	19
	LU910	LU910	0 (0)	0 (0)	19
(*c*)	CH932 (*matA2 matB2*)	RA245 (*matA2 matB1 gad-14 npfA1*)	1 (0)	0 (0)	19
	CH932	CH932 × RA245	0 (18)	0 (0)	18
	CH932	CH932	0 (1)	0 (0)	19

* Numbers for lower cultures are shown in parentheses following those for upper cultures.

selfing of at least some strains, but there is no evidence that they show the pattern of strain-specificity that would be necessary to account for the stimulated selfing we have observed (Youngman *et al.* 1977; Shipley & Holt, 1982). Indeed, there is evidence of inter-species activity of factors from *P. polycephalum* and another Myxomycete, *Didymium iridis* (Youngman, 1979).

We performed tests to determine whether clonal cultures of heterothallic, *whi-1* amoebae could be stimulated to self by exposure to extracellular factors from mating-compatible strains. These tests employed three mixtures that had previously been shown to exhibit stimulated selfing (Table 5). For each mixture, clonal cultures of the *whi-1* strain were grown on top of Nuclepore filters, beneath which were growing clonal cultures of the *whi*$^+$ strain, or mixed cultures of the *whi-1* and *whi*$^+$ strains. Control experiments were inoculated with clonal cultures of *whi-1* amoebae both above and below the filters. The pore size of the filters was 0·2 μm, which is too small to allow amoebae to pass through, but large enough to permit passage of diffusible molecules. The arrangement of filters that we used is the same as previously employed in the demonstration of extracellular factors by Youngman *et al.* (1977) and Pallotta *et al.* (1979). Although 54 of the 55 mixed, 'lower' cultures yielded white plasmodia, the results in Table 5 show that no trans-filter stimulation occurred in any of the experiments.

(v) *Nuclear behaviour during stimulated selfing*

It is necessary to consider the possibility that white plasmodia arising from stimulated selfing may be the products of both cellular and nuclear fusions. For example, if zygote formation were sometimes followed by mitotic crossing over between the *whi* locus and its centromere, it would be possible to generate a *whi-1/whi-1* plasmodium from a *whi*$^+$*/whi-1* zygote. Alternatively, elimination of chromosomes from a *whi*$^+$*/whi-1* zygote might sometimes generate a haploid or aneuploid *whi-1* hemizygote. The operation of such mechanisms of mitotic instability should be evident from the behaviour of other genes for which the amoebal mixtures were heteroallelic. In white plasmodia formed from unstable zygotes, heteroallelic markers on chromosomes other than the *whi* chromosome should either be heterozygous, or be derived with roughly equal frequency from either parent. In contrast, if stimulated selfing occurs in the absence of nuclear fusion, white plasmodia should always be identical to the *whi-1* parent at all loci. We tested white plasmodia, formed as a result of stimulation, to determine their genotypes at four loci in addition to *whi*. The four loci we selected were known, from meiotic analyses, to be unlinked both to the *whi* locus and to one another, but we have no direct evidence that they do not all lie far apart from one another on the *whi* chromosome. Nevertheless, because the haploid chromosome complement of *P. polycephalum* is about forty (Mohberg, 1977), we estimate the probability of such an arrangement at only one in $(40)^4$, or approximately 4×10^{-7}.

The white plasmodia tested were the following: 103 from the cross CH322 × CH942 (*matA3 matB3 fusA2 fusC2 whi*$^+$ × *matA4 matB3 fusA1 fusC1 whi-1*) and 39 from the cross CH839 × OX115 (*matA3 matB3 fusA1 fusC1 whi*$^+$ *gad-11 npfA1* × *matA3 matB1 fusA2 fusC2 whi-1 gad*$^+$ *npf*$^+$). The plasmodia were all tested for expression of alleles at the loci *fusA* and *fusC* (Adler & Holt, 1974). All were found to express only the alleles carried by the *whi-1* parents. Since *fusA1* and *fusA2* are codominant, any white plasmodia heterozygous for *fusA* would have been distinguishable from the *whi-1* parental types. The *fusC2* allele is dominant to *fusC1*, however, and *fusC*-heterozygous white plasmodia would have been distinguishable from the *whi-1* parental type only in the cross CH322 × CH942. Six white plasmodia from the mixture CH322 × CH942 were induced to sporulate, and 51 amoebal progeny clones derived from the spores were tested for *matA* specificity. All 51 clones expressed *matA4*, the allele carried by the *whi-1* parent. Similarly seven white plasmodia from the mixture CH839 × OX115 were induced to sporulate, and 68 amoebal clones derived from the spores were tested for *matB* specificity. Again, all clones expressed the allele carried by the *whi-1* parent. Since none of the white plasmodia showed any evidence of genotypic contributions from the *whi*$^+$ parental amoebae, our results indicate that these plasmodia were not derived as a result of mitotic instability in *whi*$^+$*/whi-1* zygotes.

