THE DIVERSE MATE-KILLERS OF PARAMECIUM AURELIA, VARIETY 8: THEIR INTERRELATIONS AND GENETIC BASIS[1]

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THIS paper deals in large part with the basis of hereditary differences in a trait which is controlled by the cooperative action of a nuclear gene and visible cytoplasmic factors. As in DIPPELL'S (1950) comparable analysis of hereditary differences in the killer trait, the seat of specificity of the differences in the trait here reported upon also appears to lie in the cytoplasm, not in the genes. In general this trait is like the killer trait (SONNEBORN 1943, 1947b; PREER 1948, 1950), but in particular its distinctions are so remarkable that it has been designated by the special term "mate-killer" (SIEGEL 1953a, 1953b).

Siegel discovered mate-killing through the analysis of crosses of stock 138 to other stocks of variety 8. In each conjugant pair, the member derived from the other stock dies, but the member derived from stock 138 lives and produces normal progeny. The former are called sensitives; the latter, mate-killers. Mate-killers are distinguished from sensitives by possessing kappa-like Feulgen-positive particles in the cytoplasm which SIEGEL calls mu. As with kappa in the killer trait, mu seems to depend for its maintenance and reproduction, but not for its origin, on a nuclear gene, which SIEGEL calls *M*. The genetics of mate-killers thus appears parallel to the genetics of killers in variety 4, but the conditions for killing are different. The killing action of mate-killers depends upon prolonged pellicular contact and has not thus far been producible by fluids in which the mate-killers have lived. In variety 4, such fluids are active in killing, due to the liberation of a poison, paramecin, by killers; but contact during mating does not result in killing.

While making a survey of the intervarietal breeding relations among the stocks of varieties 4 and 8 (LEVINE 1953), two other mate-killers, stocks 130 and 131, were found in variety 8. Matings of these stocks to known sensitives of variety 8 resulted in the regular survival of the exconjugant clones descended from stocks 130 and 131 and the death of the exconjugants descended

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from the sensitives. These mate-killers also have cytoplasmic mu particles. The first part of this paper will establish that the three mate-killers are different and the last part will report attempts to establish the hereditary basis of these differences.

MATERIALS AND METHODS

The mate-killers employed in this study were stocks 130, 131, and 138, the only three found in an examination of all (11) known stocks of variety 8. The sensitives used included a " natural " sensitive, stock 130-3 (isolated from the same collection bottle as stock 130), and sensitives derived experimentally from the mate-killers by methods to be described below. Each of the latter, by reason of its mode of origin, is probably isogenic with its corresponding mate-killer.

The general methods used in this work are those described by SONNEBORN (1950). Only the methods for detecting mate-killing and for determining the cytoplasmic origin of survivors of pairs showing mate-killing will be described in detail here. Other methods will be described when necessary. Unless otherwise mentioned, all cultures were grown at 26–27°C.

The following procedure was used to detect mate-killing. The two exconjugants of each pair were followed individually by isolating them into slide depressions containing about 0.2 ml of fresh culture fluid. These were examined two days after the completion of conjugation, when the intrastock control exconjugant clones had usually produced five fissions. At this time, in crosses between mate-killers and sensitives, an obvious difference could be found between the two members of each pair of exconjugant clones: one member, like both members of control pairs, had completed five fissions, the other member had not divided or had undergone only one fission. After further feeding (addition of 0.5 to 0.6 ml of fresh culture fluid) and the lapse of one or two more days, the initial difference was found to be magnified: those exconjugants which had completed five fissions at the first examination had usually produced five to six more fissions and had exhausted their food supply. Those exconjugants which had divided only once or not at all were now dead or dying without having undergone any further divisions.

The method most commonly used to indicate the cytoplasmic origins of the survivors of pairs showing mate-killing was to determine the mating type of the majority of the survivors of a given cross and to assign their cytoplasmic origin to the parent of the same mating type. Three considerations justify this method. (1) Little change of mating type occurs following conjugation in variety 8 (SIEGEL 1953a; personal observations). The frequency of change of mating type varies from 0 to 20% in different experiments and averages about 10%. (2) High correlations between the mating type of the survivors and that of the mate-killer parent has already been demonstrated by SIEGEL (1953a) in crosses involving the mate-killer stock 138. (3) A similar correlation was found in the present study both in crosses involving stock 138 and in crosses involving the other mate-killer stocks 130 and 131. As implied by the preceding

statements, the correlation between the mating type of the survivors and mating type of the mate-killer parent is not always perfect. The relatively few exceptions have been shown by SIEGEL (1953a) to be instances in which the cytoplasmic descendants of the mate-killer parent changed mating type; and this has been confirmed in these studies. The cytoplasmic origin of the exceptions was assumed to be the same as the other survivors of a given cross, since whenever tested the exceptions were mate-killers of the parental type.

In crosses between the different mate-killer stocks, the methods of growth and identification outlined above were used along with added tests for distinguishing the diverse types of mate-killers. These tests will be described below.

