Genet. Res., Camb. (1962), **3**, *pp.* 11–23
With 1 *text-figure and* 2 *plates*
Printed in Great Britain

Recombination in a Myxomycete, *Physarum polycephalum* Schw.

By JENNIFER DEE*
Department of Genetics, University of Glasgow

(*Received* 6 *June* 1961)

1. INTRODUCTION

Since the Myxomycetes (= Mycetozoa = true slime-moulds = acellular slime-moulds), almost uniquely among micro-organisms, pass through a number of distinct and different stages in their life-cycle, the application of genetic analysis to them may provide tools for the investigation of a number of biological problems including morphogenesis. The present work reports an attempt at genetic analysis in a Myxomycete. It demonstrates recombination in the species studied, *Physarum polycephalum* Schw.

Previous work on Myxomycetes was reviewed by Martin (1940). Since then, convincing cytological studies have been reported by Wilson & Ross (1955) and Ross (1957, 1961) and biochemical studies by Rusch (1959), Nygaard, Guttes & Rusch (1960) and Daniel & Rusch (1961).

The life-cycle

The Myxomycetes differ from all other organisms, including the cellular slime-moulds (Acrasiales), in consisting, at one stage of their life-cycle, of a true plasmodium, i.e. an acellular, multinucleate, motile mass of protoplasm of indefinite form. The life-cycle is similar in all Myxomycetes: that of *Physarum polycephalum*, which was first described by Howard (1931*a*), is summarized below (see Fig. 1).

The main vegetative stage is the plasmodium, which moves by protoplasmic streaming and grows to a size dependent on the amount of food available. The plasmodium forms spores when conditions are suitable, two of the necessary conditions being illumination and absence of food. The spores, which are black, dry and resistant, are borne in large numbers on stalked fruiting bodies. In moisture, a spore, which is about 10 μ in diameter, releases one or two uninucleate cells, slightly smaller in size, which multiply rapidly to give large numbers of identical cells. The uninucleate cells, which have been given a variety of names in previous papers, have been called simply 'amoebae' throughout the present paper. On an agar medium, they are closely similar in appearance and behaviour to small soil ('limax') amoebae, though in a liquid medium they become temporarily flagellate.

As shown in a previous report (Dee, 1960*a*), plasmodia of *P. polycephalum* are formed from the amoebae only under certain conditions, one of which is the presence of amoebae of different mating-type. In the strain used in this work there appear to be two mating-types which have been called + and −. A single spore produces amoebae of one mating-type only. Thus in this strain the culture

* Present address: Department of Zoology, University College London, W.C.1.

of amoebae derived from a single spore or from a single amoeba appears to be a clone, i.e. a group whose nuclei are all derived from one nucleus. All the work reported has been done on clones, some of which have been maintained in culture for nearly three years without change to plasmodia.

