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Genic basis of the mate-killer trait in *Paramecium aurelia*, stock 540

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1. INTRODUCTION

Stock 540 of *Paramecium aurelia* (syngen or variety 1) is a 'mate-killer', i.e. individuals of this stock, when caused to conjugate with animals belonging to other ('sensitive') stocks, bring about the eventual death of all ex-conjugants deriving cytoplasm from the sensitive stocks (Beale, 1957). Such mate-killer paramecia contain in their cytoplasm large numbers of bacterium-like mu particles, and a clear association between presence of these particles and possession of the mate-killing trait was demonstrated. Details concerning the structure of the mu particles have been described by Beale & Jurand (1960), and preliminary genetic study showed that maintenance of the mu particles in stock 540 was dependent on the presence of a dominant gene *M*, which was absent in at least one sensitive stock (stock 60), lacking mu particles (Beale, 1957). Thus in essentials the mate-killing phenomenon of stock 540 conformed with what had previously been described of mate-killers in other stocks of *P. aurelia* belonging to syngen (variety) 8 (Siegel, 1953, 1954; Levine, 1953).

The object of the present investigation was to study in more detail the gene or genes necessary for maintenance of the mu particles in stock 540, and hence for the mate-killing properties of the host paramecia.

2. MATERIALS AND METHODS

The stocks used, all belonging to syngen 1 of *P. aurelia*, are listed below:

Stock No.	*Killer or sensitive*	*Origin*
540	Mate-killer	Mexico
168	Sensitive	Japan
119	,,	Pennsylvania, U.S.A.
217	,,	Florida, U.S.A.
513	,,	France
523	,,	Switzerland
544	,,	Louisiana, U.S.A.

All except stocks 513 and 523 were kindly supplied by Dr T. M. Sonneborn.

Scoring for presence of mu particles was done by crushing the paramecia between a cover-slip and a glass slide and examining the exudate under the phase-contrast

microscope, as previously described (Preer, Siegel & Stark, 1953; Beale, 1957). It has been repeatedly confirmed that presence of mu particles is a reliable indicator of the mate-killing trait of the host paramecium. In animals which had changed genotype at conjugation or autogamy, an interval of fifteen fissions was allowed to elapse before scoring for particles since Chao (1953) had found that the disappearance of kappa particles in stock 51 (syngen 4) following substitution of the allele *K* by *k* sometimes required this number of fissions, and similar evidence has been obtained by us for the mu particles of stock 540 (see below).

RESULTS

1. *Crosses between stock 540 (mate-killer) and six sensitive stocks of syngen 1*

With the aim of determining whether a number of sensitive stocks lacked the gene or genes necessary for maintenance of the mu particles, animals belonging to six stocks (all sensitive) of widely separated origins were crossed with stock 540.

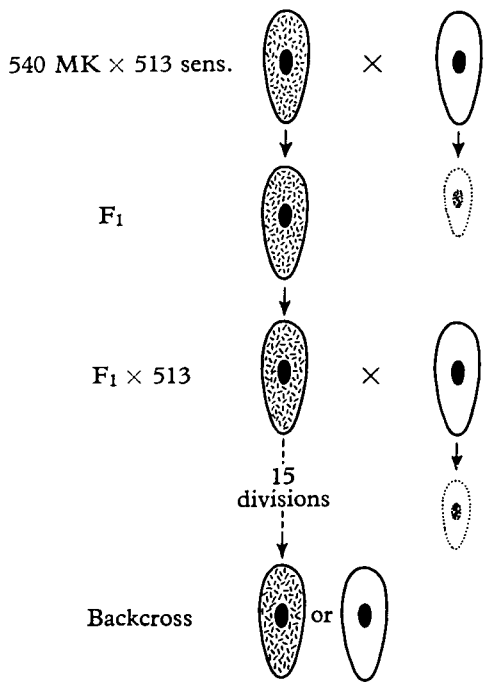

Fig. 1. Scheme of crossing stock 540 (mate-killer) and stock 513 (sensitive) to obtain segregation of *M* genes in backcross generation.