Siegel's practice of starving all mate-killer cultures for three days following a short period of rapid multiplication has been accepted in order to minimize such variations in the mate-killer effect as he describes for stock 138. The question of the existence of such variations in stocks 130 and 131 and the effects of the rate of reproduction of the mate-killers prior to mating is dealt with in the next section.

THE EFFECT OF THE RATE OF REPRODUCTION OF THE MATE-KILLERS ON THE MATE-KILLER TRAIT

The mate-killers of stock 138 (SIEGEL 1953a) have different intensities of activity on sensitives. The sensitives die without undergoing a single fission after mating with mate-killers that had previously multiplied slowly; but although doomed to eventual death, they may live and divide for a considerable period after mating with mate-killers that had previously multiplied rapidly. In either case, death is inevitable. As will be shown, the rate of multiplication prior to mating also influences the intensity of the action on sensitives by mate-killers of stocks 130 and 131. The action of slowly grown mate-killers of

TABLE 1

The effect of growth at different rates prior to conjugation on the mate-killing activity of stock 130. Character of resulting exconjugant clones from cross of mate-killers of stock 130 XV, after growth at different fission rates for four days, to stocks 131 sensitives, 130-3 sensitives, and 130 mate-killers.

Stock 130 XV mate-killer	Results of crosses to:								
Fissions per day for 4 days prior to mating	131 sensitives			130-3 sensitives			130 mate-killers		
	V-V	V-VR	V-D	V-V	V-VR	V-D	V-V	V-VR	V-D
0.5			9			9	9		
1.0			9			9	9		
1.5			9			9	9		
2.0		2	7		9		7	2	
3.0		3	5		9		5	4	
4.0		9			9		9		

V-V = Both exconjugants of a pair viable; no retardation of fission rate.
V-VR = One exconjugant of a pair viable; the other viable after a retarded period.
V-D = One exconjugant of a pair viable; the other died.

these stocks is much the same as for stock 138, but the action of rapidly grown mate-killers is very different. The latter produce only temporary effects on sensitives which completely recover and yield normal viable clones.

These relations are illustrated in table 1 which presents the results of crossing mate-killers of stock 130, previously grown for four days at different fission rates, to two different sensitives and to mate-killers of stock 130 previously grown at one fission per day. Control of the different fission rates of stock 130 was achieved in test tube cultures by the methods of PREER (1948). In this way, mate-killers were grown at 0.5, 1.0, 1.5, 2.0, 3.0 and 4.0 fissions per day, respectively, in different tubes. Each tube culture of stock 130 was initiated with about 4000 animals from one and the same source culture. In table 1 are given only the results of crosses in which the mate-killers of stock 130, grown previously at different fission rates, were mating type XV. Similar results were obtained in other experiments in which mating type XVI of stock 130 was grown at different fission rates prior to crossing.

Mate-killers of stock 130 that had been grown at fission rates of 0.5 to 1.5 fissions per day showed the mate-killer effect in its most extreme form. Their sensitive mates of stocks 130-3 and 131 died before completing two fissions. The mate-killers survived normally.

When the mate-killers were grown at 2.0 fissions per day or more rapidly, their effects on some sensitives were merely transient. This is shown most sharply by the matings of these mate-killers to sensitives of stock 130-3. On the second day after conjugation, one member of each pair had gone through five fissions while the other, in most cases, had not divided. The five fission clones then went on dividing normally. During the third day after conjugation, the other exconjugant of each pair began to divide, eventually reaching a normal fission rate and giving rise to a viable clone. Mating type tests and backcrosses showed the normally dividing exconjugant clones to be derived from stock 130 while the temporarily retarded ones were sensitives from stock 130-3.

The same kinds of effect appeared, but less regularly, in the matings to stock 131 sensitives, when the mate-killer parent had been grown at 2.0, 3.0 and 4.0 fissions per day prior to mating. Some of the mate-killer cells grown at 2.0 or 3.0 fissions per day killed the stock 131 sensitives, but some merely retarded them temporarily. Only when the mate-killers were grown at 4.0 fissions per day did they always bring about temporary retardation in the growth of the sensitive mates without killing them.

It will be noted that the results set forth above were obtained with mate-killers of mating type XV, stock 131. The results obtained with mating type XVI were irregular. This irregularity and its probable cause will be discussed later after presenting certain other facts pertinent to the problem.

The demonstration that the effects of the mate-killers on sensitives differ in dependence upon the prior growth rate of the mate-killers raises the question of whether these changes in the mate-killers are reversible. SIEGEL (1953a) showed that they are reversible in stock 138 and the observations on stocks 130 and 131 are in agreement with his. Mate-killers of stocks 130 and 131 that

produced only temporary retardation in the growth of sensitives, by reason of the prior rapid growth of the mate-killers, killed sensitives if the mate-killers were later starved for two or three days and were then given enough food for only one fission before they were mated to the sensitives.

The preceding discussion of the mate-killers of stocks 130 and 131 brought out a number of points of resemblance to the mate-killers of stock 138. The enumeration of these similarities should not, however, be allowed to obscure the important difference as to the effects of rapidly grown mate-killers. In stock 138, rapidly multiplying mate-killers doom the sensitives to ultimate death after a period of multiplication, apparently by the reason of irreversible damage to their nuclei (SIEGEL 1953a). In stocks 130 and 131, rapidly reproducing mate-killers produce brief, transient effects on the early reproduction of sensitives and recovery is rapid and complete. Whatever damage is done to sensitives by these two rapidly grown mate-killers must be temporary and reparable; and whether it is on the nuclei is not known.

