The mechanism whereby the genes M_1 and M_2 in *Paramecium aurelia*, stock 540, control growth of the mate-killer (mu) particles

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INTRODUCTION

The existence of kappa particles in the cytoplasm of *Paramecium aurelia* was first shown by Sonneborn (1943) from the breeding behaviour of certain killer stocks. Later Preer (1948) showed that these particles were Feulgen-positive bodies readily visible in the light microscope, and recently their detailed structure and that of the similar mu (mate-killer) particles has been revealed by electron microscopy (Dippell, 1958; Beale & Jurand, 1960). Most or all of these particles survive in the cytoplasm of paramecia only if the latter contain certain genes. Kappa particles of stock 51 (syngen or variety 4) require the presence of the dominant gene K (Sonneborn, 1943), the lambda particles of stock 299 (syngen 8) require another gene, L (Sonneborn, Mueller & Schneller, 1959), and mu particles in certain stocks of syngen 8 require the gene M (Siegel, 1953). We have shown that mate-killers of stock 540 (syngen 1) contain two unlinked dominant genes, M_1 and M_2, each one alone being capable of supporting growth of the mu particles associated with the mate-killer phenotype in this stock (Gibson & Beale, 1961).

Thus the various killer paramecia contain two kinds of determining factor: the genes and the cytoplasmic particles. The genes provide certain conditions essential for the maintenance and growth of the cytoplasmic particles; the particles, however, are not formed directly from the genes since introduction of, for example, the gene K into the cytoplasm of paramecia lacking kappa particles does not result in formation of any of the latter. These systems are best envisaged as comprising a symbiotic association between a paramecium and a bacterium-like micro-organism whose growth requires some essential element controlled by the genes of the paramecium. In the work to be described here we consider the question of the mechanism whereby such genes control the growth of kappa or mu particles.

The initial stimulus to these studies came from the observation, reported in our previous paper (Gibson & Beale, 1961), that replacement of the dominant genes M_1 and M_2 in stock 540, by the recessive alleles m_1 and m_2, led to a disappearance of the mu particles only after a surprisingly long delay. In different lines this delay period varied between eight and fifteen fissions after change of genotype at

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conjugation or autogamy. However, the loss of mu particles, when it did occur, seemed to be abrupt: cells either had a large number of particles or none at all, and it was therefore concluded that at varying periods after change of genotype the cell suddenly switched from a state favourable for the maintenance of mu particles to one inimical to them. The phenomenon was the more striking in that Chao (1953) had previously found a very similar situation in the killer stock 51 (syngen 4): there also, following change of genotype from Kk to kk, there was a lag of between eight and fifteen fissions before loss of kappa particles.

As a provisional hypothesis, on the basis of which our experiments were carried out, we proposed that the cytoplasm of mate-killer paramecia contained, in addition to the visible mu particles, certain other factors, here denoted 'metagons', which are assumed to be formed only in the presence of one or other of the genes M_1 and M_2. After removal of the dominant genes from a cell by conjugation or autogamy, and subsequent fission of the ex-conjugant or ex-autogamous animals, the metagons would be passively distributed to the daughter cells. Assuming an initial number of 1000 metagons per cell, animals lacking metagons would begin to appear with appreciable frequency after nine fissions, and thereafter the number of animals lacking metagons altogether would rise at each fission. Random fluctuations in the distribution of the metagons amongst the fission products would result in the fluctuation, in different lines, of the stage at which animals without metagons first appear. The aim of the experiments described below was to test the validity of this hypothesis and elucidate the nature of the metagons. A preliminary note describing some of the work has been published (Gibson, 1961).

MATERIAL AND METHODS

The stocks used (540 mate-killers and 513) and the general methods were the same as in our previous work (Gibson & Beale, 1961). Details of special techniques will be given at appropriate points in the 'Results' section. Here we mention only the following method, which was used to eliminate mu particles from paramecia containing both the genes M_1 and M_2 and the particles. A small culture (10 ml.) of mate-killer animals was placed in a 2-litre conical flask and the latter filled up with a large volume (1–2 litres) of bacterized medium. Eventually the paramecia would grow up and clear the medium, and were then found to lack mu particles. This phenomenon was accidentally discovered by Dr A. Jurand, and is presumably connected with the peculiar physiological conditions associated with a very large excess of bacteria. Mu particles in stock 540 can also be eliminated by growth at high temperature (Beale, 1957).

RESULTS

1. *Rate of loss of mu particles following loss of genes* M_2, M_1, *or of both* M_1 *and* M_2

By suitable crosses involving stock 540 mate-killers and stock 513 sensitives, as previously described (Gibson & Beale, 1961), mate-killer animals of genotype $m_1m_1M_2m_2$ were prepared and back-crossed to $m_1m_1m_2m_2$ (sensitives), yielding equal numbers of genotypes $m_1m_1M_2m_2$ and $m_1m_1m_2m_2$. Clones in the latter

class were identified and caused to pass through known numbers of fissions, between eight and fifteen, and the presence or absence of mu particles then determined. This was done as follows. First, after one fission of the ex-conjugants, one of the two daughter animals was placed in surplus culture fluid and allowed to divide about fifteen times, the sister animal being prevented from dividing by placing it in exhausted culture fluid. A sample of the animals which had passed through fifteen fissions was then tested for presence of mu particles by squashing and examination by phase-contrast microscopy. If mu particles were still present that clone was classified as $m_1 m_1 M_2 m_2$ and discarded. If mu particles were absent the clone was classified as $m_1 m_1 m_2 m_2$ and the starved animal of the same clone was then used for the main experiment.

Table 1. *Proportion of animals lacking mu particles following loss of gene* M_2

No. of fissions after change of genotype from $m_1 m_1 M_2 m_2$ to $m_1 m_1 m_2 m_2$	No. of animals tested	Percentage animals lacking mu particles	
		Observed	Estimated*
8	148	5	1·4
9	100	17	11·9
10	256	32	34·4
11	100	61	58·7
12	100	71	76·6
13	100	79	87·5
14	100	85	93·5
15	100	93	96·7

* These are maximum-likelihood estimates based on an estimated initial number of metagons (N) of 1090 ± 70. (See appendix.)

