A HOMOTHALLIC STRAIN OF THE MYXOMYCETE *PHYSARUM POLYCEPHALUM*

A. E. WHEALS

Department of Genetics, University of Leicester, England

Received May 27, 1970

THE life cycle of the Myxomycete *Physarum polycephalum* comprises two alternating phases, a macroscopic multinucleate syncytial plasmodium and small uninucleate amoebae. Meiosis occurs during the formation of spores from the plasmodium and these spores hatch to give the haploid amoebae. The formation of plasmodia from amoebae in strains investigated so far has been shown to be heterothallic (DEE 1960) involving the fusion of two haploid amoebae and the subsequent fusion of their nuclei (Ross 1957). It is controlled by a mating-type locus (*mt*) at which four alleles are known (DEE 1966). A clone of amoebae carries only one mating type and plasmodia are normally formed only when clones of different mating type are mixed.

P. polycephalum is potentially useful for the study of differentiation since it allows investigation of gene action in two distinct phases of cellular organization and during the synchronous morphogenetic process of sporulation. Unfortunately, although genetic analysis has been shown to be possible (DEE 1962), progress has been slow because of the difficulty of selecting mutants. The uninucleate amoebae can be cultured only on bacteria so that the selective procedures and biochemical analyses which can be used on this stage are limited. The plasmodium can be grown in defined medium (DANIEL *et al.* 1963), has synchronous mitosis and sporulation (HOWARD 1932) and has been the subject of many biochemical studies (RUSCH 1970). It has not seemed worthwhile to attempt isolating mutants at this stage in the life cycle because the plasmodium is multinucleate, diploid, and arises only by outcrossing. These difficulties would be removed if a strain were found which gave rise to plasmodia in clones of amoebae, since these plasmodia would have identical nuclei and be homozygous at all loci. Although such strains are known in other Myxomycete species (Ross 1957), attempts to isolate one in *P. polycephalum* have so far failed. The present paper reports a reinvestigation of strain Colonia of *P. polycephalum*, originally received from Dr. H. A. VON STOSCH and classified by him as apogamic (VON STOSCH, VAN ZUL-PISCHINGER and DERSCH 1964). The results show that Colonia amoebae give rise to plasmodia in clones and also preferentially cross with amoebae of other mating type. The Colonia strain should be particularly useful in the selection of mutants in the plasmodium and their subsequent genetic analysis.

MATERIALS AND METHODS

Strains: The strain Colonia originally came from the Botanical Institute at Cologne; it was

studied by Dr. H. A. von Stosch (Botanical Institute, University of Marburg, Germany) and given to Dr. Dee (University of Leicester, England) in 1964 as an amoebal culture. It was maintained as an amoebal clone and did not give rise to plasmodia except when crossed with other strains. In 1968 some Colonia amoebae were inoculated on a thick streak of bacteria and unexpectedly gave rise to a plasmodium in a few days. All Colonia strains used in this work were descended from this plasmodium. Other strains used have been fully described elsewhere (Dee 1966).

Amoebal culture: Amoebae were cultured on agar at 25°C with a slowly growing strain of *Escherichia coli* carrying multiple nutritional requirements (Dee and Poulter 1970).

Plasmodial culture: Plasmodia were grown at 25°C on a slightly modified version of the semi-defined medium (SDM) specified by Daniel and Baldwin (1964) as described by Dee and Poulter (1970).

Plasmodium formation: Preliminary investigations showed that plasmodium formation could be enhanced by using a slight modification of the method of Dee (1966). *E. coli* was grown overnight in broth and harvested by centrifugation. The supernatant was poured off and the pellet resuspended in the remaining drops. The resulting thick suspension was streaked on liver infusion agar, LIA (20 g agar; 1 g Oxoid Liver Infusion powder; 1 l water), containing 0.4 mM *p*-aminobenzoic acid. When the streak was dry, a loopful of a suspension of a clone of amoebae was inoculated onto one end of the streak. For mating-type tests, two amoebal clones were inoculated at the same point on the streak. The plate was incubated until a plasmodium became clearly visible to the naked eye. The plate was then subcultured onto SDM agar for further growth. The plasmodia were cleaned from *E. coli* by allowing the plasmodium to migrate twice across fresh 2% water agar plates at pH 4.6.