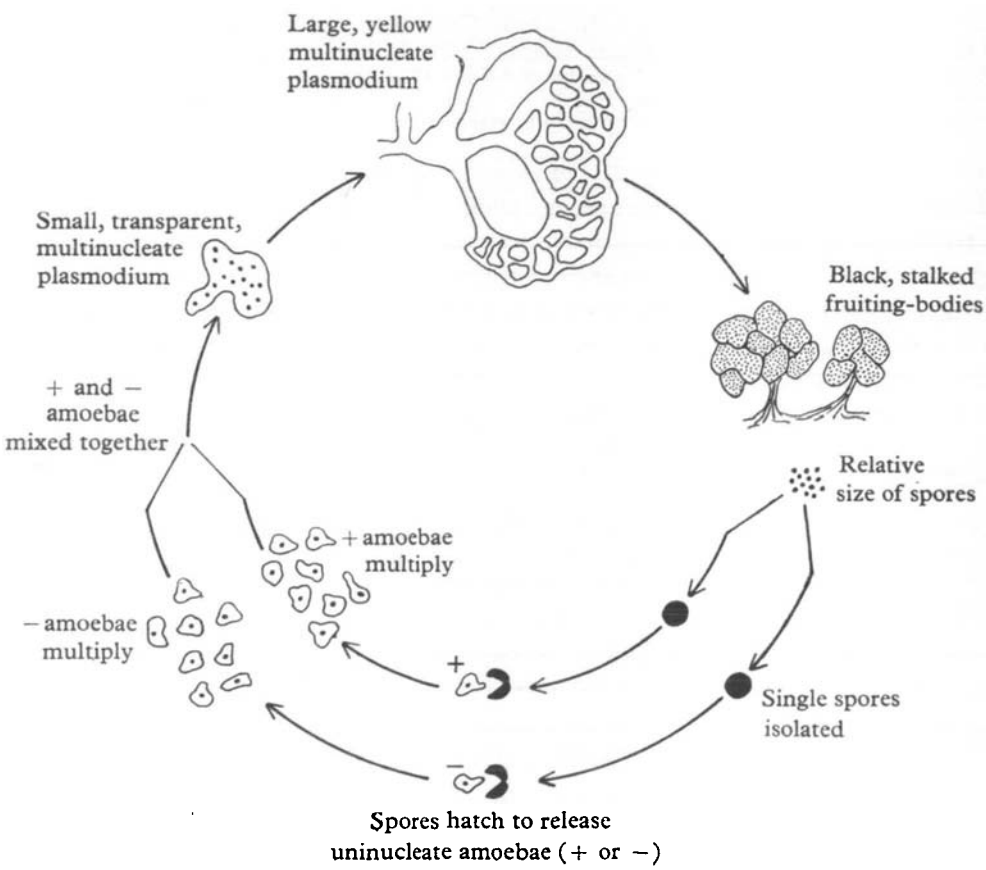


Fig. 1. The life-cycle of *Physarum polycephalum*, including the mating-type system (Dee, 1960*a*). All stages are drawn to approximately the same scale except the fruiting-bodies which are enlarged less than the other stages. The spore is about 10 μ in diameter; the plasmodium is of variable size and form. A single plasmodium will grow in a few days to cover the surface of a 9 cm. diameter Petri dish or any larger vessel in which it is cultivated.

The nuclear cycle

The nuclear cycle has not yet been completely clarified. Wilson & Ross (1955) and Ross (1961) have shown that meiosis occurs just prior to spore formation in a number of Myxomycetes, including *P. polycephalum*. The amoebae, which are uninucleate, are therefore presumably haploid. From observation of living and stained preparations of nineteen species, including *P. polycephalum*, Ross (1957) concluded that plasmodium formation began by a pair of amoebae fusing to give a zygote with a single diploid nucleus. In some species, zygotes then coalesced to

form the plasmodium, while in other species, including *P. polycephalum*, all the nuclei of the plasmodium were derived by division from one fusion nucleus. In either situation, all the nuclei of the plasmodium would presumably be diploid. Ross also concluded that multiple fusion of haploid cells did not occur and that, although haploid amoebae were ingested by growing plasmodia, their nuclei did not become active constituents of it but were digested.

For the strain of *P. polycephalum* that I have used, however, there is some evidence that nuclear fusion may not occur until just prior to meiosis (i.e. just prior to spore formation) since microdensitometer measurements of the DNA content of the nuclei in amoebae and plasmodia suggest that the ploidy of the nuclei is the same at these two stages. This work, which has been reported in detail elsewhere (Dee, 1960*b*) is certainly not conclusive. The question can be settled decisively only by further measurements of DNA content or by chromosome counts.

2. MATERIALS AND METHODS

(*a*) *Strains*

All the cultures of *P. polycephalum* used in this work were derived from a piece of plasmodium kindly supplied by Dr H. P. Rusch of Wisconsin University in October 1957. The identification of the species was confirmed by Dr G. W. Martin of the University of Iowa. (For identification, see Martin, 1949.) The plasmodium was freed from contaminants by the use of antibiotics and by migration over non-nutrient medium.

The strain of *Pseudomonas fluorescens* used as food for the Myxomycete was isolated from among contaminants associated with the plasmodium before it was purified. Stock cultures of the strain are maintained on slopes of a Czapek-Dox-glucose medium.

(*b*) *Culture methods*

All cultures were incubated at 25° C and the usual precautions to maintain sterile conditions were observed throughout. Full details of culture methods are given elsewhere (Dee, 1960*b*).

Plasmodia were cultured on autoclaved oat agar prepared by a method similar to that of Howard (1931*b*). To induce spore formation, plasmodia were cultured in flasks on a thin layer of oat agar in the presence of *Ps. fluorescens*. The cultures were kept in the dark until the food was exhausted and then exposed to fluorescent 'Daylight' illumination for short fixed periods of time, the light being switched on and off by a time switch giving alternate periods of 6 minutes' light and 14 minutes' darkness so that the cultures should not become overheated. Spores formed in the cultures usually 1–2 days after they were first exposed to light.