This animal was then placed in bacterized culture fluid and allowed to undergo precisely six fissions (making seven post-conjugational fissions altogether), yielding sixty-four animals. Of the latter, thirty-two were isolated into separate depression slides and allowed to divide once more. Thereafter thirty-two of the sixty-four animals obtained at each fission were re-isolated and allowed to divide again, the remainder being discarded. This procedure was continued until a stage was reached at which it was desired to determine the proportion of animals containing mu particles. Then, groups of fifty animals out of the sixty-four available were chosen, placed in exhausted culture fluid for 24 hours at 25° C. and allowed to starve. This starvation resulted in an increase in the number of mu particles per cell (when any were present), and also served to eliminate the bacteria whose presence sometimes confuses scoring of the mu particles. It should be added that each group of fifty animals examined, whether derived from the same or a different number of post-conjugational fissions, comprised a separate clone, formed from a separate ex-conjugant.

Table 1 shows the proportions of animals lacking mu particles at all stages from eight to fifteen fissions after loss of gene M_2. The figures were obtained by pooling the results from two or three lots of fifty animals at each fission.

In Table 2, a few results derived from a similar experiment involving loss of gene M_1 are shown. As far as can be judged from the limited data, the proportions of animals lacking mu particles after ten and eleven fissions following loss of M_1 are similar to those found following loss of M_2. At the post twelve-fission stage, however, the proportions in the M_1 experiment (82%) are somewhat higher than in the M_2 experiment (71%).

Table 2. *Proportions of animals lacking mu particles following loss of gene* M_1

No. of fissions after change of genotype from $M_1 m_1 m_2 m_2$ to $m_1 m_1 m_2 m_2$	No. of animals tested	Percentage animals lacking mu particles
10	100	33
11	100	61
12	100	82

Table 3 shows the proportions of animals with and without mu particles following change of genotype from $M_1 m_1 M_2 m_2$ to $m_1 m_1 m_2 m_2$, i.e. with simultaneous loss of both M_1 and M_2.

From the data in Tables 1 and 3, the theoretical numbers of metagons were calculated as described in the mathematical appendix, and found to be 1090 ± 70 and 1480 ± 92 respectively. Based on these values, estimates of the proportions

Table 3. *Proportion of animals lacking mu particles following simultaneous loss of genes* M_1 *and* M_2

No. of fissions after change of genotype from $M_1 m_1 M_2 m_2$ to $m_1 m_1 m_2 m_2$	No. of animals tested	Percentage animals lacking mu particles	
		Observed	Estimated*
8	100	4	0·3
9	100	11	5·5
10	100	29	23·5
11	100	34	48·5
12	100	64	69·6
13	102	75	83·4
14	100	82	91·3
15	100	92	95·6

* Maximum-likelihood estimates based on $N = 1480 \pm 92$.

of cells with and without mu particles at each fission were made. These estimates are also shown in Tables 1 and 2, and plotted graphically in Fig. 1. It will be seen that the theoretical and experimental curves coincide reasonably well except at the extremities where there are significant divergencies. At early fissions the proportions of animals lacking mu particles are greater than expected, and at later fissions fewer. These discrepancies are examined below. The maximum-likelihood estimates suggest that the genotype $M_1 M_2$ maintains only about 50% more metagons than M_2 alone, but this comparison is not reliable since the curves do
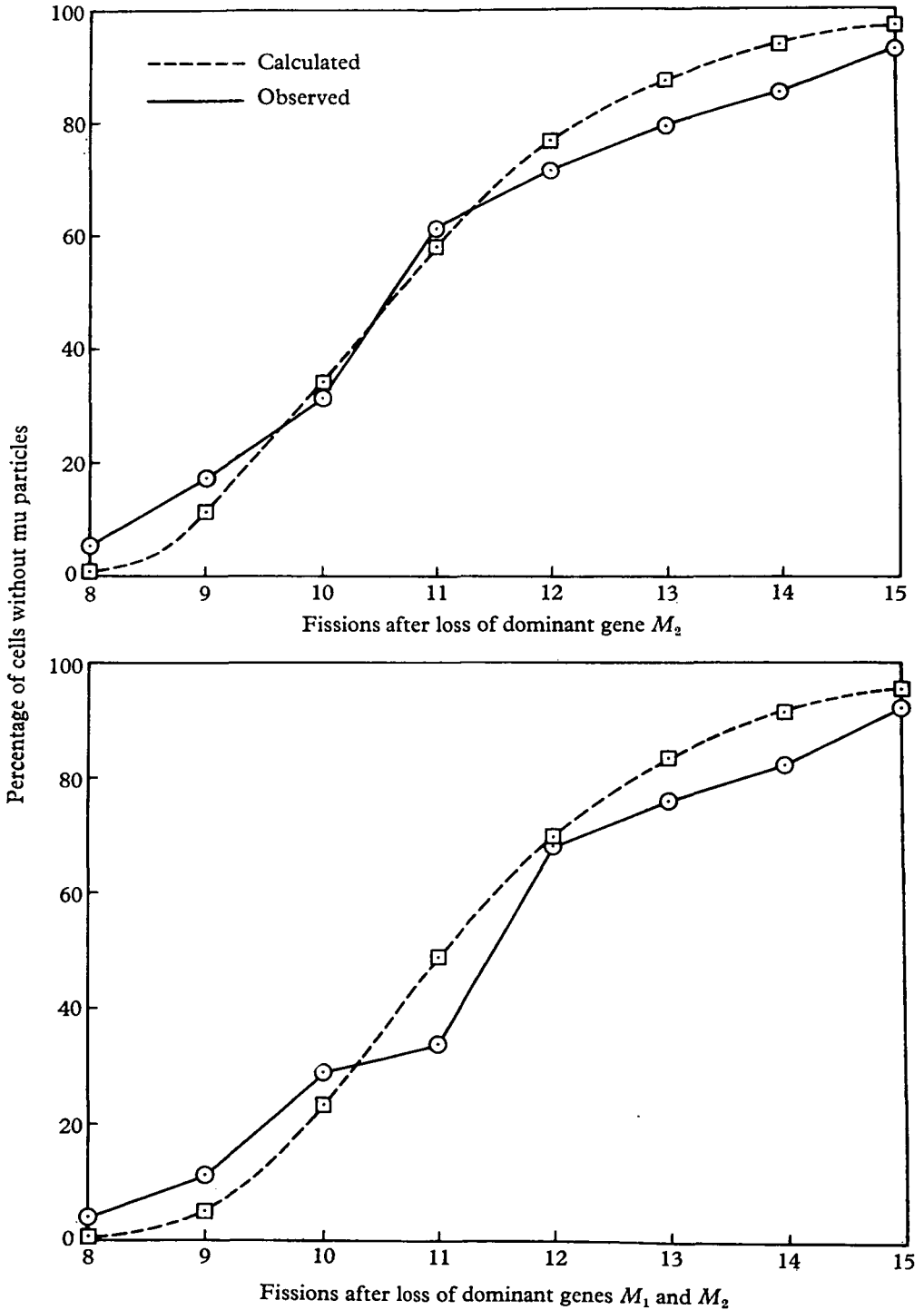
Fig. 1. Proportions of paramecia with and without mu particles after eight to fifteen fissions following loss of genes M_1 and M_2. Based on data in Table 4.