Spore formation: To produce spores, plasmodia were inoculated on SDM agar plates. After about 5 days incubation, when the plate was covered with plasmodium and exhausted of most of its nutrients, it was inverted and allowed to stand in daylight but not in direct sunlight. After 3 or 4 days, most of the plates had produced spores .

Spore plating: Fresh sporangia were crushed in a small volume of water with a blunt glass rod and vigorously agitated with a Whirlmixer (Fisons Ltd. Loughborough, England) until a homogeneous spore suspension was obtained. Spore counts were taken with a hemacytometer, appropriate dilutions made, and 0.1 ml samples of various dilutions were plated on dry LIA plates together with a drop of thick *E. coli* suspension and were spread evenly until the plate was dry. After 5 days incubation amoebal plaques became visible on the bacterial lawn. In order to pick amoebae from well-separated plaques it is necessary to have less than 50 plaques per plate. Consequently several dilutions were plated to allow for variations in spore viability amongst the various strains used.

Isolation of clones from spore platings: A loopful of amoebae was taken from a well-separated plaque, suspended in 1 ml of water and 0.1 ml plated with bacteria on LIA plates as described above. This normally yielded less than 50 plaques per plate. One of these plaques was then used to establish a clone by inoculating amoebae onto an LIA slope with bacteria. The amoebae encyst after growth on the slope and remain viable for at least a year at room temperature (20°C). By picking from a number of different isolated plaques on the original spore plate and subsequent recloning, a series of clones was established, each having arisen from a different spore.

Plasmodial fusion test: Blocks were cut from plates showing vigorous plasmodial growth and placed 2 cm apart on SDM agar. The plasmodia migrated and grew towards each other and within 24 hr were in intimate contact and could be scored unambiguously for fusion or no fusion. This has been fully described and illustrated elsewhere (Poulter and Dee 1968).

RESULTS

The Colonia plasmodium was allowed to sporulate, the spores plated, and 42 clonal isolates (*C10–C51*) were picked. Two were subsequently lost.

Plasmodial formation in clones: All 40 clones were tested for plasmodial for-

TABLE 1

Summary of plasmodial formation in clones

Clonal series	Number of clones giving plasmodia 1st test	2nd test	3rd test	At least once	Total tested
C10–C51	32	34	34	40	40
C30.1–20 *C31.1–20* *C32.1–20*	49	54	..	55	60
C30.4.1–20 *C32.3.1–20* *C32.12.1–20*	51	50	..	54	60

mation on their own and these tests were repeated three times. Plasmodia appeared from the 5th to the 14th day after inoculation. All 40 clones gave a plasmodium at least once; 21 clones in all three tests; 12 clones in two tests and 7 clones in only one test (Table 1). These plasmodia which arose in clones *C10–C51* were given the same designation as the amoebal clones from which they arose.

Mating-type test: Each of the 40 amoebal clones was crossed with 4 clones of amoebae carrying the 4 known mating types. The results are shown in Table 2. Numerous plasmodia appeared on the 3rd day after incubation and only three plasmodia appeared after the 7th day. This is significantly faster than the rate of formation of plasmodia in clones. The majority of clones formed plasmodia with three or more of the tester strains. The simplest hypothesis to explain these results

TABLE 2

Mating behavior of clones (C10–C51 *series) derived from the original Colonia plasmodium crossed with amoebae of known mating type*