Spores hatched (germinated) in the absence of free water on the surface of water agar spread with a heavy suspension of *Ps. fluorescens*, and amoebae were cultured on the same medium for all purposes including the formation of plasmodia. On water agar, the bacteria grow slightly, and the amoebae feed on them and clear them from the surface. When all the bacteria are eaten, the amoebae encyst but

remain viable for several months and resume growth and multiplication when subcultured. '*Standard Agar*' (*SA*) refers in this paper to the normal medium used, i.e. 2% (w/v) water agar spread with a suspension of *P.s. fluorescens*.

(*c*) *Plating method for amoebae*

A suspension of amoebae (active or encysted) is made in water and its density estimated by haemocytometer count. Serial dilutions are made to give a suspension of the required density. A small volume (0·1 ml. or 0·2 ml./dish) of the appropriate dilution is plated on the surface of water agar (2% w/v) in 9-cm. diameter Petri dishes and spread with a bent glass rod. After an interval of 1–5 hours, during which the dishes are allowed to stand at room temperature to enable the water to soak into the agar, a heavy suspension of *Ps. fluorescens* in saline is spread on the same dishes (0·1 ml./dish of a suspension containing approximately 1×10^{10} bacteria/ml.). When the surface of the medium has become dry, the dishes are inverted and incubated at 25° C.

During incubation, each of the amoebae plated multiplies to form a small colony which, being more transparent than the bacterial layer, shows as a plaque. After four days' incubation, the number of plaques may be counted with the naked eye (Plate 1).

The efficiency of the plating method has been thoroughly tested. The number of plaques corresponds closely to the number expected on the basis of the haemocytometer counts, and the numbers of plaques on different plates of a series show a variance no greater than expected by chance. Spores are plated by a similar method, one difference being that a second thin layer of water agar is poured over the surface of each dish after spores and bacteria have been spread. A plating method for amoebae similar to that used here was developed independently by Kerr & Sussman (1958) in work with *Didymium nigripes*, another Myxomycete.

(*d*) *Isolation of clones*

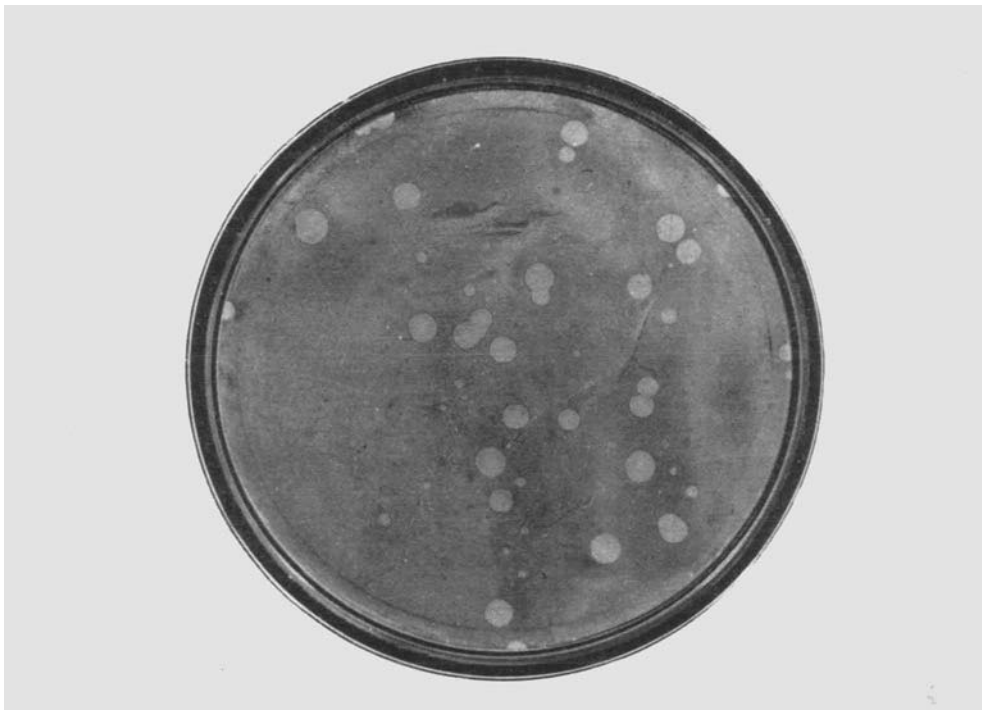
A clone of amoebae is obtained by plating spores or amoebae and isolating the amoebae from a single plaque. Clones are maintained in culture on Standard Agar slopes. Clones have also sometimes been established by isolating single spores by hand.