The surviving F_1 ex-conjugants, which were themselves all mate-killers, were then backcrossed to their respective sensitive parents, and the progeny classified in regard to possession of mu particles. The scheme of crossing is indicated in Fig. 1, and the results are summarized in Table 1. In addition to the backcross generations, some ex-autogamous F_2s were also raised, and are included in Table 1. They were

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Table 1. *Progeny of hybrids between stock 540 (mate-killer) and six sensitive stocks*

Stocks crossed	Backcrosses of hybrids to sensitive stocks			Ex-autogamous F_2s		
	Mate-killers	Sensitives	Dead*	Mate-killers	Sensitives	Dead*
540 × 217	47	11	4	—	—	—
540 × 523	23	12	10	—	—	—
540 × 119	37	19	8	—	—	—
540 × 168	35	16	3	15	9	75
540 × 513	37	13	20	52	19	197
540 × 544 (*a*)	34	34	15	22	14	136
(*b*)	65	37	11	19	23	142
(*c*)	100	50	20	—	—	—
(*d*)	70	30	10	45	14	152

* These refer to clones which died due to some lethal genetic constitution and have nothing to do with mate-killing.

less useful than the backcrosses on account of the large amount (75–90%) of post-autogamous inviability found with these inter-stock hybrids.

The results showed that a proportion of sensitive animals was obtained in the backcross and F_2 generations from all combinations of stocks tested. It is therefore assumed that none of the six sensitive stocks contained all the genes necessary for maintenance of mu particles.

Few of the backcross data given in Table 1 suggest a 1 : 1 ratio of mate-killers to sensitives, such as would occur if stock 540 contained a single dominant gene M which was lacking in the sensitive stocks, as previously proposed (Beale, 1957). Crosses involving five out of the six sensitive stocks gave proportions not significantly differing from 3 mate-killers to 1 sensitive. (The sixth, stock 544, is considered below.) Such a 3:1 ratio would be expected if stock 540 contained two unlinked duplicate genes M_1 and M_2, either one alone being capable of maintaining the mu particles. In the backcross generation there would then be four genotypes ($M_1m_1M_2m_2$, $M_1m_1m_2m_2$, $m_1m_1M_2m_2$, $m_1m_1m_2m_2$) in equal proportions, only the last producing the sensitive phenotype. To test this hypothesis a more detailed study was made of the progeny from one series of crosses, that involving stocks 540 and 513.

2. *Further study of progeny from crosses between stocks 540 (mate-killer) and 513 (sensitive)*

An ex-autogamous F_2 family consisting of fifty-two mate-killer clones and nineteen sensitives was obtained from the cross 540 × 513 (see Table 1). According to the duplicate-factor hypothesis the fifty-two mate-killer clones should fall into three genotype classes ($M_1M_1M_2M_2$, $M_1M_1m_2m_2$, $m_1m_1M_2M_2$). To test this, ten of these F_2 clones (designated 'Testers 1–10') were taken at random, and each crossed and backcrossed to stock 513. The presence or absence of mu particles in the further backcross families thus obtained was noted and the results are given in Table 2.

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Table 2. *Progeny of hybrids between stock 513 (sensitive) and ten 'tester F_2s' (mate-killers) derived from crosses between stocks 540 and 513*

Cross	Backcrosses of hybrids (tester F_2s × 513) to stock 513: Mate-killers	Sensitives	Dead†	Presumed theoretical ratio of mate-killers : sensitives
513 × Tester 1	72	28	4	3 : 1
2	79	21	5	3 : 1
3	58	42	7	1 : 1
4	62	38	10	3 : 1*
5	48	52	2	1 : 1
6	79	21	3	3 : 1
7	54	46	1	1 : 1
8	77	23	4	3 : 1
9	52	48	6	1 : 1
10	80	20	5	3 : 1

* See Table 3 for confirmation of genotype of Tester 4.
† See note in Table 1.

Five of the testers (Nos. 1, 2, 6, 8, 10) then gave backcross families in which the ratio of mate-killers to sensitives did not differ significantly from a 3:1, while four others (Nos. 3, 5, 7, 9) gave ratios not significantly different from a 1 : 1 ratio. (One tester, No. 4, gave indecisive results at this stage.) The testers 3, 5, 7, 9 were therefore presumed to contain only a single one of the dominant factors (M_1 or M_2), and testers 1, 2, 6, 8, 10 to contain both (M_1 and M_2).

Further crosses were then made between the four 'single factor' testers 3, 5, 7, 9, as shown in Table 3. The results indicated that testers 3 and 5 formed one group (denoted $M_1M_1m_2m_2$) and testers 7 and 9 a second group ($m_1m_1M_2M_2$). Inter-

Table 3. *Progeny obtained by intercrossing the 'tester' clones from an F_2 derived from stocks 540 × 513. After intercrossing the 'testers' the hybrids were passed through autogamy to yield a further ex-autogamous F_2. Some 'tester' hybrids were also backcrossed to stock 513*

Testers crossed	Nos. of mate-killer and sensitive progeny: Hybrids backcrossed to 513: Mate-killers	Sensitives	Dead*	Hybrids passed through autogamy giving F_2s: Mate-killers	Sensitives	Dead*
3 × 5				100	0	6
3 × 7				86	16	21
3 × 9				94	6	30
5 × 7	65	35	4	80	20	20
5 × 9	74	29	10	78	23	10
7 × 9				100	0	5
4 × 5	95	0	20			
4 × 9	86	0	20			

* See note in Table 1.