Tested clones	Tester strain *a* mt_1	*i* mt_2	*B173* mt_3	*B174* mt_4	Number of clones forming plasmodia
Colonia clones crossing with 4 strains	P*	P	P	P	6
Colonia clones crossing with 3 strains	—	P	P	P	4
	P	—	P	P	19
	P	P	—	P	4
	P	P	P	—	3
Colonia clones crossing with 2 strains	—	—	P	P	1
	—	P	P	—	1
	P	—	P	—	1
Colonia clones crossing with 1 strain	—	—	P	—	1
Totals for each tester					
Plasmodial formation (P)	33	20	35	34	..
No plasmodial formation (—)	7	20	5	6	..
Total tested	40	40	40	40	40

* P = plasmodial formation; — = no plasmodial formation.

is that all the clones (*C10–C51*) can cross with all four mating types and that a failure to form plasmodia is merely the result of unsuitable cultural conditions. Occasional failure to form plasmodia was observed in all tests. Three clones which failed to form plasmodia with 2 of the tester strains were retested twice against all four mating types. All three clones formed plasmodia with each of the four tester strains at least once, indicating that lack of plasmodial formation in any one test is not significant. Conclusive evidence that crossing occurs with all four mating types is presented below.

Tests of progeny of plasmodia C10–C51: All plasmodia gave rise to spores. The spores of 10 of the plasmodia *C10–C51* were plated and in all cases the viability was extremely high, always greater than 50%. This contrasts with other strains routinely used where viability is both lower and more variable. The spores from three plasmodia (*C30, C31, C32*) were recloned and 20 isolates were obtained from each plasmodium (*C30.1–20; C31.1–20; C32.1–20*). These three sets of clones were tested in the same way as the *C10–C51* series: that is, for plasmodial formation in clones and with tester strains. The results for these experiments (Tables 1,3) were essentially the same as for clones *C10–C51*. When they were tested on their own, 55 of the 60 clones gave rise to plasmodia in at least one test (Table 1). Three of these plasmodia were isolated for further tests and were given the same designation as the amoebal clones in which they arose (*C30.4; C32.3; C32.12*).

Tests of progeny from plasmodia C30.4, C32.3, *and* C32.12: Spores from these three plasmodia were plated, recloned, and 20 clones were isolated from each plasmodium (*C30.4.1–20; C32.3.1–20; C32.12.1–20*). The same experiments were performed on these clonal isolates as described above, i.e., for plasmodial formation in clones and with tester strains. The pattern of results for these experiments was essentially the same as before (Tables 1,3). Fifty-four out of 60 isolates gave plasmodia in clones. Thus the ability to form plasmodia in amoebal clones was inherited through three generations (Table 1). Table 3 shows that in all three generations of amoebae, their pattern of crossing with amoebae of known

TABLE 3

Summary of plasmodial formation in crosses between Colonia clonal isolates and amoebae of known mating type

Clonal series	Strain / Mating type	Tester amoebae: *a* mt_1	*i* mt_2	*B173* mt_3	*B174* mt_4	Total tested
C10–C51	Plasmodial formation	33	20	35	34	40
	No plasmodial formation	7	20	5	6	..
C30.1–20, *C31.1–20*, *C32.1–20*	Plasmodial formation	27	15	29	33	34
	No plasmodial formation	7	19	5	1	..
C30.4.1–20, *C32.3.1–20*, *C32.12.1–20*	Plasmodial formation	36	17	31	38	40
	No plasmodial formation	4	23	9	2	..

mating type was similar and inconsistent with the segregation of two mating types. However in all three series of clones, plasmodium formation with tester strain *i* (mt_2) was consistently lower than with the other three testers, at least half of the clones failing to cross with mt_2 in any one test (Table 3). This phenomenon is discussed below.

Plasmodial fusion tests: To confirm that crossing actually took place between particular strains, the genetically based plasmodial fusion reaction was used (POULTER and DEE 1968). By this means formation of hybrid plasmodia could be detected directly. The system has now been extensively studied (POULTER 1969) and only a summary of the genes concerned will be given here.