Since rapidly grown mate-killers of stocks 130 and 131 do not kill sensitives, the question arises as to whether they are sensitive to slowly grown mate-killers which are capable of killing sensitives. As is evident from table 1, the matings of rapidly grown mate-killers to slowly grown ones usually result in the normal survival of both. However, six exceptional pairs are listed in table 1; one exconjugant clone of each of these pairs was temporarily retarded in its growth rate and the other reproduced at the normal fission rate. Does this mean that rapidly grown mate-killers can sometimes respond as sensitives when mated to slowly grown mate-killers? A more complete answer to this question can be given after considering material to be presented in the next section. The discussion presented here is limited to the exceptions in table 1.

Three lines of evidence warrant the conclusions that these were not examples of mate-killer action, but were cases of temporary retardation in fission rate due to other unknown causes. First, the divergence in fission rates between the exconjugant clones in these six pairs was not as great as between the pair members of matings to sensitives. Second, if the retardation was an effect of mating-killing it would be expected to occur even more markedly in the matings to stock 130 mate-killers grown at 4.0 fissions per day; this did not happen. The progeny of the nine pairs in this cross reproduced normally. Third, this retardation was not found in other similar experiments. Thus, it appears that under the conditions of the experiment there was no effect of the more slowly grown mate-killers on the more rapidly grown cultures.

Since the more rapidly mate-killers are grown prior to mating, the weaker the effect they have on sensitives, the question arises as to whether long continued rapid growth could result in failure to affect sensitives at all. The next section examines this possibility.

THE TRANSFORMATION OF MATE-KILLERS INTO SENSITIVES

Growth of mate-killers of stocks 130 and 131 at rapid rates eventually results in the loss of activity on sensitives; moreover, the mate-killers them-

selves become sensitives. What is the physical basis of this transformation? It will be shown that the transformation of mate-killer into sensitive results from the loss of mu particles.

The loss of the mate-killer trait in stocks 130 and 131 has been observed following growth at the maximum fission rates at 20°, 27° and 32°C. Only the experiment carried out at 27°C is reported here; the results obtained at the other temperatures are essentially similar.

The maximum fission rate of slightly more than 5.0 fissions per day was attained in daily isolation cultures (SONNEBORN 1950) started with single mate-killer animals of stocks 130 and 131 grown in slide depressions. Each day, one animal was reisolated to start a new subculture and the remainder of the culture was retained until the food was exhausted (i.e., after about ten fissions) and the animals were starved for three days. Then enough food was added to permit one additional fission and the animals were tested on the following day for mate-killing activity. This procedure was carried out on the seven successive cultures obtained from seven daily isolations, a complete series of seven being obtained both for stock 130 and for stock 131. From the fission rate of these daily isolation series, it is clear that there had been approximately 5, 10, 15, 20, 25, 30 and 35 fissions at maximal rate in the first to seventh cultures of the series, respectively.

All the animals tested from the first and second cultures in the series were mate-killers. The third cultures in the series from both stocks were mixtures of mate-killer and sensitive animals. Practically all the animals tested in the fourth and all tested in the fifth, sixth and seventh cultures were sensitives. These were killed by mate-killers and did not affect sensitives. Cytological examination disclosed that the animals which had lost their mate-killing activity and were transformed into sensitives also lost the mu particles. The transformation of mate-killers into sensitives accompanied by the loss of mu has been confirmed by SIEGEL (1953b) working with stock 138 mate-killers, using somewhat different methods.

Again the question of the reversibility of these changes in the mate-killers must be raised. It would appear that most of these transformations are irreversible. Sensitives derived from stocks 130 and 131 have been under observation for almost two years and still have not recovered any mate-killing activity or mu particles. Neither have the stock 138 sensitives produced by Siegel reverted to mate-killers.

As these sensitives were derived from the mate-killers by environmental means and not by genic substitution, they should be isogenic with the respective mate-killers. Evidence will be presented showing that these sensitives are genically capable of supporting the mate-killer trait, ruling out the alternative that loss of mu was due to genic mutation. The correlation of loss of mu with loss of the mate-killer trait, in the absence of any genic change related to this trait, indicates strongly that the mate-killer trait cannot exist in the absence of mu. Further, the failure of these transformed sensitives to replenish the mu particles, demonstrates the inability of the genes to initiate the production of the particles.