not follow the theoretical trends closely. If there were twice as many metagons in the two-factor case, one would expect the proportions of cells without mu particles to be the same in generation n of the one-factor case as in generation $(n+1)$ of the two-factor case. This appears to be very approximately true only for the middle region of the curve; but one clearly cannot draw definite conclusions about the relative effects of one and two genes at this stage.

2. *Effect of the environment on loss of mu particles following loss of gene* M_2

For both theoretical and practical reasons it was important to know whether the environmental conditions during the period after loss of the genes M_1 and M_2 could affect the stage at which mu particles disappeared, especially since periods

Table 4. *Effect of starvation and temperature on rate of disappearance of mu particles following change of genotype from* $m_1 m_1 M_2 m_2$ *to* $m_1 m_1 m_2 m_2$

Period of starvation after first fission	Temperature during subsequent growth	No. of fissions between change of genotype and scoring for particles	Total nos. of animals scored for particles	Percentage animals lacking mu particles
24 hrs		12	102	74
	18° C			
72 hrs		12	102	71
none		12		(71)*
24 hrs		12	98	73
72 hrs	25° C	12	102	72
none		11		(61)*
96 hrs		11	50	60

* Data from Table 1 for comparison.

of starvation occurred at two places in the experiments described above. In some experiments the effect of varying periods of starvation at the stage when the animals had undergone one fission following loss of the gene M_2 was studied. After starvation the animals were transferred to fresh bacterized medium, allowed to pass through ten or eleven fissions (i.e. eleven or twelve fissions after change of genotype), and then scored for presence of mu particles as in the experiments described above.

The periods of starvation investigated were 24, 72 and 96 hours, and an additional variant was introduced by duplicating the 24- and 72-hour sets and growing the cultures at 18° C. as well as the usual 25° C. for the ten to eleven fissions between starvation and scoring. The results of these experiments are given in Table 4 and show that the varying conditions studied here had no effect on the rate at which animals devoid of mu particles appeared.

The effect of starvation on animals at another stage, namely just before the examination for presence of mu particles, was also studied, for the following reason. It had been previously shown by Preer (1948) that in certain killer stocks of syngen 2, rapid growth led to a gradual reduction in number of kappa particles per cell and their eventual elimination. Starvation—at least in our material—has the opposite effect of increasing the number of mu particles per cell. Hence it is conceivable that in our experiments the number of mu particles per cell might gradually be reduced to a very small number during the eight to fifteen fissions of rapid growth following loss of M_1 or M_2, then starvation of the animals after this process would lead to the formation of many mu particles in those cells in which only one or two mu particles had remained, and none in those cells previously containing none. If this were so, we would not be studying the reduction in numbers of metagons per cell, but reduction in numbers of the mu particles themselves.

Table 5. *Proportions of animals lacking mu particles following loss of gene M_2 and growth in yeast medium, without final starvation*

No. of fissions after change of genotype	No. of animals scored	Percentage lacking mu particles	Corresponding percentage with final starvation and growth in bacteria (from Table 1)
8	50	8	(5)
10	50	30	(32)
15	50	94	(93)

To test for such an effect an experiment was done in which the final starvation period before examination of the animals for mu particles was omitted. Since this starvation is normally interposed in order to eliminate bacteria which may confuse scoring of the mu particles, we here grew some paramecia in a medium containing yeast as food material instead of bacteria, and were then able to score for presence or absence of mu particles in actively growing paramecia.

An experiment was set up like that summarized in Table 1, but with growth in yeast and no final starvation. The fission rate in the yeast medium was about $1\frac{1}{2}$ per day at 25° C. as compared with about 3 per day in the usual bacterized medium. It was found that in the yeast experiment and without final starvation animals lacking mu particles appeared at the same rate as in the bacterial medium and with final starvation (see Table 5). Furthermore, in the yeast-medium experiments, the numbers of mu particles in animals which contained any at a stage fifteen fissions after loss of the gene M_2 were large, certainly more than one hundred per cell. Thus it was clear that loss of mu particles due to an earlier change of genotype was an abrupt event, i.e. that there was a destruction of mu particles rather than passive dilution amongst the dividing paramecia, and that the number of animals devoid of particles at a particular stage was not affected by the prior nutritional conditions.