There are two unlinked loci involved. The *f* locus has 4 alleles; the *n* locus has 2 alleles. For fusion to occur there must be identity at both loci. However n_2 is dominant to n_1, so that a plasmodium of genotype $f_1f_2n_2n_2$ will fuse with both an $f_1f_2n_2n_2$ and an $f_1f_2n_1n_2$ plasmodium. Fusion also occurs between f_3f_3 and f_4f_4 plasmodia. This anomaly has been discussed by POULTER and DEE (1968) and does not affect the ensuing genetic analysis.

To ascertain the fusion genotype of *C50* amoebae, this clone was crossed with four tester strains carrying known alleles of *f* and *n*. The fusion behavior of the resulting plasmodia was tested against several tester plasmodia of different genotypes. The system is sufficiently well analyzed to make it unnecessary to test against all 30 known genotypes. Positive fusion with one tester genotype is sufficient for unequivocal determination of fusion genotype. The plasmodia obtained were heterozygous for *f* (except from the cross with *i*) and were therefore not the result of selfing of *C50* amoebae. The fusion behavior of these plasmodia showed consistently that *C50* amoebae were f_2n_1 (Table 4). The original Colonia plasmodium and several plasmodia which formed in clones in the next three generations were tested against several tester plasmodia and shown to be $f_2f_2n_1n_1$ in all cases. This agrees with the identification of *C50* as f_2n_1 and is consistent with clonal inheritance of plasmodium formation through three generations. The results show that *C50* crosses with all four mating types since hybrid plasmodia were formed. This was further confirmed by analyzing the progeny of these hybrid plasmodia.

Test of progeny of C50 × B174: The plasmodium resulting from a cross of *B174*

TABLE 4

Analysis of fusion behavior of plasmodia formed from crosses between C50 *amoebae and 4 tester amoebal strains*

Strains crossed	Genotype of tester plasmodium which fused with crossed plasmodium	Deduced genotype of crossed plasmodium	Genotype of tester amoeba	Deduced genotype of *C50* amoebae
$a \times C50$	$f_1f_2n_1n_1$	$f_1f_2n_1n_1$	f_1n_1	f_2n_1
$i \times C50$	$f_2f_2n_1n_2/n_2n_2$	$f_2f_2n_1n_2/n_2n_2$	f_2n_2	f_2n_1 or f_2n_2
$B173 \times C50$	$f_2f_4n_1n_1$	$f_2f_4n_1n_1$	f_4n_1	f_2n_1
$B174 \times C50$	$f_2f_3n_1n_1$	$f_2f_3n_1n_1$	f_3n_1	f_2n_1

TABLE 5

Tests for plasmodial formation by progeny clones of B174 + C50

Tests showing plasmodium formation:			Deduced *mt* of clone	Number of clones in each class
In clones	With *i* (mt_2)	With *B174* (mt_4)		
—*	P	—	mt_4	15
P	—	P	mt_h	11
P	P	P	mt_h	3
			Total tested	29

* P = plasmodial formation; — = no plasmodial formation.

and *C50* amoebae (plasmodium *B174 + C50*, genotype $f_2f_3n_1n_1$) was sporulated and the spores plated and recloned, 29 clones being isolated (*BC1–29*). These 29 clones were tested for plasmodium formation on their own and with *B174* and *i* tester amoebae. These particular tester strains were used in order to distinguish between mt_4 and mt_h progeny clones. Progeny clones carrying mt_4 would be expected to cross with *i* and not with *B174*; progeny clones carrying mt_h would be expected to cross with *B174* but not readily with *i* (see Table 2). Strain *i* also carried suitable *f* and *n* alleles for plasmodial fusion analysis. The results are shown in Table 5. The clones that failed to cross with *B174* (mt_4) but did cross with *i* (mt_2) are deduced to be mt_4. From the data in Table 5 it can be seen that all the mt_4 progeny (15) have failed to give plasmodia in clones and all the progeny that have given plasmodia in clones (14) have also crossed with mt_4. In other words, the ability to form plasmodia in clones is segregating (1:1) from mt_4. The simplest explanation is that this behavior is due to an allele of *mt* which will be referred to as mt_h (Table 5). The possibility of other genes being involved cannot be excluded however.

