GENETIC ANALYSIS IN THE COLONIA STRAIN OF *PHYSARUM POLYCEPHALUM*: HETEROTHALLIC STRAINS THAT MATE WITH AND ARE PARTIALLY ISOGENIC TO THE COLONIA STRAIN

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ABSTRACT

Amoebae of the Colonia (CL) strain, which normally produce plasmodia in clones, are shown to cross with *mt*3 and *mt*4 amoebae under appropriate conditions. Strains carrying *mt*3 and *mt*4 in a CL genetic background were constructed and used in the genetic analysis of mutants isolated in CL.

Physarum polycephalum, an acellular slime mold or Myxomycete, has two vegetative stages. In one stage the organism exists as small uninucleate amoebae and in the other as large multinucleate plasmodia (GRAY and ALEXOPOULOS 1968). Amoebae of all isolates that have been studied are ordinarily haploid (MOHBERG *et al.* 1973). In heterothallic isolates (Figure 1), plasmodia are diploid and are formed by the fusion of amoebae carrying different alleles (e.g., *mt*3 and *mt*4) of the mating type locus (DEE 1966a). The plasmodia undergo sporulation and meiosis, producing spores that release haploid amoebae upon germination. In the Colonia isolate, plasmodia are haploid (COOKE and DEE 1974a) and form within clones of amoebae (WHEALS 1970); thus, the plasmodia are hemizygous at all loci and express recessive mutations. Sporulation and spore germination resemble the same processes in heterothallic isolates; however, it is not known whether meiosis (which would have to be preceded by chromosome doubling) occurs. As shown here and by others (WHEALS 1970; COOKE and DEE 1974b), Colonia amoebae cross with heterothallic amoebae under certain conditions, producing a diploid plasmodium (Figure 1). The progeny of such a hybrid include amoebae that form plasmodia in clones and amoebae of the heterothallic mating type in a 1:1 ratio. Thus, the clonal plasmodium forming (CPF) property of Colonia amoebae is controlled by an allele, designated *mth*, of the mating type locus. (We use both the functional term, CPF, and the genotypic term, *mth*, because certain mutants carry *mth* and are not CPF (WHEALS 1973) and mating type heterozygotes can be isolated that are CPF and not *mth* (ADLER and HOLT, 1975).)

Direct descendants of the Colonia amoebae originally used by WHEALS (1970) form plasmodia in clones much more rapidly than the original line. The rapid plasmodium formation of the recent sublines is essential in certain selection

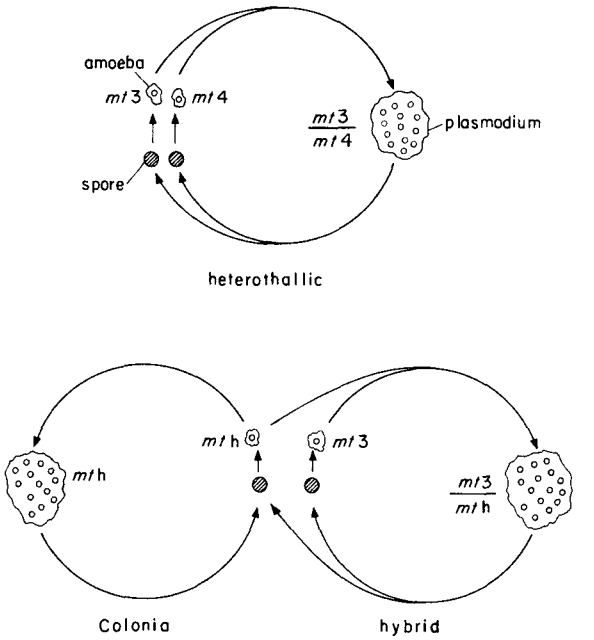

FIGURE 1.—Life cycles of *P. polycephalum.*

procedures (WHEALS 1973). However, the sublines do not cross readily (WHEALS 1973; DEE, WHEALS and HOLT 1973; DEE 1973), which seems to preclude the possibility of genetic analysis.