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crossing of clones belonging to the different groups gave rise, after autogamy, to both mate-killers and sensitives.

Tester 4, whose genotype had been undecided from the results given in Table 2, was then crossed to each of testers 5 and 9, now known to belong to different groups. When these hybrids were backcrossed to a sensitive clone, only mate-killer progeny were obtained, indicating that tester 4 contained both dominant genes M_1 and M_2.

Since all the results from crosses between stocks 540 and 513 conformed with expectations based on the two-factor hypothesis (apart from a few minor numerical deviations), the hypothesis was considered proved.

3. *Further study of progeny from crosses between stocks 540 and 544*

Reference to Table 1 will show that while five of the sensitive stocks (217, 523, 119, 168, 513), after crossing to the mate-killer stock 540, gave results in good numerical agreement with the duplicate-factor hypothesis, stock 544 did not: there was a significant excess of sensitive clones over the one-quarter expected in backcrosses of 540/544 × 544. Further study was therefore given to these progeny. Ten F_2 clones (mate-killers) obtained by passage through autogamy of the hybrid 540/544 were designated 'testers 11–20' inclusive. The latter were each crossed to stock 544 and the hybrids backcrossed once more to stock 544 (Table 4). Four of these testers

Table 4. *Progeny obtained from 'tester' clones derived from an ex-autogamous F_2 from stocks 540 × 544. Each tester was crossed to stock 544 and the hybrid backcrossed to stock 544*

Stocks crossed	Nos. of progeny in backcross of hybrid to stock 544			Presumed theoretical ratio of killers : sensitives
	Mate-killers	Sensitives	Dead*	
Tester 11 × stock 544	64	35	4	2 : 1
12 ,,	70	28	6	2 : 1
13 ,,	48	49	3	1 : 1
14 ,,	65	34	0	2 : 1
15 ,,	69	31	1	2 : 1
16 ,,	60	35	2	2 : 1
17 ,,	52	48	8	1 : 1
18 ,,	51	46	9	1 : 1
19 ,,	44	43	14	1 : 1
20 ,,	76	35	3	2 : 1

* See note in Table 1.

(Nos. 13, 17, 18, 19) yielded 1 : 1 ratios of mate-killers to sensitives; the remaining six (Nos. 11, 12, 14, 15, 16, 20) (like the original F_2 from stocks 540 × 544) gave an excess of sensitives on an expectation of 3 mate-killers : 1 sensitive. These 'aberrant' figures suggested a 2:1 ratio. It was therefore postulated that one or other of the recombination classes, either $M_1M_1m_2m_2$ or $m_1m_1M_2M_2$, was lacking in these progeny. This supposition was checked by crossing each of the testers 11–20 with the two testers 5 and 9 derived earlier from crosses between stocks 540 and 513.

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Tester 5 had previously been assigned the genotype $M_1M_1m_2m_2$ and tester 9 $m_1m_1M_2M_2$. Table 5 gives these results, which show that it is the group $m_1m_1M_2M_2$ which is missing. Six of the testers 11–20 belonged to group $M_1M_1M_2M_2$ and four to $M_1M_1m_2m_2$. While the numbers are not great, these results, taken together with the previous ones involving stocks 544 and 540, are consistent with the view that stock 544, though it evidently has the genotype $m_1m_1m_2m_2$, on crossing with stock 540 ($M_1M_1M_2M_2$) yields only three of the four expected classes, thus giving a 2:1 ratio of mate-killers to sensitives in ex-autogamous F_2s and backcrosses to the sensitive parent.

Table 5. *Progeny obtained by crossing testers 11–20 (from stocks 540 × 544) with testers 5 ($M_1M_1m_2m_2$) and 9 ($m_1m_1M_2M_2$)*

Cross	Backcross of hybrid to stock 544			Cross	Backcross of hybrid to stock 544			Inferred genotype of testers 11–20
Tester 5 ×	Mate-killers	Sensitives	Dead*	Tester 9 ×	Mate-killers	Sensitives	Dead*	
tester 11	62	0	33	tester 11	90	0	10	$M_1M_1M_2M_2$
12	75	0	25	12	76	0	24	$M_1M_1M_2M_2$
13	90	0	10	13	55	13	20	$M_1M_1m_2m_2$
14	84	0	16	14	58	0	22	$M_1M_1M_2M_2$
15	80	0	20	15	80	0	20	$M_1M_1M_2M_2$
16	90	0	10	16	81	0	19	$M_1M_1M_2M_2$
17	82	0	18	17	66	22	20	$M_1M_1m_2m_2$
18	58	0	42	18	46	6	50	$M_1M_1m_2m_2$
19	65	0	35	19	74	28	55	$M_1M_1m_2m_2$
20	81	0	19	20	76	0	22	$M_1M_1M_2M_2$

* See note in Table 1.