The facts described and the conclusions drawn from the mate-killer trait agree with, in general, those described for the killer trait (SONNEBORN 1943, 1947a; PREER 1948). PREER has shown that some kappas of variety 2 killers are not able to increase as rapidly as the cells when the latter increase at maximal rate over a wide range of temperatures. Consequently the kappa concentration progressively decreases and the killer character undergoes a series of changes. A small reduction in kappa concentration changes the cell from a strong killer to a weaker one. A greater reduction in kappa results in the loss of killing activity, but the cell remains resistant to paramecin. With still further reduction, the cell becomes sensitive to paramecin. These changes are reversible until the last particle of kappa is removed from the cell; then the animal is irreversibly transformed into a sensitive. Since kappa in stock 51, variety 4, can multiply as rapidly as the cells over a wide range of temperatures, growth at maximal fission rates at these temperatures is not effective in producing sensitives. However, at temperatures between 29° and 34°, the cells can multiply more rapidly than kappa and at still higher temperatures kappa is destroyed. In these ways there can be produced a series of changes similar to that described for variety 2 killers (SONNEBORN 1947a and later abstracts).

The progressive changes in mate-killing activity are similarly interpreted as due to the decrease in the concentration of mu because of its slower rate of reproduction than that of the cells. SIEGEL (1953a), using stock 138, showed that the intensity of mate-killing is directly related to the concentration of mu particles in the mate-killers. Preliminary experiments (SIEGEL personal communication) have not demonstrated any action of slowly grown mate-killers on rapidly grown mate-killers before the latter are completely denuded of particles.

In sharp contrast with the large numbers of sensitives obtainable from stocks 130 and 131, preliminary evidence (SIEGEL unpublished) indicates that sensitives are not as readily derived from mate-killers of stock 138. This may mean that just as different kappas are known to multiply at different rates (SONNEBORN 1947a; PREER 1948; DIPPELL 1950), stock 138 mu may increase more rapidly than mu from stocks 130 and 131.

SIEGEL (1953a) reports that some of the stock 138 sensitives obtained by loss of mu through environmental means, have permanently damaged nuclei or are amicronucleate. SIEGEL interprets the damage to the nuclei as self-inflicted, when the mu concentration falls below a critical level. He has shown that the damage to the micronuclei of "doomed" sensitives, produced as a consequence of mating with rapidly grown mate-killers of stock 138, can also be correlated with a decrease in the concentration of mu in the mate-killers. The phenomena in sensitives obtained from mate-killers of stocks 130 and 131 are in marked contrast with those reported by SIEGEL for stock 138. The sensitives of the two former stocks show no evidence of being amicronucleate or of possessing damaged nuclei. This is correlated with similar lack of nuclear damage when sensitives are mated to rapidly grown mate-killers of these two stocks.

The loss of mu as a result of the differential rates of cell and mu reproduction in mating type XV of stocks 130 and 131 requires, as set forth above, at least three days of growth at maximal rate. Indications are that similar results are obtained with even shorter periods of rapid growth of mating type XVI, especially so in stock 131. The period of rapid growth required for the latter may be so short that there is danger of losing mu if these animals are grown at maximal rate for as short a period as one or two days and even if the growth rate is less than maximal. Because of this and because this problem was outside the scope of the present study, it was put aside for later investigation, and type XVI mate-killers of stock 131 were not used in this study except when necessary. However, the more rapid loss of mu from type XVI than from type XV is to be expected in the light of knowledge of kappa in variety 4. CHAO (1953) found that the amount of kappa is twice as great in killers of mating type VII as in killers of type VIII. Since type XVI is the homologue of type VIII, it is not unlikely that it contains much less mu than does type XV. Hence, it might be expected to lose its mu by shorter periods of growth at rapid rates than those required to eliminate mu from mating type XV.

In the discussion up to this point, we have stressed on the one hand the correlation between the presence of mu and possession of the mate-killer trait and, on the other hand, the correlation between the absence of mu and sensitivity to mate-killers. This implies that possession of mu not only determines mate-killing, but also resistance to mate-killing. The implication is correct with reference to the relations between animals of the same stock. However, as will be set forth in the next section, the implication is *not* correct with reference to relations between mate-killers of different stocks.

THE INTERACTION OF THE MATE-KILLERS WITH EACH OTHER

Previous work on varieties 2 and 4 (SONNEBORN 1939, for variety 2; DIPPELL 1950, for variety 4) has shown that different kinds of killers may kill each other. The mate-killers of variety 8 may also, as will appear, affect each other to some extent; but in matings between any two of the three diverse mate-killer stocks, one and only one of the mate-killers is killed, the other survives. Moreover, when the cytoplasmic origins of the survivors are determined, it is found that in each cross one of the mate-killer stocks is regularly resistant to the action of the other, and a "peck-order" of mate-killing emerges. The peck-order, according to strength of mate-killing, is: 138—130—131.

The data to be presented now as evidence for the peck-order are a sample of a larger group leading to the same conclusions. The mate-killers used in these experiments were properly starved to permit the sharpest expression of mate-killing. Reciprocal crosses showed the same results.

Stock 131 is placed at the bottom of the peck-order because it was killed by the other two mate-killers and had no effect on either. Of 30 unilateral survivors of the cross of mate-killers of stock 138 to those of stock 131, 28 were the mating type of the stock 138 parent and two were of the comple-

mentary mating type. Twenty-seven of the survivors of a cross of stock 130 mate-killers to mate-killers of stock 131 were of the mating type of the stock 130 parent and three were not.