(*e*) *Test plates*

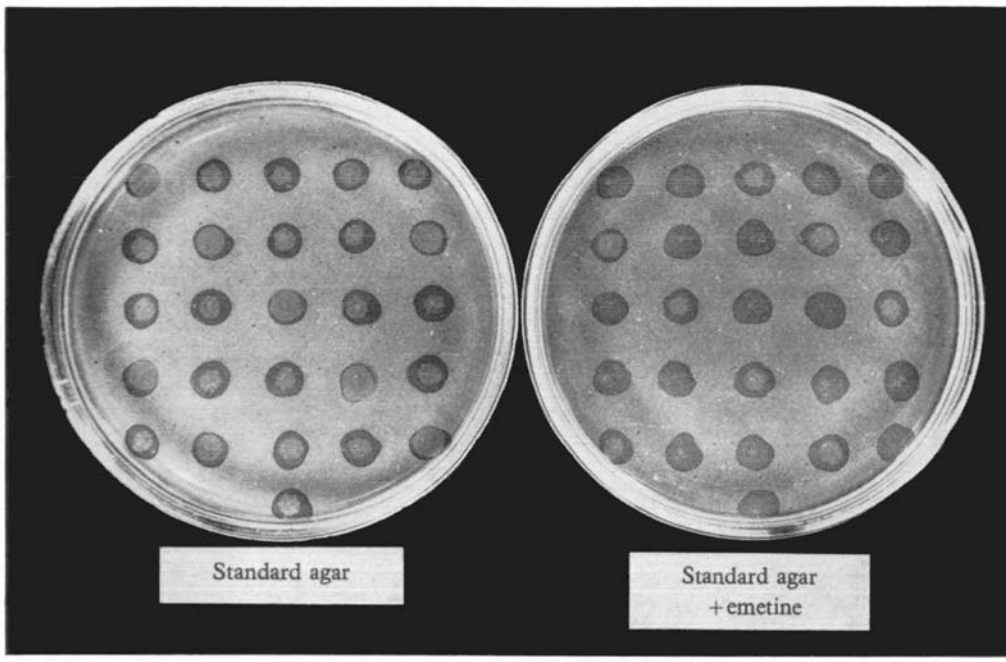
To determine whether or not a given culture of amoebae will grow on a particular medium, the plating method is unnecessarily complicated and test plates are used instead. A sample of amoebae of each strain is spot-inoculated on a spot of bacteria on the surface of water agar. Many strains may be tested on a single dish (Plate II).

3. THE DETECTION OF RECOMBINATION

The amoebae of *P. polycephalum* can be handled by the standard techniques of microbial genetics. They are uninucleate and probably haploid; they multiply quickly but survive for long periods without subculturing; they can be plated;



Plaques on a Petri dish after six days' incubation ($\frac{4}{5}$ natural size). The plate has been spread with a dilute suspension of amoebae and a heavy suspension of bacteria and incubated. The dark background is the layer of bacteria; the circular lighter areas are the 'plaques'. Each plaque has been formed by the multiplication of one amoeba in the original suspension. Plaques are first scored after four days' incubation, but new plaques continue to appear for several days after this and scoring is continued until the tenth day after incubation started. Fifty plaques may be counted on the photographed plate.



Test plates ($\frac{2}{3}$ × natural size). Loopsful of bacterial suspension were placed at twenty-six points on each plate. A loopful of a suspension of amoebae was then inoculated in the bacterial patch at each point. The plates are seen after three days' incubation. The bacterial patches appear dark, but those in which amoebae have multiplied have a clear area at the centre.

The patches were numbered 1–26 from left to right along successive rows and the following three strains of amoebae were inoculated at corresponding points on the two plates:

a (sensitive to emetine, mating-type +) on patches 1, 4, 7, etc.
i (sensitive to emetine, mating-type −) on patches 2, 5, 8, etc.
E19 (resistant to emetine, mating-type −) on patches 3, 6, 9, etc.

On Standard Agar, all strains have multiplied; on Standard Agar containing emetine, only strain *E19* has multiplied.

JENNIFER DEE

https://doi.org/10.1017/S0016672300003086 Published online by Cambridge University Press

clones can be isolated by plating; and many strains can be tested on a single dish. It was obvious that this was the stage of the life-cycle at which genetic markers might be most easily found and used. Two markers have so far been identified and these are described below.

(*a*) *The mating-type system*

The discovery of a mating-type system in *P. polycephalum* has already been reported (Dee, 1960*a*). It was found that when cultures of amoebae derived from ten single spores were mixed together in pairs in all possible combinations, only certain pairs gave rise to plasmodia. The data indicated that plasmodia were formed only when amoebae of different mating-type were mixed, that only two mating-types (+ and −) occurred in the spores investigated, and that a single spore produced a culture of amoebae of one mating-type only. A similar mating-type system was discovered in the Myxomycete *Didymium nigripes* by Von Stosch (1935).