We show here that *mt*3 and *mt*4 strains from the Indiana isolate of *P. polycephalum* (DEE 1966a) will cross with the recent, rapid-plasmodium-forming Colonia amoebae under appropriate conditions. Published work on plaque size (HAUGLI and DOVE 1972) and nutritional (DEE, WHEALS and HOLT 1973) variants in *P. polycephalum* as well as work with other organisms (FINCHAM and DAY 1963) demonstrate the desirability of using isogenic strains in genetic analysis. Thus, we carried out a series of backcrosses in an attempt to construct amoebal strains that carry the *mt*3 and *mt*4 alleles, mate with rapid-plasmodium-forming Colonia amoebae, and are partially isogenic with Colonia.

MATERIALS AND METHODS

Culture procedures: All cultures were maintained at 26° unless otherwise stated. Amoebae were routinely grown on a lawn of live *E. coli* on liver infusion agar (LIA) (WHEALS 1970, 1973). Plasmodia were grown on plasmodial rich medium agar (PRM-agar). PRM is the standard casein hydrolysate-yeast extract medium (HORWITZ and HOLT 1971) supplemented with 0.5 g/l glycine and is solidified by mixing with an equal volume of 3% agar. Crossing was carried out by mixing two amoebal strains in a small "puddle" of 0.05–0.1 ml *E. coli* suspension (HAUGLI 1971) on dPRM agar (PRM diluted 20-fold in 1.5% agar). About 10^3 amoebae of each strain were used. Some crosses were performed at 30°. Plasmobia derived from amoebae with the *mt*h allele were allowed to form by a similar procedure and are referred to as "CPF plasmodia."

Sporulation and spore germination were carried out as described by WHEALS (1970). Amoebae from well-separated plaques on germination plates were recloned before they were analyzed.

Amoebal strains: A variety of systems have been used to describe strains used in Physarum genetics. For ease of reference, we have numbered serially the strains used in our laboratory. These numbers are cross referenced to other systems in Table 1. We use the abbreviation CL to refer to current isolates of the Colonial strain, which is treated as wild type in several laboratories. Descriptions of the various alleles may be found in the text and references. The plasmodial fusion loci *f* and *n* (DEE 1973) have been renamed *fusA* and *fusB*, respectively. The same allele numbers have been retained. i.e. *f*1 is now *fusA*1. All strains used in this paper except CH3 and CH7 are *fusB*1.

Determination of phenotype: The crosses described in this paper are of the "hybrid" type (Figure 1). The CPF amoebal progeny from such a cross were routinely designated *mt*h. The non-CPF amoebae were routinely assigned the heterothallic mating type that had been used to form the crossed plasmodium from which they were derived. In several hundred cases such non-CPF amoebae have been tested for mating by mixing with heterothallic amoebae of the mating type used to form the crossed plasmodia and with heterothallic amoebae of a different mating type. In every case, plasmodia were not formed with amoebae of the same mating type and were formed with amoebae of the different mating type.

Emetine chloride (Mann) was sterilized by filtration and incorporated into liver infusion agar plates. Tests for antibiotic resistance and for plaque size were carried out by the methods of HAUGLI, DOVE and JIMINEZ (1972) and HAUGLI and DOVE (1972). The measurement of mean plaque size was reproducible from plate to plate and experiment to experiment (ADLER 1975).

The genes that influence plasmodial somatic fusion are used in this paper as markers only. These genes do not influence mating and the *mt* genes do not influence plasmodial fusion. Two genetically identical plasmodia fuse on contact to form a heterokaryon. Plasmodia formed clonally by *m*th amoebae bearing different alleles at plasmodial fusion loci do not fuse. Hemizygotes and homozygotes behave identically with respect to fusion. Plasmodia which are heterozygous for fusion alleles may or may not fuse with a plasmodium of a different genotype depending on the specific dominance relationships for the alleles. Plasmodial fusion phenotypes were determined by placing two agar blocks (from previous PRM-agar plates) bearing growing plasmodia onto a fresh PRM-agar plate (CARLILE and DEE 1967). The new plate was examined at about 24 hours and at intervals thereafter. If at one of the times of observation the two plasmodia were in intimate contact but had not established veins linking the two plasmodia, the pair of plasmodia were called non-fusing. If two well-defined plasmodia were linked by veins, the pair was called fusing. In some cases, several hours after vein formation a cytotoxic reaction occurred