Stock 138 is placed higher in the peck-order than stock 130, because in matings between these two stocks the exconjugant clones cytoplasmically descended from stock 138 survived and those from stock 130 died. Forty-four of 50 surviving exconjugants (one from each pair) were of the mating type of stock 138, five were not and the mating type of one was not determined. However, these surviving exconjugant clones, derived cytoplasmically from stock 138, were retarded in their early reproduction in much the same way as were sensitive clones mated to rapidly grown mate-killers of stocks 130 and 131. Two days after the completion of conjugation, these clones had passed through only two to three fissions whereas the intrastock controls had completed five fissions. The retarded clones then recovered the normal fission rate and gave rise to viable cultures.

TABLE 2

Classification of unknown cultures as to grade or type of mate-killing. Alternative characteristics of resulting exconjugant clones from crosses of an unclassified culture to mate-killers of stocks 138 and 130 and to a sensitive.

Testers	Classification of unknowns:			
	Like 138 MK	Like 130 MK	Like 131 MK	Like a sensitive
Stock 138 MK	V-V	VR-D	V-D	V-D
Stock 130 MK	VR-D	V-V	V-D	V-D
Sensitive stock	V-D	V-D	V-D	V-V

MK = mate killers.
V-V = both exconjugants of a pair viable; no retardation of fission rate.
VR-D = One exconjugant of a pair viable after a retarded period; the other died.
V-D = One exconjugant of a pair viable; the other died.

Growing stock 138 and 130 mate-killers under conditions other than those described in this section prior to matings between them might result in the death of both exconjugants. It is conceivable even if highly improbable, that conditions in nature might result in the death of both exconjugants following matings between two adjacent mate-killer populations. Such a lethal interaction, due to the action of mu particles, might act as an isolating mechanism if maintained long enough.

The peck-order provides a means of defining the phenotypes of the mate-killers on the basis of their sensitivity to one another. From the results of crosses to three tester stocks (table 2), any culture can be classified as a sensitive or mate-killer and the grade or type of mate-killing determined. This fact makes a study of the hereditary basis of the different grades of mate-killers possible.

GENIC ANALYSIS OF THE DIFFERENT GRADES OF MATE-KILLING

The analysis of the mate-killer trait has raised three important genetic problems. (1) What is the hereditary basis of the differences between mate-killers and sensitives of the same stock? (2) Are there genic differences between

mate-killers and naturally occurring sensitives in respect to the mate-killer trait? (3) What is the genetic control of the differences in mate-killing as manifested in the peck-order by the three mate-killer stocks?

The first question was answered by the demonstration, for all three mate-killers, that the trait depends on the presence of mu in the cytoplasm. The second question has been partially answered by SIEGEL's (1953b) discovery that animals of sensitive stock 151 not only have no mu, but are genically unable to maintain mu, being homozygous for the recessive *m* gene. The third question is the subject of the rest of this paper.

Genetic control of the grades of mate-killing might be visualized in three ways. (1) Differences in both genes and mu might result in different types. Different genes in the three mate-killer stocks may control different modifications of the mu particles. (2) Mu could be identical in the three mate-killers, different genes determining the different types. (3) The three stocks may be genically identical in the control of mate-killing; the genetic basis of the three diverse mate-killers would then rest in the different mu particles. Should one of the first two possibilities be correct, the mate-killer types would be expected to segregate in Mendelian fashion in appropriate crosses.

The experimental procedure

Three series of three crosses were undertaken to test for the genic determination of the mate-killer diversities. In each series, mate-killers of one stock were crossed to sensitives derived from each of the mate-killers. The sensitive conjugant of each pair died or showed one of the other effects of mate-killers on sensitives, but not before providing the mate-killer conjugant with a set of genes. The action of the hybrid genome on the mate-killer trait was tested by determining the grade of mate-killing of the F_1 according to the scheme outlined in table 2. Finally the same testing procedure was used to check for segregation of the mate-killer types among the backcross progeny of the F_1. The backcross progeny were obtained by crossing the F_1 to the sensitive tester stocks which were used in the original cross. Approximately 40 backcross clones from each of the nine crosses in the three series were individually tested for their grade of mate-killing. The general scheme of the experimental analysis is presented in table 3.

Sensitives, isogenic with the mate-killers, were used so that the genes of the mate-killer stocks could be exchanged without complicating the experiment with the possible mixture of mu particles. Sensitives were derived from the mate-killers of stocks 130 and 131 by the growth rate methods previously described. The author is indebted to DR. R. W. SIEGEL for supplying him with a culture of isogenic sensitives derived from mate-killers of stock 138.

The F_1 mate-killers

The tests of the grades of mate-killing of the F_1 clones can be summarized as follows: The three different genotypes in any one series show the same grade of mate-killing. On the other hand, clones in different series, but hav-

TABLE 3

Breeding scheme for the genic analysis of the control of the grades of mate-killing.

Table 3a. The F_1 crosses.

Series	Mate-killer parents	Sensitive parents		
		A 138	B 130	C 131
I	138 XV	6 prs.	9 prs.	9 prs.
II	130 XV	9 prs.	9 prs.	9 prs.
III	131 XV	9 prs.	9 prs.	9 prs.