The spores used for the tests described above (Dee 1960*a*) were derived directly from the single plasmodium received from Dr Rusch. The ten cultures of amoebae produced by these spores were called *a*, *b* ... *j*. Two of these, *a* (+) and *i* (−), which have now been maintained in culture for nearly three years, were used as a basis for all the work reported below.

Isolates of amoebae derived from a plasmodium of known composition are classified for mating-type by backcrossing to each of the two parent strains (i.e. the two from which the plasmodium was formed). A cross is made simply by mixing samples of amoebae of two cultures together in a wet spot of bacterial suspension on the surface of water agar in a Petri dish. If plasmodia are formed, they are clearly visible on the spot after a few days' incubation.

A strain of amoebae that gives plasmodia when crossed to the + parent is classified as − and one that gives plasmodia when crossed to the − parent is classified as +. No strain has yet been found to give plasmodia in combination with both parent strains. From every cross, however, a large proportion of the progeny has been found to give plasmodia with neither parent strain, and these cannot be classified for mating-type by the backcross tests alone. Although this may suggest that more than one pair of major mating-type factors are involved in determining plasmodium formation, various crossing tests have failed to demonstrate the presence of more than one pair. Since this pair (+ and −), as tested by backcrossing to the parents, has segregated as 1:1 in all crosses so far made (see Table 4), the presence of unknown factors influencing plasmodium formation does not affect its usefulness as a genetic marker.

(*b*) *Isolation of an emetine-resistant strain*

When emetine hydrochloride (an alkaloid active against *Entamoeba histolytica*) is incorporated in water agar at concentrations above 0·015% (w/v), amoebae plated or inoculated on this medium fail to multiply, although the *Pseudomonas fluorescens* spread with them as food grow normally. Dense platings of amoebae of

clone *i* were made on such medium. Of 3×10^8 amoebae plated, one produced a plaque of amoebae resistant to emetine. A strain (*E19*) was established from this plaque and purified by repeated cloning (i.e. plating and picking from single plaques) on medium containing emetine. A plating test showed clearly that this strain was more viable on emetine-containing medium than *a* (Table 1) and further tests revealed that it maintained its resistance to emetine through many successive subcultures in the absence of the drug. The isolation of this strain provided a second marker, so that doubly-marked strains could now be crossed in order to test for recombination.

Table 1. *Effect of emetine concentration on growth of resistant and sensitive strains. Platings were made on Standard Agar and on Standard Agar supplemented with various concentrations of emetine*

	No. of plaques (average of 3 dishes)		
		Strain *E19*	
% (w/v) emetine in medium	Strain *a*	Line 1*	Line 2*
0·0	92	84	83
0·019	0	98	94
0·025	0	91	93
0·031	0	89	79
Average no. of amoebae plated per dish:	120	96	100

* Line 1 had been cultured on SA.
Line 2 had been cultured on SA containing 0·019% emetine.

(*c*) *Cross of* a *and* E19

A cross was made between the following two strains:

a: sensitive to emetine, mating-type +.
E19: resistant to emetine, mating-type −.

Both strains were purified by two successive single-plaque isolations immediately before the cross was made. Amoebae of the two strains were then mixed together and a plasmodium (*a*+*E19*) was formed which was found to show no greater resistance to emetine than the plasmodium (*a*+*i*).

Plasmodium (*a*+*E19*) was induced to form spores. Samples of the spores were plated on Standard Agar and clones of amoebae were isolated by picking from single plaques which were selected at random. Five sets of such isolates were picked at different times from spores of the same plasmodium (see Table 2). Four of these five sets were tested for resistance to emetine and for mating-type.

Resistance to emetine was scored in the following way (Plate II). After preliminary culture on Standard Agar, each isolate was transferred to bacterial spots at corresponding points on two plates, one of water agar + emetine and the other

of water agar. Isolates that multiplied on the water agar plate only were classified as sensitive; those that multiplied on both plates as resistant. Those that failed to grow on the water agar plate were discarded. Strains *a* and *E19* were used as controls in all tests, to ensure that the emetine medium used would discriminate between resistant and sensitive strains. Slightly different concentrations of emetine medium were used for different sets: 0·019% (w/v) for Set 1, 0·031% (w/v) for Sets 2 and 4, 0·016% (w/v) for Set 5. The distinction between ability and inability to grow on emetine was clear for most isolates, since, where multiplication occurred, many amoebae on a spot started to multiply at once, giving a large patch of amoebae, but a few isolates gave only slight multiplication on the emetine medium and these were classified as 'doubtful'. Segregation for resistance was found in each set (Table 3).