TABLE 6

Fusion behavior of plasmodia formed by crossing progeny clones of B174+C50 *with* i *and* B174

Mating type of progeny clone of *B174+C50**	Tester amoeba used to form plasmodium	Fusion behavior with tester plasmodia†						Deduced fusion type of crossed plasmodium‡	Deduced genotype of progeny clone‡	Number of clones in each class*
		$f_2f_2n_1n_1$	$f_2f_2n_2n_2$	$f_2f_3n_1n_1$	$f_2f_3n_2n_2$	$f_3f_3n_1n_1$	$f_3f_3n_2n_2$			
mt_4	*i* ($mt_2f_2n_2$)	—	F	—	—	—	—	$f_2f_2n_1n_2$	$mt_4f_2n_1$	9
		—	—	—	F	—	—	$f_2f_3n_1n_2$	$mt_4f_3n_1$	6
mt_h	*i* ($mt_2f_2n_2$)	—	F	—	—	—	—	$f_2f_2n_1n_2$	$mt_hf_2n_1$	2
		—	—	—	F	—	—	$f_2f_3n_1n_2$	$mt_hf_3n_1$	1
	B174 ($mt_4f_3n_1$)	—	—	F	—	—	—	$f_2f_3n_1n_1$	$mt_hf_2n_1$	10
		—	—	—	—	F	—	$f_3f_3n_1n_1$	$mt_hf_3n_1$	4

* Mating type of clone deduced from Table 5. Note that 3 of the mt_h clones formed plasmodia with *i* and *B174* and all these plasmodia are included in this table. These 3 clones are therefore included twice in the totals on the right.

† Each plasmodium was tested for fusion with all six tester plasmodia shown. F = fusion; — = no fusion.

‡ *B174+C50* was homozygous n_1n_1, so all progeny clones must be n_1.

TABLE 7

Recombination between mt *and* f *in progeny clones of* B174+C50

Genotype*	Number of clones	Total recombinants	Recombination frequency
mt_4f_3	6		
mt_4f_2	9	13	$\frac{13}{29} \times 100 = 44.8\%$
mt_hf_3	4		
mt_hf_2	10		

* Deduced from Table 6.

All plasmodia formed in these tests were isolated and analyzed for fusion behavior with a range of tester plasmodia. The results (Table 6) showed that hybrid plasmodia were formed when mt_h amoebae were crossed with *i* or *B174*; for example, f_2f_3 plasmodia were formed in crosses between mt_h clones and *B174*. From the fusion tests, the fusion type of all the plasmodia could be found (Table 6) and, since the genotypes of *i* and *B174* are known, it was possible to deduce the genotypes of the 29 progeny clones of *B174+C50*. Three clones crossed with both tester strains. The genotype of each of these three clones was deduced from fusion analysis of the two plasmodia formed in each case. For any one of the three clones, the genotype deduced from the cross with *i* was the same as that deduced from the cross with *B174* (Table 7). The results clearly show segregation and recombination of fusion-type and mating-type alleles consistent with previous results.

Pairwise crosses of progeny clones of B174+C50: Ten of the 29 clones were selected for crosses with each other in all pairwise combinations. Clones of each of the four putative genotypes were selected—f_2mt_4, f_3mt_4, f_2mt_h, f_3mt_h.