*Table 3b. Test for the grade of mate-killing manifested by the F_1 survivors from series I.**

No. of F_1 tested	Crossed to the following tester stocks:		
	138 MK	130 MK	
5 from I-A	9 prs./F_1	9 prs./F_1	138 sens. / 9 prs./F_1
5 from I-B	9 prs./F_1	6 prs./F_1	130 sens. / 9 prs./F_1
3 from I-C	9 prs./F_1	9 prs./F_1	131 sens. / 15 prs./F_1

*Table 3c. Test for the grade of mate-killing manifested by the backcross of clones from series I.**

The backcross clones from	Crossed to the following tester stocks:		
	138 MK	130 MK	
I-A	6 prs./clone	6 prs./clone	138 sens. / 6 prs./clone
I-B	6 prs./clone	6 prs./clone	130 sens. / 6 prs./clone
I-C	6 prs./clone	6 prs./clone	131 sens. / 6 prs./clone

MK = mate-killers.
Sens. = sensitives.
*The F_1 and backcross generation survivors from series II and III were tested for their grade of mate-killing in the same manner as were those of series I.

ing the same genotypes, show different grades; the grade of mate-killing always being the same as that of the parent mate-killer.

The F_1 results are not readily compatible with the hypothesis of genic determination. To explain the fact that hybrids of the same genotype exhibit different types of mate-killing, one would have to make the unlikely assumption (in the absence of a phenomic lag) that the genes involved in the mate-

killer trait are both dominant and recessive to each other under the same conditions. If the mate-killers do indeed have different genes in control of the trait, then the backcross generation derived from the mate-killers should show a segregation of the types. If the genes in control of the mate-killers are the same in the different stocks, then the backcross clones should show

TABLE 4

The grades or types of mate-killing manifested by the backcross clones derived from crosses between the various mate-killer stocks (table 3).

Parental mate-killers	Classification of backcross clones	Sensitive parents used in F_1 and backcrosses		
		A 138	B 130	C 131
Series I 138	No. F_1	5	5	3
	No. Backcross clones	42	42	45
	Phenotype of backcross clones:			
	Mate-killer, parental type	37	15	31
	Mate-killer, other types	0	0	0
	Sensitives	0	0	0
	Undetermined	3	21	9
	Not tested {Died	1	4	1
	Not tested {Selfers	1	2	4
Series II 130	No. F_1	3	5	3
	No. Backcross clones	43	45	45
	Phenotype of backcross clones:			
	Mate-killer, parental type	19	27	20
	Mate-killer, other types	0	0	0
	Sensitives	0	0	0
	Undetermined	7	14	10
	Not tested {Died	15	1	15
	Not tested {Selfers	2	3	0
Series III 131	No. F_1	3	5	3
	No. Backcross clones	42	42	45
	Phenotype of backcross clones:			
	Mate-killer, parental type	31	24	36
	Mate-killer, other types	0	0	0
	Sensitives	1	1	0
	Undetermined	7	8	4
	Not tested {Died	1	6	1
	Not tested {Selfers	2	3	4

the same type of mate-killing as the parental and F_1 cultures from which they are cytoplasmically derived.

The backcross generation mate-killers

Table 4 gives the grades of mate-killing shown by the backcross clones as determined from the crosses to the tester stocks. All clones which died before they could be tested or which were not tested because of selfing (mating between the members of a clone) are listed separately in the table.

Verification can now be given for the statements made earlier that sensitives and mate-killers of the same stocks are genically identical with respect to mate-killing, thus justifying the use of these sensitives as donors of genes. There was no case of segregation of the grades of mate-killing or of sensitives among the backcross progeny of intrastock crosses of mate-killers and sensitives (crosses I-A, II-B, and III-C, table 4). (Ignore for the moment those clones classified as undetermined.) Cross I-A (table 4) confirms SIEGEL's work with stock 138. These crosses served as controls for the crosses among the three stocks.

Two sensitive clones appeared among the backcross progeny of the mate-killers of stock 131 to the other two stocks (series III-A and III-B, table 4), but their occurrence is interpreted as not due to genic recombinations. One of these sensitive clones was mating type XVI. The irregularity of mate-killing among mating type XVI cultures of stock 131 was mentioned above. Since no other sensitive clones appeared in the other series, genic recombination is considered highly unlikely.

The striking thing about the information in table 4 is the remarkable agreement between the mate-killing of the backcross clones and those of the parental and F_1 cultures (again ignoring those clones classified as undetermined). Everyone of these backcross clones was a mate-killer (except for the two sensitive clones from the progeny of mate-killers of stock 131 discussed above) typical of the parental and F_1 mate-killer used. In other words, no segregation of diverse mate-killers occurred.

Before it can be concluded that the absence of segregation is a real phenomenon, it must be demonstrated that the backcross clones classified as undetermined do not represent a class of segregants and that the widespread occurrence of cytogamy (pairing of opposite mating types as in conjugation without the exchange of nuclei taking place between the mates) is not masking the presence of genic differences. These considerations will now be discussed and evidence presented minimizing their importance.