Table 2. *Origin of isolates from the cross* a × E19 *used in subsequent tests*

Details of spores		Details of plating			Isolates	
Flask No.	Age when plated (weeks)	Spores plated per dish	No. of dishes	Plaques per 100 spores plated	Total no.	Set
14	1	230	19	6·3	104	1
7	5	150	8	4·0	104	2
7	5	300	8	6·8		
6	10	70	8	3·0	37	3
6	10	140	8	2·1	(discarded)	
6	12	400	16	3·4	100	4
7	30	400	16	0·5	54	5
7	32	500	8	0·6		
7	32	1000	8	0·4		

All spores were formed by the same plasmodium but those in different flasks were formed on different dates. The spores were plated on SA. Each 'isolate' is a culture of amoebae obtained from a single plaque. Set 3 was discarded without testing.

Table 3. *Isolates from cross* a × E19. *Segregation for emetine resistance*

	Set 1	Set 2	Set 4	Set 5
Resistant	76	61	62	21
Sensitive	20	34	33	25
Doubtful	6	3	2	3
Total	102	98	97	49

See text for method of scoring.

Mating-type was tested by backcrossing the isolates to the strains *a* and *i* (Sets 1 and 2) or *a* and *E19* (Sets 4 and 5). (Since *E19* was derived directly from *i*, either may be regarded as a 'parent' strain of plasmodium (*a* + *E19*).) As in previous

tests for segregation of mating-type, many of the isolates could not be classified for this marker by the backcross tests since they failed to form plasmodia with either parent strain. These are classified as 'No result' (Table 4). However, the

Table 4. *Isolates from cross* a × E19. *Segregation for mating-type*

	Set 1	Set 2	Set 4	Set 5
Mating-type +	14	11	17	11
Mating-type −	11	12	25	6
No result	77	75	55	32
Total	102	98	97	49

See text for method of scoring.

number that could be classified as + or − was sufficient to show that recombination had occurred between mating-type and resistance to emetine (Table 5). The total samples shown in these tables are slightly smaller than the numbers originally isolated because in each set of isolates a few were lost.

Table 5. *Isolates from cross* a × E19. *Full classification*

	Set 1	Set 2	Set 4	Set 5
Parental types				
Resistant −	9	9	14	3
Sensitive +	1	5	6	7
Recombinant types				
Resistant +	13	5	10	4
Sensitive −	2	3	11	3
Total parental types	10	14	20	10
Total recombinant types	15	8	21	7
Other classes				
Resistant No result	54	47	38	14
Sensitive No result	17	26	16	15
Doubtful +	0	1	1	0
Doubtful −	0	0	0	0
Doubtful No result	6	2	1	3
Total classified	102	98	97	49

Analysis of the results shown in Tables 3–5 led to the following conclusions: the ratio emetine-resistant : emetine-sensitive deviates significantly from 1 : 1 in three of the four sets (Sets 1, 2 and 4) and the results for the four sets are heterogeneous. The ratio mating-type + : mating-type − does not deviate significantly from 1 : 1 in any of the four sets and the results for the four sets are homogeneous. The ratio recombinants : parentals also agrees with 1 : 1 and the results for the four sets are homogeneous.

(*d*) *Results of re-testing Sets 1 and 4 for emetine resistance*

The isolates of Sets 1 and 4 were subcultured to Standard Agar slopes at the time of their first test for emetine resistance. One month later, samples from these slopes were tested for emetine resistance by the same method as before. Set 4 isolates were again subcultured to Standard Agar slopes at the time of their second test. Four months later, subcultures were made to a third set of Standard

Table 6. *Results of re-testing Sets 1 and 4 on emetine*

	Set 1		Set 4		
	Test 1	Test 2	Test 1	Test 2	Test 3
Resistant	76	68	62	51	64
Sensitive	20	31	33	38	30
Doubtful	6	2	2	8	3
Total	102	101*	97	97	97

* One isolate produced plasmodia and was discarded.
See text for details of subculturing between tests.

Agar slopes and samples from these slopes were used for a third test, which was performed five months after the second. The results of these tests are shown in Tables 6–7. The total numbers classified as resistant, sensitive and doubtful in each test are given in Table 6 and the various changes in classification that occurred are shown more precisely in Table 7. In Set 1, 88 of the 101 isolates were classified in the same way in both tests, and in Set 4, 77 of the 97 isolates were classified in the same way in all three tests.