4. *Effect of mate-killer genotype on speed of killing*

As described above, any paramecium containing mu particles has been found to act as a mate-killer. However, it is possible that the number of particles present is to some extent related to the number of dominant *M* genes present. It has not been possible to count the particles in individual paramecia, but it is relatively easy to determine the time between conjugation of mate-killers with sensitive animals and death of the sensitive ex-conjugants. Levine (1953) had evidence that where the number of mu particles was large death of a sensitive animal was more rapid than where the number was small.

In Table 6, six different genotypes of mate-killers are compared in regard to the speed at which they killed sensitive paramecia following conjugation. Each combination was tested in two ways: (1) the mate-killer conjugant being mating type I and the sensitive being mating type II, and (2) the reverse.

After conjugation the sensitive ex-conjugant became gradually smaller and eventually vanished completely. The first observation when no animal could be seen was recorded as the 'time of death'. For times above 24 hours the results were not accurate to more than 12 hours.

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Table 6. *Number of hours between conjugation and death of sensitive ex-conjugant (stock 544) following conjugation with mate-killers of various genotypes*

Mate-killer conjugant		Mate-killer conjugant mating type I Sensitive conjugant mating type II								Mate-killer conjugant mating type II Sensitive conjugant mating type I								Mean
Description	Genotype																	
Stock 540	$M_1M_1M_2M_2$	4	6	10	8	11	10	10	10	11	10	8	10	10	12	12	10	9·5
Tester 9	$m_1m_1M_2M_2$	22	20	24	48	20	24	32	26	28	24	24	30	42	48			29
Tester 5	$M_1M_1m_2m_2$	20	24	32	22	24	30	22	36	40	48	26	22	24	34	30		29
F_1 540 × 544	$M_1m_1M_2m_2$	15	18	22	24	20	20	20	18	22	24	22	18	15	24	48	32	23
F_1 tester 5 × 544	$M_1m_1m_2m_2$	48	36	44	46	32	48	36	48	24	36	38	42	50				41
F_1 tester 9 × 544	$m_1m_1M_2m_2$	51	48	51	51	51	48	50	50	50	48	36	36	20	24			44

Notes: 1. Each figure in the Table refers to time for one ex-conjugant animal.
2. Most times above 24 hours accurate to nearest 12 hours only.

In these experiments occasional pairs were obtained apparently yielding two viable ex-conjugants, but by studying the behaviour of marker genes (controlling the antigens) it could be shown that in these cases no exchange of nuclear material had taken place. Wherever there was true conjugation, not merely an abortive contact between a mate-killer and a sensitive paramecium, unilateral death followed.

The results given in Table 6 show clearly a proportionality between number of M genes and speed of killing, from 9·5 hours with four M genes to about 44 hours with one M gene. There was no difference in killing effect of particular mating-types, of given genotype.

6. *Number of fissions between loss of* M *genes and disappearance of mu particles*

When heterozygous mate-killers (of genotype $M_1m_1M_2m_2$ or $M_1m_1m_2m_2$) were passed through autogamy or backcrossed to sensitive animals of genotype $m_1m_1m_2m_2$, the surviving animals having the genotype $m_1m_1m_2m_2$ (and cytoplasm derived from the mate-killer parent) were found not to lose the mu particles immediately, but only after a delay lasting an appreciable number of fissions. However, when the loss of particles did occur it seemed to take place abruptly: cells either had a large number (> 100) of particles or none at all.

A detailed study of the rate of appearance of animals lacking particles due to change in genotype will be the subject of a future publication. All that we wish to state here is that, from preliminary tests, the mu-less animals appeared at times between the eighth and fifteenth post-conjugational fissions for crosses of the type $m_1m_1M_2m_2 \times m_1m_1m_2m_2$, the mean being about twelve fissions, and following crosses of the type $M_1m_1M_2m_2 \times m_1m_1m_2m_2$, the mu-less animals appeared between eleven and fifteen fissions after conjugation, with a mean of 13·5 fissions.