TABLE 1

Amoebal strains

Designation here	Other designations	Progeny (or mutant) of	Genotype	Source and reference
CL		C50	*mth fusA*2 *fusC*1	COOKE and DEE 1947b
CH2	a		*mt*1 *fusA*1	DEE 1962
CH3	i		*mt*2 *fusA*2	DEE 1962
CH4	B173		*mt*3 *fusA*4 *fusC*2	DEE 1956a
CH5	B174		*mt*4 *fusA*3 *fusC*2	DEE 1966a
CH7	A7138		*mt*1 *fusA*2 *act*	DEE and POULTER 1970
CH9	APT1	(C50)	*mth fusA*2 *fusC*1 *apt*1	WHEALS 1973
CH10,11		CH2 × CH9	*mt*1 *fusA*1	
CH36		(CL)	*mth fusA*2 *fusC*1 *eme* E4	
CH45		CL × CH4, fourth generation (see Figure 2)	*mt*3 *fusA*2 *fusC*2	
CH111		CL × CH45	*mth fusA*2 *fusC*2	

resulting in the cessation of streaming in an area of several cm^2; this reaction is referred to as fusion killing (CARLILE and DEE 1967). Microfusions (CLARK and COLLINS 1973) were not observed in these strains, although an extensive search for them was not undertaken.

RESULTS

Conditions for mating CH4 and CH5 with CL: We repeated earlier observations that the rapid plasmodium forming sublines of Colonia (CL) do not cross readily. Heterothallic strains that we tried to cross to CL included CH2,3,4,5,7,10 and 11 (Table 1). Crossing was attempted on liver infusion agar plates supplemented with 0.4 mM para-aminobenzoic acid (DEE 1966b), nutrient broth-glucose-agar plates (POULTER 1969), and on dPRM-agar plates (DEE, WHEALS and HOLT 1973). We tried cross streaking the two strains to be crossed, spreading the two strains in different absolute and relative numbers over the whole plate, and mixing the amoebae in a small area ("puddle mating") in the center of the plate. Most of these attempted matings were performed at 26° and at 29.5°. Plasmodia that arose were tested for their ability to fuse with CL plasmodia. In nearly all cases fusion was observed, indicating that plasmodia had arisen directly from CL amoebae without crossing. However, when CH4 (*mt*3) and CH5 (*mt*4) were "puddle mated" with CL on dPRM-agar plates at 26° and 29.5°, the resulting plasmodia would not fuse with CL plasmodia. The putative CH4 × CL plasmodia fused with CH4 × CH9, and the putative CH5 × CL plasmodia fused with CH5 × CH9. (Strain CH9 is an amoebal-plasmodial-transition mutant of Colonia that has the genotype *mth apt*1, does not form plasmodia in clones, crosses reliably with heterothallic strains, and carries the same plasmodial fusion alleles as CL (WHEALS 1973).) These results strongly suggest that CH4 and CH5 crossed with CL under the conditions used.

We tried 29.5° for crossing because we found that CL forms plasmodia in clones slowly at this temperature. Neither crossing of CH4 with CH9 nor the growth rate of CL amoebae was influenced by raising the temperature from 26° to 29.5°. Although the higher temperature was not required for crosses with CH4 and CH5, it has proven valuable in crossing inbred *mt*3 and *mt*4 strains with CL. The utility of dPRM- agar for crossing may derive from its low pH (COLLINS and TANG 1973).