4. DISCUSSION

In order to discuss the results, we must make some assumptions about the nuclear cycle. It is reasonable to assume that the amoebae are haploid. The plasmodium may be a haploid heterokaryon or a diploid, but we assume that fusion of nuclei in pairs and, subsequently, meiosis occur at some time before spore formation.

When these assumptions are made, and if it is further assumed that only nuclei of opposite mating-type can fuse (i.e. that no selfing occurs), the results are found to agree with the hypothesis that mating-type is determined by a single pair of alleles and that the factors determining mating-type and emetine resistance recombine freely. Emetine resistance may also be determined by a single pair of alleles, but if it is, some factor has disturbed the segregation of the alleles in three of the four samples tested. If it is assumed that mating-type and emetine resistance are determined by two pairs of alleles, at two unlinked loci, then whether the cause of disturbed segregation for emetine resistance is viability or misclassification or both, the ratio recombinants:parentals is still expected to be 1:1, and this has in

https://doi.org/10.1017/S0016672300003086 Published online by Cambridge University Press

Table 7. *Changes in classification of Sets 1 and 4 after re-testing on emetine*
(+ = resistant; − = sensitive; (+) = doubtful. The column of figures shows the numbers of isolates classified in the various ways indicated on the left.)

(*a*) *Set 1*

Classification		No. of isolates
Test 1	Test 2	
+	+	68
−	−	19
(+)	(+)	1
Total classified the same way in Tests 1 and 2		88
+	−	7
−	(+)	1
(+)	−	5
Total classified differently in Tests 1 and 2		13
	Total	101

(*b*) *Set 4*

Classification			No. of isolates
Test 1	Test 2	Test 3	
+	+	+	50
−	−	−	27
(+)	(+)	(+)	0
Total classified the same way in Tests 1, 2, 3			77
+	−	+	6
+	(+)	+	4
−	(+)	−	3
Total classified the same way in Tests 1 and 3 only			13
−	−	+	2
−	−	(+)	1
+	+	(+)	1
Total classified the same way in Tests 1 and 2 only			4
+	(+)	(+)	1
Total classified the same way in Tests 2 and 3 only			1
(+)	−	+	2
Total classified differently in Tests 1, 2 and 3			2
		Total	97

https://doi.org/10.1017/S0016672300003086 Published online by Cambridge University Press

fact been found. Thus, if a disturbing cause is assumed to be present, the results are consistent with the hypothesis that mating-type and emetine resistance are determined by single pairs of alleles at two unlinked loci.

The factors most commonly found to cause deviations from 1:1 of allele ratios in other organisms are selfing, viability differences and misclassification. Selfing has clearly not occurred in the cross described since there is no deviation from 1:1 of the ratio recombinants:parentals. A viability difference may exist between strains resistant and sensitive to emetine, although there is no direct evidence that this is so (see below).

Several results, however, suggest that misclassification for emetine resistance occurred, the principal of these of course being the changes in classification on re-testing. In Set 1, 13 isolates out of 101 'changed' in classification and in Set 4, 20 out of 97. If these changes had all occurred in one direction, for example if many isolates originally classified as resistant had later been found to be sensitive, the changes in classification might have been taken as evidence of real changes in the constitution of the amoebae. But in Set 4 (Table 7), which was tested three times, many of the isolates that had apparently lost their resistance in the second test were found to have gained it again in the third. The existence of 'doubtful' isolates is of course additional evidence of uncertainty in the classification.

The most likely cause of the uncertainty in classification and of the deviation from 1:1 of the segregation for emetine resistance is the isolation of mixed clones.* Isolates that consisted of mixtures of resistant and sensitive amoebae would usually show growth on emetine and would be classified as 'resistant', so that more resistant than sensitive isolates would be scored. If there were very few resistant amoebae in a sample of the mixture, only slight growth might occur and the classification would be 'doubtful'. When a mixed isolate was subcultured to a slope of water agar, allowed to multiply and then sampled again, as in the re-tests of Sets 1 and 4, differences in classification might result from local differences in the proportions of the two types of amoebae in the culture.

There is in fact independent evidence that some of the isolates contained more than one clone. In Set 1, 2 out of 104 isolates and, in Set 5, 3 out of 54 isolates had to be discarded before testing because they had produced plasmodia. These isolates presumably contained mixtures of amoebae of different mating-type. The total number of isolates containing mixtures of more than one clone should be expected to be greater than this since mixtures of amoebae of the same mating-type and some of the mixtures of amoebae of different mating-type must have failed to produce plasmodia and must therefore have remained undetected.