Since it had been observed that hybrid plasmodia appear earlier than selfed plasmodia (see above), conditions were adjusted to reduce the number of plasmodia forming late. Plasmodium formation normally occurs only in fairly moist conditions and becomes less frequent as the agar dries up during incubation, so the plates were partially dried before being inoculated with amoebae. Under these conditions, no plasmodia appeared after the 6th day of incubation. The results are shown in Table 8. As before, strains of amoebae able to form plasmodia in clones are denoted by mt_h. In the pairwise crosses shown in Table 8, all the plasmodia except two appeared in crosses between mt_4 and mt_h amoebae. In all crosses in which the amoebae carried different fusion alleles (13 out of 25), it was shown by fusion tests that the plasmodia formed were hybrid. For the other crosses, fusion analysis could not distinguish between hybrid and selfed plasmodia. Similarly, it was not possible to deduce by fusion analysis whether the plasmodia which appeared in crosses between strains of amoebae both carrying mt_h were hybrid or selfed. A second crossing test on moist conditions was performed between amoebae both carrying mt_h but differing in their fusion alleles. Two separate crosses involving four different clones produced plasmodia which were hybrid for the *f* allele, demonstrating that strains of amoebae carrying mt_h and

TABLE 8

Plasmodial formation in pairwise crosses of progeny clones of B174+C50

Clone number	Genotype	Clone number: 1	2	3	9	15	6	7	8	21	26
	Genotype	mt_lf_3	mt_lf_3	mt_lf_2	mt_lf_3	mt_lf_2	mt_hf_2	mt_hf_2	mt_hf_3	mt_hf_3	mt_hf_2
1	mt_lf_3	—*	—	—	—	—	P	P	P	P	P
2	mt_lf_3		—	—	—	—	P	P	P	P	P
3	mt_lf_2			—	—	—	P	P	P	P	P
9	mt_lf_3				—	—	P	P	P	P	P
15	mt_lf_2					—	P	P	P	P	P
6	mt_hf_2						—	P	—	—	—
7	mt_hf_2							—	—	—	—
8	mt_hf_3								—	—	—
21	mt_hf_3									P	—
26	mt_hf_2										—

* P = plasmodial formation; — = No plasmodial formation.

capable of producing plasmodia in clones could also produce hybrid plasmodia when crossed.

DISCUSSION

The mode of action of the mating-type locus is not known; it could be affecting amoebal fusion, or nuclear fusion, or both. Analysis of previously known strains of *P. polycephalum* has shown that identity at the *mt* locus prevents plasmodium formation; this is an example of homogenic incompatibility (ESSER and KUENEN 1963).

The investigations reported here on the strain Colonia show conclusively that pure clones of amoebae of this strain can give rise to plasmodia which can sporulate and produce amoebae which themselves behave in the same way. This ability has been shown to be inherited through three generations. All the amoebae in this line will be referred to here as Colonia amoebae. Many Myxomycete species have been induced to complete their life cycle in single-spore culture, but there has been much disagreement regarding the underlying mechanisms (GRAY and ALEXOPOULOS 1968). On the basis of current evidence, three possibilities should be considered to explain plasmodium formation in clones of Colonia amoebae:

1. A single amoeba can develop into a plasmodium without cell or nuclear fusion and without change of ploidy (apogamy or apomixis, see RIEGER, MICHAELIS and GREEN 1968). The life cycle is therefore completely haploid or completely diploid.

2. A single amoeba can develop into a plasmodium as a result of a spontaneous doubling of its chromosome number. Amoebae are regularly haploid and plasmodia are diploid.

3. Two genetically identical amoebae can fuse to give rise to a plasmodium (homothallism). Amoebae are haploid and plasmodia are diploid.

The hypothesis of mutability at the *mt* locus proposed by COLLINS (1965) to explain occasional plasmodial formation in single-spore cultures of the hetero-

thallic Myxomycete *Didymium iridis* seems unlikely to account for the repeated formation of plasmodia in all clones of Colonia amoebae.