3. *Effect of starvation of ex-conjugant animals on macronuclear fragments*

In view of the possibility that delay between loss of the genes M_1 and M_2 and loss of mu particles might be connected with the behaviour of the macronuclear fragments which are formed by the breaking-up of the old macronucleus at conjugation or autogamy, some observations were made on the numbers of such fragments at various numbers of fissions after conjugation, and following various degrees of starvation of the ex-conjugants. Sonneborn (1947) had shown that starvation markedly favoured the disappearance of macronuclear fragments, and his findings were confirmed here, as shown by data summarized in Table 6. After

Table 6. *Numbers of macronuclear fragments after various numbers of post-conjugational fissions, and with various degrees of starvation*

No. of post-conjugational fissions	No. of hours' starvation of ex-conjugants	No. of animals examined	Mean no. of macronuclear fragments per animal
	0	32	8·6
	13	48	7·9
3	38	43	6·9
	48	49	5·2
	72	450	0·01 (i.e. 4 animals each had one fragment)
4	0	32	4·6
6	0	84	1·1
	48	34	0·67
7	0	308	0·57
8	0	560	0·20

three days' starvation of ex-conjugants followed by growth through three fissions, only 4 animals out of 450 had a single fragment each and the remaining animals had none. Unstarved ex-conjugants yielded eight to nine fragments per animal at the same stage. These data also show that even in the absence of starvation, by the eighth post-conjugational fission—i.e. the stage at which animals lacking mu particles first appear—the mean number of macronuclear fragments per animal was only 0·2 (i.e. eight out of ten animals had no fragments at all). Thus there could be no direct association between disappearance of the macronuclear fragments and loss of the mu particles.

4. *Further study of animals which have passed through fifteen fissions following change of genotype from* $m_1 m_1 M_2 m_2$ *to* $m_1 m_1 m_2 m_2$

Amongst animals which had undergone fifteen fissions following loss of the gene M_2, the proportion retaining mu particles was found to be about 8% (see Fig. 1). By this stage, therefore, most of the animals belonging to that 8% would be expected, according to the hypothesis, to contain only a single metagon. To test

for this, it was necessary to grow some animals to the 'fifteen-fission' stage, identify those which contained mu particles, then allow such animals to pass through a few further fissions and determine how many of the descendants of each 'fifteen-fission' animal contained mu particles. The procedure was as follows. Heterozygotes of genotype $m_1 m_1 M_2 m_2$ were passed through autogamy and some ex-autogamous clones of genotype $m_1 m_1 m_2 m_2$ identified as previously described. Sister animals were then carried through a total of fifteen post-autogamous fissions, and 478 animals at this stage were then made to form conjugating pairs with sensitive animals (of genotype $m_1 m_1 m_2 m_2$). Unilateral death was found with thirty-eight pairs (i.e. 7·9%) and these were assumed to consist of one mate-killer and one sensitive conjugant. Thus it was concluded that the presence of mu particles, even in the absence of the genes M_1 and M_2, produced the mate-killing phenotype, and further that the proportion of mate-killers at the 'fifteen-fission' stage was the same when determined by this method as by the earlier procedure involving loss of M_2 by conjugation and direct observation of mu particles.

Table 7. *Analysis of groups of eight animals obtained by three fissions of 'fifteen-fission' animals. (Only mate-killers grown on)*

	Numbers of octets	
Proportions with : without particles	Obtained	Estimated
0 : 8	0*	0
1 : 7	32	36·8
2 : 6	5	1·2
3 : 5	1	0·0
4 : 4	0	0·0

* This zero value is to be expected here since all 'fifteen-fission' animals lacking mu particles were excluded from the experiment by the technique of first mating the 'fifteen-fission' animals with sensitives and only growing on those shown to be mate-killers.

The surviving ex-conjugants from the thirty-eight pairs mentioned above were then isolated and passed through three further fissions. The thirty-eight groups of eight animals were separately examined for presence of mu particles by squashing and examination by phase-contrast microscopy. These results are shown in Table 7, the estimated values being obtained as described in the Appendix. The majority of 'fifteen-fission' animals which were mate-killers yielded mate-killers and sensitives in the proportion of 1:7 in the groups of eight; a few gave 2:6 or 3:5; none gave 0:8. The latter result is especially significant since it shows that the change from mate-killer to sensitive following change of genotype is caused by the loss of a unit factor (or metagon), and cannot be due to the reduction in amount of some quantitative determinant below a threshold. Thus the metagon hypothesis receives strong support.

The process whereby the mu particles disappeared was further studied in the following way. Animals at the 'fifteen-fission' stage were made to conjugate with sensitives and the mate-killer survivors picked out as before. The latter were

then removed from the bacterized medium, placed in separate drops of yeast medium and allowed to divide once more. Each of the two daughter animals thus obtained was separately squashed and examined for mu particles, some immediately after fission, some at later stages during the next inter-fission period (without starvation, of course). It was observed that immediately after fission both daughter animals still had many mu particles, but as the inter-fission period went on, one of each pair of daughters had fewer and fewer particles. The reduction in numbers of mu particles was first apparent at about 1 hour after fission, and by 6 hours the process was complete, i.e. no particles remained. During the period of reduction in number of particles, those that remained were found still to be aggregated into the clumps characteristic of mu particles (see Siegel, 1953; Beale & Jurand, 1960), though the outlines of individual particles seemed to become rather indistinct.

These findings confirm that active destruction of mu particles takes place in animals which have recently been deprived of metagons, and further, since the number of mu particles per cell in those cells which remain mate-killers does not diminish, there must be a continuous multiplication of mu particles even at these late stages. For greater clarity, the various stages of these experiments are illustrated in a diagram (Fig. 2).