The deviation from 1:1 of the segregation for emetine resistance could also have been caused by viability differences. The segregation in Set 5 is interesting in this respect, since, although it does not disagree with 1:1, it is inconsistent with the segregation found in Set 2, which was derived from the same batch of spores. Set

* Since only a low proportion of spores hatched, it is unlikely that mixed clones arose from the proximity of some of these, unless, as is possible, a hatching spore stimulates spores near it to hatch. It is possible that some spores carry more than one nuclear type.

2 was isolated from the spores when they were 5 weeks old and Set 5 when they were 7 months old. The change in the segregation might perhaps be associated with this age difference; if so, the operation of viability factors is indicated.

SUMMARY

1. Techniques were developed to facilitate genetic analysis of a Myxomycete, *Physarum polycephalum*. The uninucleate cells that form part of the life-cycle (the 'amoebae'), in two-membered culture with *Pseudomonas fluorescens*, were found to be suitable for handling by some of the standard techniques of microbial genetics, including plating.

2. As reported previously, a single-factor (+/−) mating-type system was discovered in *P. polycephalum* controlling the formation of plasmodia by the amoebae. Plasmodia are formed only when amoebae of different mating-type are mixed together. A single spore gives a culture of amoebae of one mating-type only, which may be assumed to be a clone.

3. A strain of amoebae resistant to emetine hydrochloride was isolated and was found to maintain its resistance through many subcultures in the absence of the drug.

4. A cross was made between the emetine-resistant strain (mating-type −) and an emetine-sensitive strain (mating-type +). Spores obtained from the hybrid plasmodium were plated and four sets of isolates of amoebae were made from these spores, and separately tested for mating-type and for emetine resistance.

5. The results of testing these isolates agree with the hypothesis that mating-type and emetine resistance are determined by pairs of alleles at two unlinked loci provided that factors disturbing the segregation for emetine resistance are assumed to be present.

6. Consideration of factors that could have disturbed the segregation for emetine resistance shows that selfing did not occur in the cross but that some misclassification for emetine resistance probably did occur.

I wish to thank Professor G. Pontecorvo, F.R.S., for his constant encouragement and help in this work, and Mr E. C. Forbes, for his many useful suggestions of possible techniques. The work was carried out during the tenure of a D.S.I.R. Research Studentship, which I acknowledge with thanks.

REFERENCES

DANIEL, J. W. & RUSCH, H. P. (1961). The pure culture of *Physarum polycephalum* on a partially defined soluble medium. *J. gen. Microbiol.* **25**, 47–59.

DEE, J. (1960*a*). A mating-type system in an acellular slime mould. *Nature, Lond.*, **185**, 780–781.

DEE, J. (1960*b*). *Genetic Analysis of a Myxomycete*. Ph.D. thesis. (University of Glasgow).

HOWARD, F. L. (1931*a*). The life history of *Physarum polycephalum*. *Amer. J. Bot.* **18**, 116–133.

HOWARD, F. L. (1931*b*). Laboratory cultivation of myxomycete plasmodia. *Amer. J. Bot.* **18**, 624–628.

KERR, N. S. & SUSSMAN, M. (1958). Clonal development of the true slime mould, *Didymium nigripes*. *J. gen. Microbiol.* **19**, 173–177.

MARTIN, G. W. (1940). The Myxomycetes. *Bot. Rev.* **6**, 356–388.
MARTIN, G. W. (1949). The Myxomycetes. *N. Amer. Flora*, I, **1**, 1–190.
NYGAARD, O. F., GUTTES, S. & RUSCH, H. P. (1960). Nucleic acid metabolism in a slime mould with synchronous mitosis. *Biochim. biophys. Acta*, **38**, 298–306.
ROSS, I. K. (1957). Syngamy and plasmodium formation in the Myxogastres. *Amer. J. Bot.* **44**, 843–850.
ROSS, I. K. (1961). Further studies on meiosis in the Myxomycetes. *Amer. J. Bot.* **48**, 244–248.
RUSCH, H. P. (1959). Organization of growth processes. *UNESCO Symposium on Biological Organization* (Pergamon Press), pp. 263–274.
STOSCH, H. A. von (1935). Untersuchungen über die Entwicklungsgeschichte der Myxomyceten. Sexualität und Apogamie bei Didymiaceen. *Planta*, **23**, 623–656.
WILSON, C. M. & ROSS, I. K. (1955). Meiosis in the Myxomycetes. *Amer. J. Bot.* **42**, 743–749.