The results reported in Table 2 show that clones of Colonia amoebae are capable of crossing with clones of all four mating types. Crossing was proved by the detection of hybrid plasmodia and by analysis of the amoebal progeny of these plasmodia which showed recombination of parental markers (Table 6). Plasmodia appeared in these crosses two days earlier than in the control cultures of Colonia amoebae alone and all plasmodia isolated from these crosses proved to be hybrid. Thus it seems that in mixed culture, Colonia amoebae form crossed plasmodia more readily than selfed ones. This situation resembles "relative heterothallism" in *Aspergillus nidulans* (PONTECORVO 1953), and suggests homothallism as the explanation of plasmodia formation in clones. Crossing was also proved between amoebae capable of plasmodium formation in clones when progeny of the cross *C50* × *B174* carrying different *f* alleles were mixed and shown to give hybrid plasmodia. This strongly suggests that plasmodium formation within clones also occurs by amoebal fusion, i.e., homothallism (Hypothesis 3). Also normal 1:1 segregation of genetic markers was observed in the progeny of crosses involving Colonia amoebae. The possibility that the life cycle of Colonia is apogamic can therefore be excluded.

VON STOSCH *et al.* (1964) did comparative chromosome counts and studies on nuclear division in the Colonia strain and seem to favor apogamy as the explanation for their results. Unfortunately this work has not been published in full. Since the Colonia strain was cloned many times before the present work was initiated, and since different culture conditions were used in the present work, the results may not be directly comparable. THERRIEN (1966) has made measurements of the nuclear DNA content in the amoebae and plasmodia of *D. iridis* and has shown that the DNA content of plasmodial nuclei is twice that of amoebal nuclei. Similar experiments on the Colonia strain would also distinguish between homothallism and apogamy.

Colonia amoebae (as defined above under DISCUSSION) showed consistently a low frequency of crossing with *i* in all three generations tested. Colonia amoebae crossed with normal high frequency with all other tester strains. In addition, both strain *i* and mt_2 strains derived from it cross with normal high frequency when tested against other strains of different mating type. The phenomenon is thus not environmental but a specific interaction between Colonia and *i*. It could be due to the *mt* alleles involved or due to modifying genes in one or both of the strains. All crosses of Colonia amoebae with mt_2 involved the same two genotypes since (a) all Colonia clones tested arose clonally from the original homozygous Colonia plasmodium and were thus genetically identical, and (b) strain *i* was always used as the tester strain for mt_2. Strain-dependent low frequency of crossing has been noted before (DEE 1966).

However, there is some evidence to suggest that the *mt* locus rather than modifying genes is the cause of low frequency of crossing. Firstly, DEE (1966) concluded that low frequency of crossing between certain strains only occurred when they were genetically closely related, for example, between parent and

progeny. Since strains *a* and *i* are sibling clones and Colonia is unrelated, this explanation seems unlikely to account for the low frequency of crossing between *i* and Colonia. Secondly, half of the progeny clones of the plasmodium *B174+C50* carried the mt_h allele. Although cultural conditions were not the same, these clones still had a low frequency of crossing with *i* even though they now carried a random assortment of *B174* and *C50* genes. No experiments have been done to analyze the situation further.

The Colonia strain will be of undoubted use in isolating recessive mutants affecting the plasmodium. Amoebae will be mutagenized and cloned, and plasmodia isolated from these clones. Since the amoebae are haploid, a mutation induced in an amoeba will be inherited by all amoebae of the clone and will be homozygous in all nuclei of the plasmodium arising from that clone. Thus recessive mutations affecting the plasmodium can be selected. To this end, work is in progress in selecting and analyzing mutants affecting plasmodial formation.

I should like to thank Dr. H. A. VON STOSCH for supplying the Colonia strain, Dr. R. T. M. POULTER for providing some of the tester plasmodia and Dr. JENNIFER DEE for helpful discussion during the progress of this work and for much advice during preparation of this paper. I would like to acknowledge receipt of a research scholarship from the Medical Research Council.