The germination efficiency of spores derived from haploid plasmodia is much lower than that of spores from diploid plasmodia (Laffler & Dove, 1977), typically 0·1 % versus 10 % respectively. Thus tests of germination efficiency provide a simple indication of plasmodial ploidy. The thirteen spore batches we analysed all showed germination efficiencies that suggested they were derived, as expected, from haploid rather than diploid plasmodia (data not shown).

4. DISCUSSION

We have shown that crossing-compatible mixtures of haploid, heterothallic amoebae yield not only diploid, crossed plasmodia, but also plasmodia that have arisen by selfing. Furthermore, the frequency of selfing in crossing-compatible mixtures is significantly higher than the frequency of selfing in clonal cultures, or in crossing-incompatible mixtures. It is thus desirable that the design of experiments should always anticipate the possibility that some of the plasmodia formed in a mixture will not have resulted from crossing. In extreme cases, all the plasmodia formed in a culture may be attributable to stimulated selfing.

We have failed to detect any filter-transmissible factor that might be responsible for stimulating unfused, heterothallic amoebae to self at the frequencies detectable in mating-compatible mixtures. Nevertheless, a negative result in experiments of this type cannot be regarded as entirely conclusive, and the existence of extracellular stimulators of selfing must remain an open question. The plasmodia formed as a result of stimulated selfing appear to be genetically identical to those arising from clonal selfing, and this seems to rule out the possibility that cellular and nuclear fusion might be followed by development of zygotes into stimulated selfs. We found that the extent of stimulated selfing was greater in mixtures heteroallelic for *matB* than in homoallelic mixtures. Since the only known effect of *matB* is to influence the frequency of amoebal fusions, and since stimulated selfing was greater in those mixtures in which amoebal fusion was favoured, our results are consistent with the idea that stimulated selfing might involve fusion of amoebal cells and subsequent development of the fused cells into plasmodia in the absence of nuclear fusion. Evidence from other sources makes this an attractive possibility.

Youngman *et al.* (1981) constructed diploid amoebae that were homozygous for *matA* alleles and did not carry *gad* or *npf* mutations. Clonal cultures of these diploid amoebae showed no greater tendency to form plasmodia than did clonal cultures of the relevant haploid strains, but plasmodia readily formed when diploid strains homozygous for different *matA* alleles were mixed. Unexpectedly, these plasmodia proved not to be tetraploid, but to be heterokaryons containing mostly unfused nuclei of the two diploid, parental types. The apparent failure of nuclear fusion in diploid × diploid 'crosses' is not fully understood. Nevertheless, as Youngman *et al.* pointed out, such behaviour suggests that plasmodium formation may be 'switched on' as soon as two different *matA* alleles are present within the same amoebal cell. Support for this view of *matA* function comes from recent experiments which indicate that fusion between haploid amoebae of unlike *matA* genotype is followed immediately by commitment of the fusion cell to plasmodial development, and only later by fusion of the two haploid nuclei to generate a diploid zygote (Shipley & Holt, 1982). These observations, together with our own results, lead us to suggest a possible sequence of events to account for stimulated selfing in *matA*-heteroallelic mixtures. Once fusion has taken place between haploid amoebae, the presence of unlike *matA* alleles within a binucleate fusion cell may cause the cell to become committed to plasmodium formation. If some committed fusion cells undergo binary fission before their haploid nuclei can fuse, these cells will not become zygotes, but will yield haploid daughters that may sometimes remain

committed to plasmodial development, and may ultimately become visible as selfed plasmodia. Our suggestion that stimulated selfing may depend on fission of binucleate cells is supported by observations of fission in binucleate cells that appeared to be plasmodial precursors (Anderson, Cooke & Dee, 1976).

The precise events of plasmodium formation are less well understood in crosses involving *gad*$^-$ *npf*$^-$ amoebae than they are in *matA*-heteroallelic crosses. Nevertheless, the mechanism that we have proposed to account for stimulated selfing in *matA*-heteroallelic crosses could also operate in mutant crosses if plasmodium formation in these, too, is 'switched on' while fusion cells are still binucleate, rather than later, when uninucleate zygotes have formed. The absence of crossing in some mutant mixtures that exhibited stimulated selfing is not necessarily inconsistent with the suggestion we have made, but further speculation here would be inappropriate. The properties of *gad* and *npf* mutations are detailed elsewhere (Anderson & Holt, 1981; Dee, 1982; Shinnick *et al.* 1983).

A phenomenon that may be similar to stimulated selfing in *P. polycephalum* has been observed in mixtures of haploid and polyploid amoebae of *D. iridis* (Therrien & Collins, 1976; Collins, Therrien & Betterley, 1978). Collins *et al.* interpreted their results in terms of a mechanism that does not seem applicable to the stimulated selfing we have observed in *P. polycephalum*: cellular and nuclear fusion followed by chromosomal elimination.

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