The undetermined backcross clones as a factor in the lack of segregation

A backcross clone was classified as a particular mate-killer if no more than one of the six pairs in any of the three matings to testers gave aberrant results. If two or more pairs gave aberrant results, it was decided to waive judgment and classify these clones as undetermined types. By aberrant is meant results in which not all the pairs in a mating show the same pattern of viability. For instance, a backcross clone cytoplasmically descended from mate-killers of stock 130 reacted as a stock 130 mate-killer in the matings to stock 138 and 130 mate-killer testers. However, in the mating to the sensitive testers only four pairs showed unilateral survival, both exconjugants of the other two pairs were nonviable. Another type of aberrant result was the normal survival of both members of a pair in two or more pairs of a test of six, when the other pairs exhibited the typical mate-killer reaction. Both of these backcross clones were classified as undetermined.

Three lines of evidence suggest that the undetermined backcross clones of the first type are not segregants demonstrating genic control of the mate-killer types.

(1) A regularity in the proportions of the undetermined clones in the different crosses would be expected if they represent segregants. No such regularity was evident. Neither was there any regularity in the occurrence of lethality in the matings to the testers which resulted in most of the undetermined clones being classified as such.

(2) An examination of table 4 shows that the majority of the undetermined clones were found in the crosses involving stock 130. Most of the lethality which resulted in the undetermined clones was probably traceable to the sporadic mortality occurring in intrastock 130 crosses rather than to segregation of new types of mate-killers. A large number of intrastock 130 matings were followed in the course of the experiment as controls for the matings to the testers and a great deal of nonviability occurred. Twenty-two sets of six pairs each were followed from the matings between the two mating types of stock 130, both being mate-killers. Five of these would have to be classified as undetermined because of nonviability among the exconjugant clones. All six pairs of one set were aberrant. Six sets of six pairs each from the mating of the two mating types of stock 130 when both were sensitives were similarly followed. Two of these six sets would be classified as undetermined because of nonviability obviously not connected with mate-killing since both conjugants were sensitives. In the matings between stock 130 mate-killers and sensitives, 13 sets of six pairs each were observed. Two of these would be undetermined as some of the mate-killer conjugants were nonviable in addition to the sensitives. The contention that the sporadic mortality in stock 130 is responsible for most of the undetermined clones is supported by the fact that almost a third of the backcross progeny of the intrastock 130 cross (cross II-B, table 4) were undetermined because of excessive mortality in the crosses to testers. Segregation of new types of mate-killers would hardly be expected in this intrastock cross.

(3) The cross of stock 138 mate-killers to stock 130 sensitives (cross I-B, table 3) was repeated and 10 backcross clones were tested for their grade of mate-killing. Eight were stock 138 mate-killers and two were undetermined by our standards. The frequency of undetermined clones in this experiment is significantly different from that of the undetermined clones in cross I-B (table 4): 2 out of 10 as compared to 21 out of 42. This indication that the frequency of the undetermined clones may be variable, would not be expected of a genically controlled trait. Use of cultures which do not show intrastock mortality should greatly diminish the frequency of these undetermined clones.

Very few clones were classified as undetermined because of viability of both members of a pair where it was not expected. The few cases which did occur are explicable on any of the following three possibilities: (1) mating between the members of a clone, (2) both conjugants of a pair becoming mate-killers as a result of cytoplasmic exchange (SIEGEL 1953b), or (3) by the

separation of the mates before the completion of conjugation and the transmission of the lethal effect (SIEGEL 1953a).

Cytogamy as a factor in the lack of segregation

Should cytogamy be of widespread occurrence in crosses to mate-killers, the lack of segregation would be due to the failure of reciprocal fertilization instead of the presence of the same genes for mate-killing in the three stocks. Three lines of evidence minimize the importance of cytogamy in this experiment. First, SIEGEL (1953b) demonstrated reciprocal fertilization in crosses of stock 138 mate-killers to stock 151 sensitives by Mendelian segregation of mate-killers and sensitives in the backcross progeny. Second, a considerable proportion of nonviability is always characteristic of the F_2 by autogamy after interstock crosses in *P. aurelia* and is itself an indication of true crosses in F_1. This was always obtained following crosses among the three stocks. Third, consistent results were obtained for all F_1 clones followed in each cross. Thus cytogamy cannot be an important factor in these experiments.

DISCUSSION

The genetic control of the different grades of mate-killing

The results of the crosses presented in the previous section lead to the conclusion that there are no simple gene differences between the three mate-killer stocks in the maintenance of the mate-killer trait and in the control of the specificity of mate-killing. (This conclusion assumes that a very long phenomic lag is not operating.) It should be pointed out that the relatively small number of backcross clones tested does not rigorously exclude the control of mate-killing specificity by multiple genes, any one of which could be effective in perpetuating the parental type of mate-killing. This could be excluded by demonstrating the direct control of the grade of mate-killing by the mu particles.

DIPPELL (1950) analyzed five mutant killers from two stocks of variety 4 and established that the genes in these mutants are the same in respect to the killer trait. She further demonstrated that the differences between the mutants are due to differences between the kappa particles. This was done by the dilution of the kappa concentration to a single particle per cell, followed by slow growth of the animals to permit cells to regain the original kappa concentration. It was found that some mutant killers contained two different types of kappa particles, each with a particular phenotype associated with it. In all she found three distinct forms of kappa particles, which she concludes must have arisen by mutation of one kind of particle to another kind.