5. *Further study of animals which have passed through eleven fissions following change of genotype from* $m_1 m_1 M_2 m_2$ *to* $m_1 m_1 m_2 m_2$

In a similar manner to the experiments with the 'fifteen-fission' animals (described in the previous section), some 'eleven-fission' animals were also allowed to pass through three further fissions and the proportions of mate-killers and

Table 8. *Analysis of groups of eight animals obtained by three fissions of 'eleven-fission' animals. (Only mate-killers grown on)*

	Numbers of octets	
Proportions with : without particles	Obtained	Estimated
0 : 8	0*	0
1 : 7	54	132
2 : 6	46	30
3 : 5	45	4
4 : 6	8	0
5 : 3	6	0
6 : 2	4	0
7 : 1	3	0
8 : 0	0	0

* See note to Table 7.

sensitives in the groups of eight from each 'eleven-fission' animal determined. The results are given in Table 8. It was again found that there were no sets in the 0:8 (mate-killer:sensitive) class. However, these results markedly deviated from expectation in regard to the numbers of 'eleven-fission' animals yielding groups of
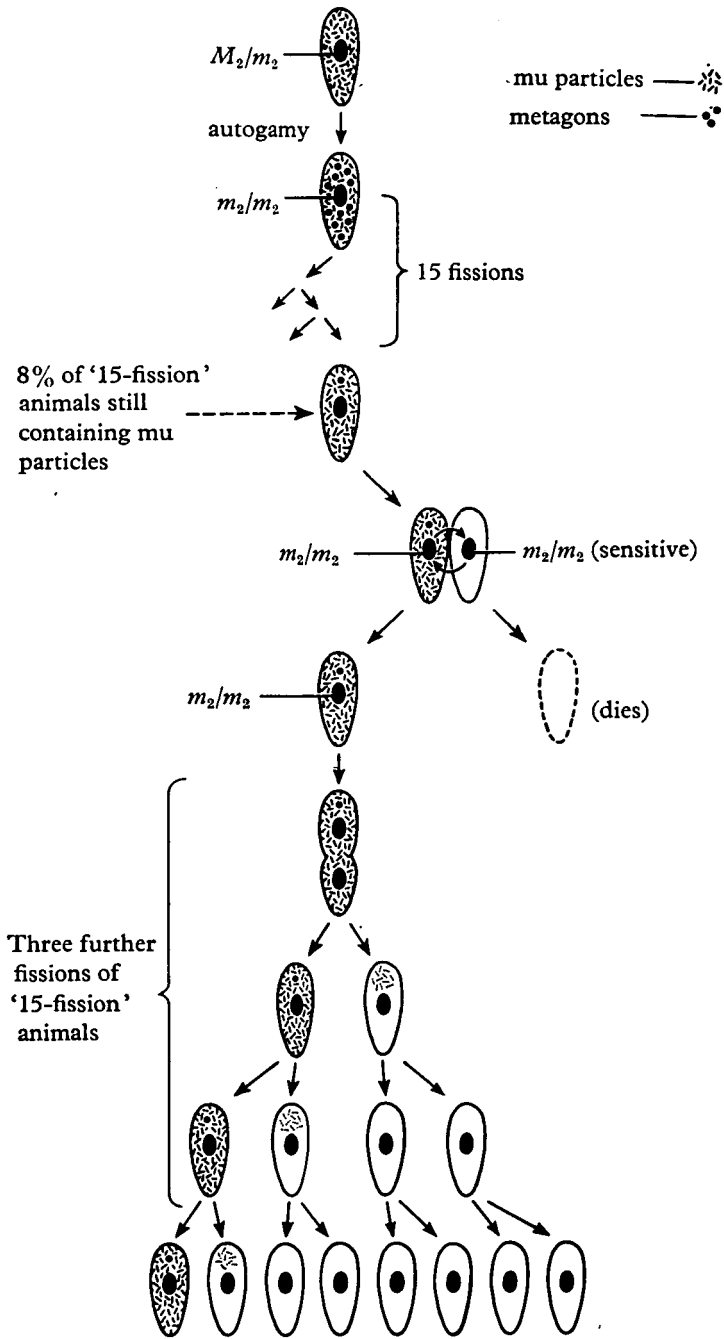
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Fig. 2. Method of analysis of paramecia assumed to contain a single metagon after fifteen fissions following loss of gene M_2.

eight with two, three or higher numbers of animals containing mu particles. As can be seen from Fig. 1, after eleven fissions the proportion of animals lacking mu particles is 61%; at this stage therefore one would expect the majority of animals which did contain mu particles to have only a single metagon. In fact the data indicate that there were nearly as many animals with two or three metagons as with one, and some 'eleven-fission' animals contained at least seven metagons. These inconsistencies are examined in the next section.

6. *Demonstration of non-random distribution of mesosomes*

In view of the discrepancies appearing in the previous section, and also of the deviations of the experimental results from the theoretical curves given in Fig. 1, it seemed likely that the metagons were not being distributed at random in the dividing animals. One possible explanation was that two or more metagons remained associated together more often than would be expected by chance, due to 'clumping', fixation at some site in the cell, or other cause. This would result in the appearance at the early fissions of more sensitives than expected and of more mate-killers at the late fissions, as was found (see Fig. 1). It would also account for the unexpectedly high numbers of 'eleven-fission' animals containing more than a single metagon, as shown in Table 8.

To obtain more definite information on this point, the following experiment was carried out. Animals were grown through eleven fissions after autogamy as before, the mate-killers were identified by conjugation, and again passed through three further fissions. The latter process was so carried out, however, that the exact pedigree of each member of the groups of eight animals was known. Hence the segregation of metagons at the twelfth, thirteenth and fourteenth fissions could be inferred. The results are shown in Table 9. It will be seen that of the groups derived from animals containing more than one metagon, two are by far the most frequent, namely the twenty-four of group MSMS SSSS (with two metagons), and the twenty-four of group MSMS MSSS (with three metagons). The results suggest that there is a very strong tendency for pairs of metagons to remain together at the twelfth fission and to separate at the thirteenth, a result which one would not have expected on any theory or random or non-random distribution of metagons! This raises the question whether some undetected factor in the experimental conditions may have been responsible, but we have no evidence of any such factor at present.