Fusion phenotypes of progeny of CH4 × CL: Because of evidence that CH5 is diploid (MOHBERG *et al.* 1973), we first studied the CH4 × CL plasmodium. To confirm that the plasmodium was a cross, we induced sporulation, isolated amoebal clones from the spores, and looked for evidence of recombination between mating type and fusion type. Among the 100 progeny examined, 48 formed plasmodia in clones. Forty of the CPF plasmodia were fusion tested with CL plasmodia, and, in certain combinations, with one another. The results (Table 2) show that most of the plasmodia did not fuse with CL and those that did fuse with CL produced fusion killing. These results strongly suggest that recombination between mating type and fusion type has occurred. On the basis of published models (POULTER and DEE 1968; WHEALS 1970), we expected only two fusion classes, *fusA*2 and *fusA*4. However, our results do not directly contradict any

TABLE 2

Plasmodial fusion behavior of CPF progeny of CL × CH4

Class	Tester strains						Number in class
	CL	CL·CH4:74	CL·CH4:47	CL·CH4:94	CL·CH4:88	CL·CH4:92	
1	—	(+)	—	—	+	—	1
2	—	—	(+)	—	—	—	1
3	—	—	—	(+)	—	—	1
4	*	+	—	—	(+)	—	1
5	—	—	—	—	—	(+)	1
6	*	—	—	—	*	—	1
7	*	*	—	*	+	—	1
8	*	*	—	—	*	—	1
9	*	*	—	—	—	—	2
10	*	—	—	*	—	—	1
11	*	—	—	*	*	—	1
12	—	*	—	*	*	—	2
13	—	*	—	—	*	—	6
14	—	+	—	—	*	—	3
15	—	*	—	—	—	—	1
16	—	+	—	—	*	*	1
17	—	*	—	—	*	*	1
18	—	—	*	—	—	—	2
19	—	—	*	—	—	*	1
20	—	—	*	*	—	+	1
21	—	—	—	*	—	—	1
22	—	—	—	+	—	*	1
23	—	—	—	—	*	—	1
24	—	—	—	—	+	*	1
25	—	—	—	—	*	*	1
26	—	—	—	—	—	*	2
27	—	—	—	—	—	—	2
28	—	*	—	—	—	*	1
						Total tested	40

+ = fusion; * = fusion followed by killing; — = non-fusion; (+) = a plasmodium tested against itself. A plasmodium is placed in a particular class if it displays the pattern of —, +, and * shown in the corresponding row of the table. Classes 1–5 contain, as shown in the table, only one plasmodium each and these plasmodia are identical to the 5 CL·CH4 progeny used as testers.

published experiments, and complex fusion behavior involving dominance and multiple loci has been observed in the isolate (Indiana or Turtox) from which CH4 is derived (COLLINS 1972), as well as in another isolate (COLLINS and HASKINS 1972).

Successive backcrosses of mt3 *strains to CL*: Since strains CH4 and CL are derived from different natural isolates (DEE 1973), they may carry different alleles at many loci and they may differ chromosomally as well. The existence of many such differences between the strains is suggested by the variety of fusion and other (see below) phenotypes among their progeny. Thus, a series of backcrosses to CL was carried out, selecting for a *mt3* clone at each stage (see Figure 2).

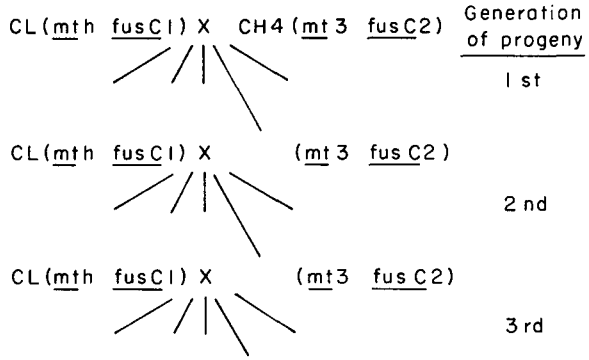

FIGURE 2.—Scheme for backcrossing and designation of generation number (*mt3* series).