SUMMARY

Plasmodium formation in strain Colonia of the Myxomycete *Physarium polycephalum* has been investigated and has been concluded to be homothallic. Using well-analyzed genetic markers, the following observations have been made on this strain. All Colonia amoebae can give rise to plasmodia within clones. This ability has been shown to be inherited through three successive life cycles. Amoebae of one Colonia clone can cross with amoebae of any other Colonia clone. Colonia amoebae can also cross with amoebae carrying any of the four known mating-type (*mt*) alleles. In the progeny of these crosses, the ability to form plasmodia within clones segregates 1:1 from mating type and it is therefore postulated to be due to an allele of the *mt* locus (mt_h). The use of this homothallic strain in isolating recessive mutants affecting the plasmodium is discussed.

LITERATURE CITED

COLLINS, O. R., 1965 Evidence for a mutation at the incompatibility locus in the slime mold, *Didymium iridis*. Mycologia **57**: 314–315.

DANIEL, J. W., K. L. BABCOCK, A. H. SIEVERT and H. P. RUSCH, 1963 Organic requirements and synthetic media for growth of the Myxomycete *Physarum polycephalum*. J. Bacteriol. **86**: 324–331.

DANIEL, J. W. and H. H. BALDWIN, 1964 Methods of culture for plasmodial Myxomycetes. pp. 9–41. In: *Methods in Cell Physiology 1*. Edited by D. M. PRESCOTT. Academic Press, New York and London.

DEE, J., 1960 A mating-type system in an acellular slime-mould. Nature **185**: 780–781. ——, 1962 Recombination in a Myxomycete, *Physarum polycephalum* Schw. Genet. Res. **3**: 11–23. ——, 1966 Multiple alleles and other factors affecting plasmodium formation in the true slime mould *Physarum polycephalum* Schw. J. Protozool. **13**: 610–616.

DEE, J. and R. T. M. POULTER, 1970 A gene conferring actidione resistance and abnormal morphology on *Physarum polycephalum* plasmodia. Genet. Res. **15**: 35–41.

ESSER, K. and R. KUENEN, 1963 *Genetics of Fungi.* English translation, 1967. Springer-Verlag, Berlin and New York.

GRAY, W. D. and C. J. ALEXOPOULOS, 1968 *Biology of the Myxomycetes.* Ronald Press, New York.

HOWARD, F. L., 1932 Nuclear division in plasmodia of Physarum. Ann. Botan. **46**: 461–477.

PONTECORVO, G., 1953 The genetics of *Aspergillus nidulans.* Advan. Genet. **5**: 141–238.

POULTER, R. T. M., 1969 Senescence in the Myxomycete *Physarum polycephalum.* Ph.D. Thesis, Univ. of Leicester.

POULTER, R. T. M. and J. DEE, 1968 Segregation of factors controlling fusion between plasmodia of the true slime mould *Physarum polycephalum.* Genet. Res. **12**: 71–79.

RIEGER, R., A. MICHAELIS and M. M. GREEN, 1968 *A Glossary of Genetics and Cytogenetics.* English edition. Allen & Unwin, London.

ROSS, I. K., 1957 Syngamy and plasmodial formation in the Myxogastres. Am. J. Botan. **44**: 843–850.

RUSCH, H. P., 1970 Some biochemical events in the life cycle of *Physarum polycephalum.* pp. 297–327. In: *Advances in Cell Biology 1.* Edited by D. M. PRESCOTT. Appleton-Century-Crofts, New York.

THERRIEN, C. D., 1966 Microspectrophotometric measurement of nuclear deoxyribonucleic acid content in two Myxomycetes. Canad. J. Botan. **44**: 1667–1675.

VON STOSCH, H. A., M. VAN ZUL-PISCHINGER and G. DERSCH, 1964 Nuclear phase alternance in the Myxomycete *Physarum polycephalum.* pp. 481–482. In: *Abstracts 10th Intern. Botan. Congr.*, Edinburgh 1964.