7. *Transfer of metagons between conjugants by cytoplasmic bridges*

Utilizing the technique of Sonneborn (1950) whereby broad cytoplasmic bridges can be induced to form between conjugants, an experiment was done to follow the effect of cytoplasmic exchange between paramecia which had passed through seven fissions after loss of the gene M_2, and others which had passed through fifteen fissions. The former animals would be expected to contain a considerable number of metagons, the latter usually none, but occasionally one. In this experiment the 'seven-fission' animals were marked with immobilization antigen 90G and the

Table 9. *Distribution of animals with and without mu particles in octets obtained by three fissions of 'eleven-fission' animals. (Only mate-killers grown on)*

M = with mu particles;   S = without mu particles

The diagram shows the favoured interpretation of the commonest arrangements in the 2:6 and 3:5 classes

Proportions with : without particles	No. of octets found	Arrangement
0 : 8	0	SSSS SSSS
1 : 7	35	MSSS SSSS
2 : 6	1	MSSS MSSS
	24	MSMS SSSS*
	0	MMSS SSSS
3 : 5	24	MSMS MSSS*
	1	MMMS SSSS
	0	other arrangements
4 : 4	3	MMMS MSSS
	1	MMSS MSMS
	0	other arrangements
5 : 3	1	MMMS MMSS
	0	MMMM MSSS
6 : 2	2	not analysed
7 : 1	2	not analysed

* Shown in diagram.
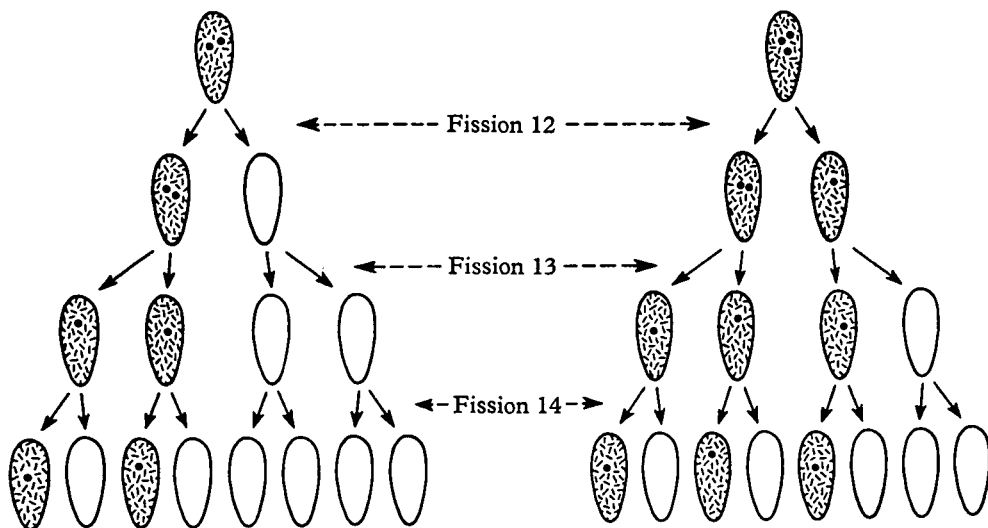
'fifteen-fission' animals with antigen 60G, thus making it possible to identify the origin of the surface structures of the ex-conjugants, and also—by examination of hybrids at a stage five or more fissions after conjugation—to check that nuclear exchange had taken place. (For the behaviour of the antigens in crosses, see Beale (1954).)

Cytoplasmic exchange was induced by treating the conjugating pairs with dilute anti-60G and anti-90G sera at a time about 1 hour before the normal end of conjugation, and removing the pairs from the antiserum some 6 hours later. In about 10% of the treated pairs, broad cytoplasmic bridges had formed after about 4 hours in the serum, and remained for about another 6 hours before final separation of the conjugants. As 'controls', in which cytoplasmic exchange did not occur, some pairs were isolated without any serum treatment.

Following conjugation the ex-conjugants were passed through three fissions, yielding groups of eight animals, of which seven were examined directly for mu particles and the eighth allowed to pass through a further three fissions. The animals thus obtained were in their turn examined for mu particles. The results are shown in Fig. 3. It should be explained that in the 'control' group of ten pairs, the latter were selected on a basis of survival of both ex-conjugants, i.e. when mate-killing did not occur, due to presence of mu particles in the 'fifteen-fission' conjugant. Another eighty-one pairs gave unilateral death of the 60G ex-conjugant, derived from 'fifteen-fission' animals lacking mu particles, but these eighty-one were discarded. In the 'experimental' group of matings with cytoplasmic exchange, however, there was no such selection of pairs for analysis. Six pairs were obtained and all showed survival of both ex-conjugants. In view of the low frequency (8%) of animals with mu particles at the 'fifteen-fission' stage, it is likely that most or all of these six used here lacked mu particles. Thus, the 'fifteenth-fission' conjugants contained at least one metagon in the 'control' series, but probably none in the 'experimental' series.

The results shown in Fig. 3 clearly establish that passage of metagons took place when there was cytoplasmic exchange. Many of the progeny of the 'fifteenth-fission' animals which had received cytoplasm from a 'seven-fission' animal contained mu particles: after three fissions of these ex-conjugants, in all six clones all the animals contained mu particles, in seven out of eight being directly observed and the eighth inferred from presence of mu particles in later progeny. In the progeny of the 'fifteen-fission' conjugants which did not receive any cytoplasm from the 'seven-fission' animals, the numbers of animals containing mu particles were no more than would be expected on the supposition of one or two metagons being originally present in the 'fifteenth-fission' conjugant.

One surprising result in these experiments was the larger total numbers of animals with mu particles found in all the progeny of the pairs showing cytoplasmic exchange. In the control series a total of 32 out of 80 (i.e. 40%) animals had particles after six fissions of the ex-conjugants derived from the 'seven-fission' conjugant, and 0% from the 'fifteenth-fission' conjugant. In the series with cytoplasmic exchange the corresponding percentages were 71 and 73 respectively. This discrepancy cannot be explained without further study. Possibly it is due to break-up of 'clumps' during cytoplasmic exchange between conjugants.