To begin the backcross series, an additional 100 CH4 × CL amoebal progeny strains were isolated and analyzed for mating type (Table 3). Attempts were made to cross 10 of the *mt3* progeny with CL. Plasmodia arose in all of the attempted matings, and samples of the plasmodia were fusion tested with CL. Six of the plasmodial strains failed to fuse (Table 3), suggesting that these plasmodia arose from crossing rather than clonally. One of the non-fusing plasmodia was selected for further backcrossing and sporulated. The spores produced both *mth* and *mt3* amoebal strains (Table 3). Attempts were made to cross 15 of the *mt3* strains, as above, and the procedure was repeated. The scheme for the crosses is given in Figure 2 and data on the segregation of mating and fusion behavior in Table 3.

A plasmodium that did not fuse with CL was selected at each step of backcrossing to ensure that the selected plasmodium was crossed rather than formed clonally from CL. Thus we anticipated that at least two genes would be retained from CH4: one controlling mating type and one controlling plasmodial fusion. No other intentional selection was applied during the backcrossing procedure. Two is a minimum for the number of cistrons necessarily retained: either or both

TABLE 3

Segregation of mating type and fusion behavior in the mt3 *backcross series*

Amoebal generation	Mating type		Fusion behavior of *mt3* × CL plasmodia with CL plasmodia			
	mth	*mt3*	Number tested	Fusion	Fusion + killing	Non-fusion
1	57	43	10	1	3	6
2	14	26	15	9	1	5
3	22	20	10	6	0	4
4	7	18	8	5	0	3
5	58	42	9	3	0	6
6	12	8	7	5	0	2
7	45	55	6	4	0	2
8	21	19	6	4	0	2

Data are number of progeny strains in each category.

mating and plasmodial fusion may be controlled by more than one gene, and genes controlling these phenotypes may be linked to unrelated genes that cannot, for reasons of non-homology, be exchanged between the two natural isolates by crossing over. Thus we considered it essential to obtain information relative to the extent to which the *mt*3 strains were becoming more like CL.

Plasmodial fusion behavior of backcrossed strains: Of 10 plasmodia produced by mixing first generation *mt*3 amoebae and CL, only 1 fused compatibly with CL and 3 gave fusion killing with CL (Table 3). Similarly formed plasmodia from the second generation gave 9/15 fusions and 1/15 fusion killing. In the third and subsequent generations, fusion and non-fusion appeared in about a 1:1 ratio, and fusion killing was absent (Table 3). These observations suggest that alleles resulting in fusion killing were eliminated by the third generation. In addition, they suggest that at least in the later generations, only one pair of alleles controlling plasmodial fusion segregated. To test these suggestions further, plasmodia were formed clonally from the 58 *mt*h fifth generation progeny and their fusion behavior with one another and with CL was determined (Table 4). As expected, the plasmodia fell into two approximately equal classes and no fusion killing was observed. Thus, only one CH4 allele affecting plasmoidal fusion remained in the fifth generation. The locus is unlinked to mating type and is designated *fusC*. The allele from CL is designated *fusC*1 and that from CH4, *fusC*2. A heterozygote for *fusC*1 and *fusC*2 (CL × CH45) was fusion tested against several *fusC*1 and *fusC*2 hemizygotes and homozygotes. The heterozygote did not fuse with *fusC*1 hemizygotes and homozygotes but did fuse with the *fusC*2 hemizygotes and homozygotes; *fusC*2 is therefore dominant to *fusC*1.

Behavior of CPF progeny on a defined medium: Plasmodia produced clonally from *mt*h progeny were tested at 29.5° for growth on a defined medium (DM-1) containing glucose, glutamate, methionine, glycine, thiamine, biotin, hematin and salts (DEE, WHEALS and HOLT 1973). Twenty-three of 47 first generation CPF plasmodia failed to grow on DM-1 and among these were 6 that grew poorly on PRM. Although the genetic basis for the nutritional differences among the plasmodia is not known, the fact that a large fraction (24/47) grew on the minimal medium suggests that few loci are involved. All of the plasmodia from the third (20 cases) and fifth (56 cases) generations grew on both DM-1 and PRM.