It is not known whether the populations of mu in the three mate-killers are homogeneous or mixed. The determination of the homogeneity of the populations of mu may be very difficult due to the aggregation of the particles (SIEGEL 1953a for stock 138 and personal observations on stocks 130 and 131).

The dilution to a single particle per cell depends on the random distribution and dilution of the particles. If the distribution of the mu particles is markedly nonrandom, it may prove difficult or impossible to dilute the concentration to one particle per cell.

Should the mate-killers not lend themselves to the type of analysis carried out by Dippell on the killers, evidence as to the role of particles in determining the specificity might come from mixing mu particles during crosses between the mate-killers (by inducing cytoplasmic exchange between the mates). It might be expected that the F_1 clones produced would be different from those described in the genic analysis experiment.

The evidence that the genes play no role in the control of the grades of mate-killing, DIPPELL's findings for the killer trait, and the discovery that the mu particles of the different mate-killers are morphologically distinguishable (SIEGEL and PREER personal communication) all speak strongly for the mu particles controlling the specificity of the mate-killer phenotypes.

Stock differences in sensitivity to mate-killing

The peck-order of mate-killing demonstrates that not all the mate-killers are equally sensitive to other mate-killers. There is some evidence that not all the sensitive stocks are equally sensitive to a particular mate-killer. Evidence for this contention is given in table 1. Stock 130-3 sensitives were only temporarily retarded in fission rate by the same stock 130 mate-killers which killed stock 131 sensitives. Results of this sort were frequently seen in these same crosses. Death of stock 130-3 sensitives and survival of stock 131 was never observed. This difference in resistance to mate-killing may be due to genetic differences between the two stocks or to the phenomenon discovered by AUSTIN in connection with differences in sensitivity to killing. AUSTIN (1951) found that different serotypes of the same stock may vary in sensitivity to paramecin killing. The observed difference in sensitivity to mate-killing may be a manifestation of such a serotype effect. A careful study of variety 8 sensitives should be made in this respect.

Mate-killers and killers

The similarities and dissimilarities of the mate-killer and killer traits have been mentioned throughout the paper and need not be repeated. Only a few points previously not mentioned will be discussed.

The particles determining one trait offer no protection against the action of the other. The variety 8 mate-killers are killed in typical fashion by the variety 4 killers. In intervarietal matings between varieties 4 and 8, both exconjugants of a pair undergo four to six fissions before dying if the variety 8 stock is not a mate-killer. In intervarietal matings between mate-killers and killers or sensitives of variety 4, one member of a pair undergoes the expected four to six fissions whereas the other dies without dividing more than once. The exconjugant which dies sooner is the variety 4 killer or sensitive which has been stricken by the mate-killer.

Attempts to determine whether maintenance of the mate-killer and killer traits is dependent on the same locus have so far proved inconclusive. Attempts to introduce kappa particles into variety 8 during conjugation have not been fruitful because of the high mortality occurring in the intervarietal crosses (LEVINE unpublished). LEVINE and SIEGEL (unpublished), using SONNEBORN'S (1950) infection technique, have tried unsuccessfully to infect variety 8 with kappa by exposing stock 138 sensitives of the M genotype to breis of killer animals. Using the same technique, variety 4 sensitives with the dominant gene, K, were exposed to breis of mate-killers of stock 138, again with no success. Efforts to combine the particles of killers and mate-killers should be continued, since if accomplished, not only could the question of genic control of the maintenance of the traits be settled, but studies on competition and genetic exchanges between the particles as are found in the bacteriophages (LURIA 1947) might become feasible.

SUMMARY

Stocks 130, 131, and 138 of variety 8, *Paramecium aurelia,* have been examined for hereditary differences in the mate-killer trait. This trait is known to be controlled by the cooperative action of a nuclear gene and visible cytoplasmic particles called mu. Sensitives mated to slowly reproducing animals of these stocks regularly die before completing two fissions. The exconjugants cytoplasmically descended from stocks 130, 131 and 138 produce viable cultures. When mated to rapidly grown mate-killers of stock 138, sensitives produce a limited number of daughter cells, all of which are doomed to die. In contrast, sensitives mated to rapidly grown mate-killers of stocks 130 and 131 are only temporarily retarded in their early reproduction, then recover and produce normal viable cultures. Prolonged rapid growth of mate-killers of stocks 130 and 131 results in the loss of mu and in the irreversible transformation of these animals into sensitives.

When crossed to one another, the mate-killers exhibit a system of striking interactions. Each cross exhibits the mate-killing reaction: the exconjugants from one of the stocks regularly die and the others survive. This system of interactions gives rise to a "peck-order" of mate-killing which according to strength of mate-killing is: 138—130—131.

A breeding analysis has failed to demonstrate genic control of the grades of mate-killing as manifested in the peck-order. This strongly suggests that the seat of specificity of the differences in mate-killing between the three stocks resides in the cytoplasmic particles.

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