Some further data on the effects of induced cytoplasmic exchange were obtained by study of conjugation between 'eleven-fission' animals and stock 513 sensitives. The object here was to follow the effect of cytoplasmic exchange where there were
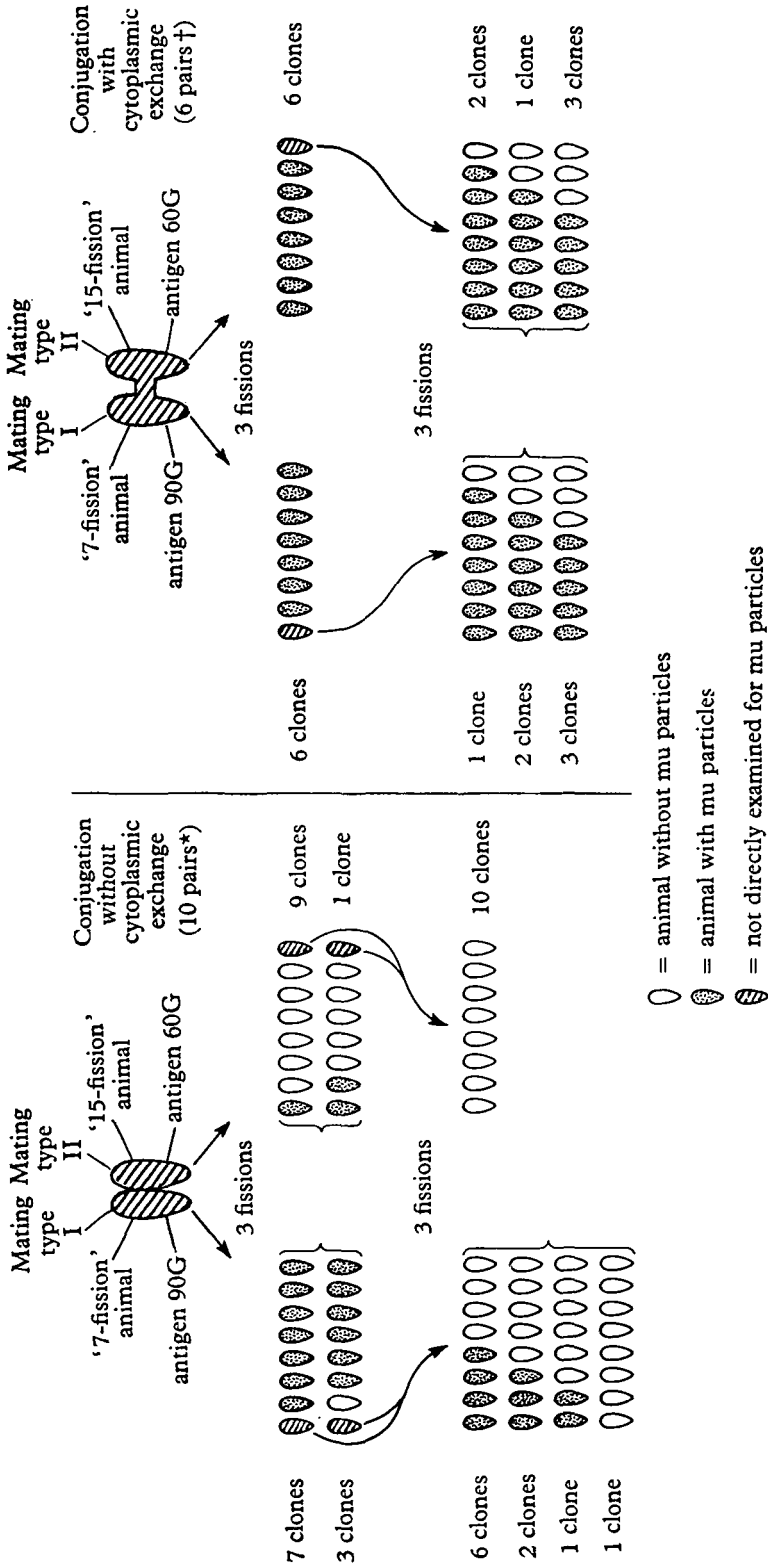
Fig. 3. Comparison of the effect of conjugations with and without cytoplasmic exchange between paramecia at a stage seven fissions after loss of gene M_2 and others at a stage fifteen fissions after such loss.

* As described in the text, these ten pairs were selected by taking those showing survival of both ex-conjugant clones. Both conjugants in each pair therefore contained mu particles.

† Here there was no selection, and most or all of the six 'fifteen-fission' conjugants lacked mu particles.

only a few metagons in one conjugant and none in the other. The results are summarized in Table 10. Study of these results shows that in fifteen pairs one of the ex-conjugants yielded all eight animals without mu particles and the other gave one or more animals with mu particles. This is interpreted to mean that where there is massive cytoplasmic exchange between these mate-killer and sensitive conjugants, the latter usually are not killed but survive to give rise after three fissions to eight animals all of which may be sensitive. In this connection it is recalled that Siegel (1953) found that cytoplasmic exchange between mate-killers

Table 10. *Analysis of groups of eight cells following conjugation of 'eleven-fission' animals with stock 513 (sensitive) and induced cytoplasmic exchange*

Proportions with : without mu particles after three fissions of both ex-conjugants		
Progeny* of 'eleven-fission' conjugant	Progeny* of stock 513 (sensitive)	Nos. found
0 : 8	0 : 8	22
1 : 7	0 : 8	11
0 : 8	1 : 7	2
1 : 7	1 : 7	8
0 : 8	2 : 6	1
1 : 7	2 : 6	4
2 : 6	1 : 7	3
0 : 8	3 : 5	1
1 : 7	dead	1
2 : 8	dead	1

Note. By 'progeny' we here refer to the clones derived from a conjugant marked with antigen 90G (the 'eleven-fission' animals) or antigen 60G (stock 513). Cytoplasmic and nuclear contents of the two ex-conjugants from a pair would be the same.

and sensitives of syngen 8 sometimes resulted in death of the sensitive conjugants, sometimes did not. In our experience the latter is by far the commoner effect, since out of thirty-two pairs involving conjugation between a mate-killer and a sensitive (followed by cytoplasmic exchange), only two showed unilateral death of the sensitive animals (see Table 10).