TABLE 4

Plasmodial fusion behavior of CPF progeny of CL × CH45

Tester strains			Number of progeny
CL	CH111		
+	—		31
—	+		27
+	+		0
—	—		0
		Total tested	58

Symbols as in Table 2. Strain CH111 is one of the 58 plasmodia tested.

Plaque sizes of first and fifth generation heterothallic progeny: Strains CH4 and CL produce plaques of markedly different mean diameters, 6.5 mm and 2.6 mm, respectively, after 6 days of growth. The heterothallic progeny of CH4 × CL produced a wide distribution of mean plaque sizes with a peak in the 3–3.5 mm class (Figure 3). Heterothallic progeny of the fifth generation showed a much narrower distribution of sizes, with the average size (2.76 mm) slightly greater than the mean for CL (2.65 mm). An analysis of variance showed that the inter-strain variance was significantly greater than the intra-strain variance. Thus, it appears that the *mt*3 parent of the fifth generation progeny and CL still differ in genes controlling plaque size. However, it is clear that any such difference is much less than the difference between CH4 and CL, and in any case the variability has not interfered with the genetic analysis of plaque size mutants in this laboratory (S. SELVIG and P. ADLER, unpublished data).

Our finding that CL plaques are much smaller than CH4 plaques raised the possibility that the strain chosen for wild type grows relatively slowly. Consequently, we measured amoebal growth rate in exponential phase on plates inoculated with 10^4 amoebae per plate. Growth was exponential for 2–3 days and the

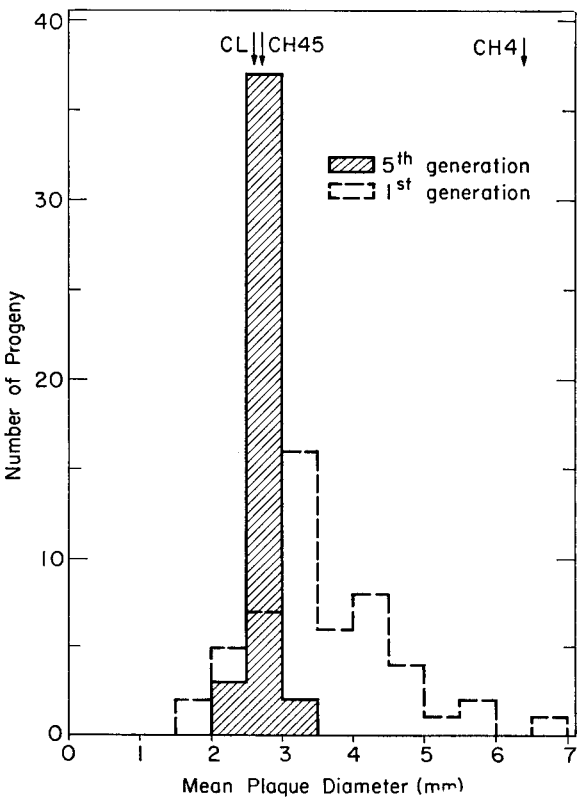

FIGURE 3.—Distribution of mean plaque diameters for first and fifth generation *mt*3 progeny. Non-encysted amoebae were plated with *E. coli* on LIA. The plates were scored after 6 days' incubation at 26°.

doubling time for both strains was in the range 6 to 6.5 hours. Thus, the strain chosen for wild type will grow as fast or nearly as fast as CH4.