DISCUSSION

The relationship between the mu particles and the two dominant duplicate genes M_1 and M_2 has no exact parallel in other types of killer paramecia. In syngen 4, Sonneborn (1947) and Balbinder (1956) have shown that certain stocks contain, in addition to the gene K, genes denoted S_1 and S_2 which modify the capacity of the animals to maintain kappa particles. Paramecia containing K and in addition S_1 or S_2 are more likely to lose the particles than animals containing the alleles s_1 and s_2; but here K is the only gene known to be essential for maintenance of kappa. The existence in stock 540 of two genes, M_1 and M_2, either one of which is capable of supporting the growth of mu particles, is difficult to understand from an evolutionary standpoint, especially since so far no other stocks of syngen 1 have been found to contain either of these genes. One interpretation is that these genes have some function unconnected with maintenance of mu particles, and it is on the basis of this other function that natural selection has operated to secure the establishment of the genes in stock 540. Then at some later period infection by mu particles took place, and it became apparent that these genes produced a satisfactory 'cytoplasmic environment' for the particles.

Examples of duplicate factors are rather rare in the genetical literature (apart from polyploids), and it may be, as Sonneborn (personal communication) has pointed out, that stock 540 has one pair of chromosomes duplicated. The diploid chromosome number of *P. aurelia* is known to vary considerably between different stocks of a single syngen (Dippell, 1954). However, this interpretation becomes more difficult to accept when one considers that there is independent assortment of the two pairs of alleles M_1–m_1 and M_2–m_2, indicating a lack of homology of the chromosomes concerned.

The anomalous behaviour of stock 544, following crosses with stock 540, may be interpreted as follows. It is assumed that the chromosomes of stock 544 are involved in a translocation, whereby the two recessive alleles m_1 and m_2 are linked together. On crossing with stock 540, which bears the alleles M_1 and M_2 on separate chromosomes, and subsequently backcrossing to 544, individuals will be expected to arise containing duplications or deficiencies. Depending upon the relative lengths of the segments containing m_1 and m_2, positions of the centromeres, etc., one might expect the occurrence of unbalanced types $M_1m_1m_2$ (deficiency) and $M_2m_1m_2m_1m_2$ (duplication). It is possible that the former would be inviable and the latter viable. In view of the unsuitability of *Paramecium* for cytogenetic work, it does not seem profitable to pursue this matter further.

Turning now to the relationship between genes and particles, and between genes and their phenotypic effect, we find somewhat similar results to those of Chao (1953), working with killer paramecia in syngen 4. Chao found that the homozygotes KK contained approximately double the number of kappa particles found in the heterozygotes Kk. Although we were not able to count the mu particles in paramecia of different genotypes, a proportionality between number of M genes and speed of killing was found, and the latter is probably related to the number of particles. These variations in particle number have to be considered against a standard environment, since it is known that factors such as temperature and growth rate markedly affect the number of particles. In stock 540, starvation, for example, results in a dramatic increase in the concentration of mu particles.

The disappearance of mu particles which takes place when an animal of genotype $m_1m_1m_2m_2$ is derived from $M_1m_1m_2m_2$ at conjugation or autogamy, apparently occurs only after a delay of 8–15 fissions, which agrees remarkably well with the range found by Chao (1953) for the disappearance of kappa particles in stock 51, syngen 4. Moreover, in both cases loss of particles, when it does occur, is abrupt. There is not, as might be expected, a simple dilution of the particles from cell generation to generation, whereby the number per animal is halved at each fission.

These facts bear on the manner in which the genes M_1 and M_2 support growth of the mu particles, and a fuller discussion of this important problem will be given later.

SUMMARY

1. Stock 540 of *Paramecium aurelia* (syngen or variety 1) contains two duplicate genes M_1 and M_2, each of which is capable of supporting growth of mu particles in the cytoplasm, thus producing the mate-killer phenotype.

2. Of six sensitive stocks of *P. aurelia* syngen 1, collected from widely separated localities, none contained either M_1 or M_2.

3. One sensitive stock (544), though proved to contain both recessive genes m_1 and m_2, gave aberrant ratios of mate-killers and sensitives following hybridization with stock 540. Evidence has been adduced consistent with the hypothesis that the m_1- and m_2-bearing chromosomes are involved in a translocation, by comparison with stock 540.

4. There is a proportionality between numbers of M genes and speed of killing, and presumably therefore with the number of mu particles.

5. Loss of mu particles following substitution of M genes by their recessive alleles occurs at times varying between eight and fifteen fissions after change of genotype.

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