Attempt to detect segregation of mating ability: It was not difficult, at each stage of backcrossing, to find a *mt*3 strain that would mate with CL amoebae as judged by fusion criteria. Thus, it appeared that the ability to mate with CL did not segregate among the progeny. On the other hand, we would not have detected *mt*3 *fusC*2 strains that had lost the ability to mate with CL, since such strains would have been indistinguishable from *mt*3 *fusC*1 amoebae. Thus, we independently determined the *fusC* allele in 9 unselected *mt*3 fifth generation progeny. Each progeny strain was crossed with CH9 (*mt*h *fusC*1 *apt*1) and the resulting plasmodia were scored for fusion or non-fusion with CL. Six of the 9 plasmodia failed to fuse, showing that the *mt*3 parent carried *fusC*2. The same 6 strains were also mixed with CL amoebae, and in each case, a plasmodium arose that would not fuse with CL. (As expected, the remaining three strains gave plasmodia that fused with CL when crossed with CL.) Thus, there was no segregation of mating ability among the *mt*3 *fusC*2 progeny. If one pair of alleles affecting mating and unlinked to *mt* and *fusC* had segregated, the probability of obtaining the above results would be 1/64; if there were two or more alleles, the probability would be negligible.

Reduction in mating ability during backcrossing: Although we were not able to detect variation of mating behavior within a generation, a gradual reduction in mating ability is apparent when heterothallic amoebae from the various generations are compared. If CH4 and CL are mixed at 26° or 29.5°, macroscopic plasmodia appear in 3 to 4 days. At 26°, the ratio of crossed to clonally formed plasmodia arising from such mating plates is greater than 100:1. At 29.5° a clonally formed plasmodium has never been recovered. First, second, and third generation *mt*3 strains behave similarly to CH4, except that a slightly longer time (about 12 hours with the third generation) is required for plasmodium formation. With a fourth generation *mt*3 strain (CH45) the results are more complex. Crossing is still very efficient at 30°, although it takes from 5–8 days for plasmodium formation. Crossing is erratic at 26°, with the ratio of crossed plasmodia to clonal plasmodia summed over many experiments being about one. In any particular experiment, however, it is usual to find all crossed plasmodia or all clonal plasmodia. When fifth through eighth generation *mt*3 amoebae are are mixed with CL at 26°, usually only clonal plasmodia arise, while at 29.5° mostly crossed plasmodia are found. As in the case of attempted crosses between CH45 and CL at 26°, within any one experiment it is common to find all crossed or all clonal plasmodia. We have not succeeded in determining a variable or variables that cause one set to produce mostly clonally formed plasmodia. In any case, the erratic crossing behavior exhibited by the later generations at 29.5° has not seriously hindered the utility of these strains for genetic analysis.

Use of backcrossed mt3 *strains in mutant analysis*: Strain CL amoebae were mutagenized by ethyl methanesulfonate (HAUGLI 1971), grown for 2 days on LIA, and then plated on LIA plates containing 100 μg/ml emetine chloride. Several mutant colonies appeared, and one (CH36) was selected for detailed analysis. Amoebae of this strain form 2 mm plaques after 7 days at 100 μg/ml

TABLE 5

*Analysis of progeny of CH36(*mt*h* eme fusC*1) × CH45(*mt*3* eme+ fusC*2)*

Genotype	Number of progeny
mth eme	27
mth eme+	26
mt3 eme	21
mt3 eme+	26
Total analyzed	100
mth fusC1 eme	15
mth fusC1 eme+	11
mth fusC2 eme	11
mth fusC2 eme+	10
mth eme not fusion tested	1
mth eme+ not fusion tested	5

Emetine resistance was determined on LIA with 100 μg/ml emetine.

emetine, whereas CL does not produce plaques at 75 μg/ml emetine in 2 weeks. Strain CH36 was mated with CH45, the fourth generation *mt3* strain selected for further analysis, and the resulting plasmodium was sporulated. Amoebal strains derived from the spores were *mt3*:*mth* in a ratio of 53:47 (Table 5). Emetine resistance (*eme*) segregated 1:1 and independently of mating type. The CPF progeny were analyzed for fusion behavior (Table 5, lower half) The results show segregation of *fusC* independent of emetine resistance. Strain CH36 carries, in addition to a gene conferring emetine resistance, a lesion that leads to non-growth on DM-1. This lesion has been found to segregate in a 1:1 fashion independently of *mt*, *fusC* and *eme*.

A number of amoebal strains showing reduced growth at 30° have been isolated from mutagenized amoebae (S. SELVIG, unpublished). We attempted to cross 10 of these strains at 26° with CH45. Five of the 10 crossed on both of the first two attempts and the remaining strains were successfully crossed within 2 to 4 attempts.

Backcross series with mt4: We have also carried out a similar series of backcrosses starting with CH5 × CL and designed to construct a strain bearing *mt4* in a Colonia background. So far, seventh generation *mt4* amoebae have been obtained and crossed to CL. As in the *mt3* series, convergence to CL phenotypes was observed for both plaque size and plasmodial growth properties. The plasmodial fusion allele retained from CH5 is phenotypically the same as that in a Colonia background. So far, seventh generation *mt4* amoebae have been retained from CH4, i.e., *fusC2*. In addition, a plasmodial-fusion-killing allele has been retained. The allele in CL is called *kilA1* and the allele retained from CH5 is called *kilA2*. The *kilA* locus is unlinked to *mt* or *fusC*. The *kilA* locus influences not whether two plasmodia fuse but the behavior of a heterokaryon after fusion has occurred. When two plasmodia differing in their *kilA* genotypes fuse, a region of killed plasmodium results (CARLILE and DEE 1967). The fifth generation *mt4* strain selected for further analysis had the same DNA content by Feulgen staining and microspectrophotometry (COOKE and DEE 1974a) as CL and fifth generation *mt3* strains.

DISCUSSION

The inbred *mt*3 and *mt*4 strains described in this paper provide the means for genetic analysis of mutants isolated in the Colonia strain (Table 5). The method has the advantage that it can be applied directly to mutants isolated in the Colonia amoebae that form plasmodia rapidly. An alternative method, which utilizes amoebae that form plasmodia slowly, is applicable when rapid plasmodium formation is not required, or when a mutant strain is converted, by selection, to slow plasmodium formation (COOKE and DEE 1974b).

It was not evident *a priori* that it would be possible to construct a strain that contained mostly Colonia genes and displayed heterothallic mating behavior. Even though heterothallic amoebae were known to be produced from a Colonia by heterothallic cross (WHEALS 1970, 1973), the low spore viability often found in *P. polycephalum* would permit these heterothallics to be highly selected for retention of genes from the heterothallic parent. For several reasons, we believe that backcrossing produced strains containing mostly Colonia genes. (1) Backcrossing resulted in dramatic reductions in heterogeneity of fusion (Tables 2 and 4) and plaque size (Figure 3) phenotypes, elimination of heterogeneity in plasmodial growth on DM-1 and PRM, and a convergence to the CL phenotypes. Spores from the cross CL by CH20 (*mt*3, third generation) were essentially 100% viable (number of plaques/hemacytometer count); that is, it appears that a particular set of chromosomes from CH4 is not required for viability of *mt*3 (or *mt*h) strains (3) The fact that clonal and heterothallic behavior continued to segregate approximately 1:1 through the series of backcrosses (Table 3) supports the hypothesis that mating behavior is primarily due to a single locus or to a group of loci on a single chromosome.

The fusion allele that we retained from CH4 and CH5 does not appear to be at either the *fusA* or *fusB* loci previously described in these strains (WHEALS 1970). Strains CL, CH4 and CH5 carry the same allele at the *fusB* locus (WHEALS 1970). In the model proposed for control of fusion by alleles of the *fusA* locus, the various alleles do not display dominance with respect to one another (POULTER and DEE 1968).

Mating type h amoebae, when mixed with heterothallic amoebae, face two competing developmental pathways (Figure 1). The increased proportion of crosses at 30° results, presumably, from selective inhibition of the clonal pathway at this temperature. The cellular basis for this selective effect, which occurs even within highly inbred strains, is obscure. The cellular basis of reduced ability of the highly inbred *mt*3 strains to cross with CL is similarly obscure. The reduction in crossing ability could have resulted from an effect on one or more of the steps in crossed plasmodium formation.

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