The data in Table 10 also show that the metagons sometimes all passed from the 'eleven-fission' conjugants into their stock 513 mates, so that the former then gave rise to progeny all of which lacked mu particles. In fact the total numbers of metagons were twenty-nine for the progeny of the 'eleven-fission' conjugant and twenty-six for the progeny of the 'stock 513' conjugant, showing how thorough must have been the mixing of the two cytoplasms.

8. *Reintroduction of the gene* M_2 *after eleven and fifteen fissions following its removal*

In all the previous work we have been studying the effect of loss of the dominant genes M_1 and M_2. It is now important to consider the effect of the opposite change of genotype, from $m_1 m_1$ or $m_2 m_2$ to one containing the respective dominant alleles, and to determine whether here also there is a prolonged phenotypic lag. Some crosses were therefore made as shown diagrammatically in Fig. 4. In these
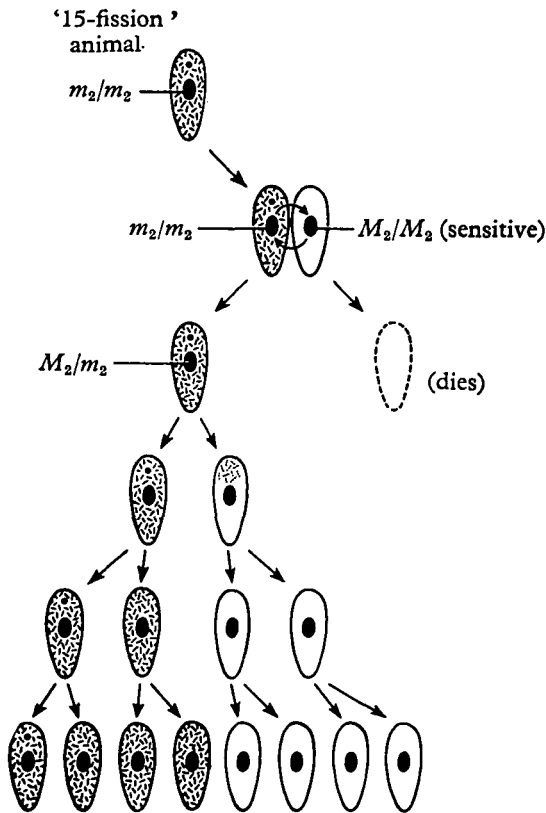

Fig. 4. Effect of reintroducing the gene M_2 into $m_2 m_2$ animals containing mu particles and one metagon. It is probable that many metagons are formed during the third and fourth post-conjugational cell generations, but this is hypothetical at present. Hence they have not been indicated in the diagram.

experiments it was essential to ensure that some mu particles were still present in the cytoplasm of the animals into which the dominant gene (M_2) was introduced, since, once devoid of particles, a paramecium could not be expected to start forming them again merely following reintroduction of the necessary gene (as shown by Sonneborn (1943) for the gene K and kappa particles in *P. aurelia* syngen 4).

As shown in Fig. 4, conjugation was brought about between 'fifteen-fission' animals of genotype $m_1 m_1 m_2 m_2$ but containing mu particles, and sensitive animals of genotype $m_1 m_1 M_2 M_2$ from which the mu particles had been eliminated by

environmental treatment, as described in the 'Methods' section. The occurrence of unilateral death amongst some of these pairs was used both to identify the 'fifteen-fission' animals which contained mu particles, and also to confirm that true conjugation had taken place. Experience showed that incomplete conjugation between pairs of animals, without exchange of genes, did not result in mate-killing, presumably because the period of contact was not long enough.

Table 11. *Effect of reintroduction of gene* M_2 *into 'fifteen-fission' animals (mate-killers)*

No. of fissions after reintroduction of gene M_2	Proportions of animals with:without mu particles*	No. of clones found
2	2 : 2	3
3	4 : 4	6
4	{ 8 : 8	5
	9 : 7	1 (?)

* No other ratios were obtained.

Following conjugation, the surviving ex-conjugants were passed through two, three or four fissions, and then all the progeny were tested for presence of mu particles. It was found that at any of the stages studied half the descendants of one ex-conjugant contained mu particles and half did not (see Table 11). (The only exception was one clone which yielded nine animals with particles and seven without after four fissions. This is presumably due to experimental error.)

Table 12. *Effect of reintroduction of gene* M_2 *into 'eleven-fission' animals (mate-killers)*

No. of fissions after reintroduction of gene M_2	Proportions of animals with:without mu particles	No. of clones found
3	4 : 4	7
4	{ 8 : 8	6
	16 : 0	1

The effect of the introduced dominant gene becomes clear when one compares these results with those of Table 7, where usually only a single animal in any one clone contained mu particles. Following reintroduction of M_2 there is evidently a delay of only one fission before completion of the manifestation of the gene's activity. Between the first and second fissions mu particles disappear in the animal lacking the metagon derived from the 'fifteen-fission' conjugant. After the second fission all animals which have received mu particles from their parental cells are able to maintain them. These may be mediated through the production of new metagons, but the exact mechanism is not yet known.

Similar tests were made in which the gene M_2 was introduced into 'eleventh-fission' animals, and the results are shown in Table 12. Here again most of the ex-conjugants gave clones comprising 50% mate-killer and 50% sensitive animals. The one exception containing 16 out of 16 mate-killers is assumed to be due to the presence of two metagons in the original 'eleventh-fission' animal, and their disjunction at the first fission after the eleventh.

DISCUSSION

The experimental evidence given in the preceding sections supports the hypothesis that the maintenance of mu particles in *P. aurelia* is dependent on the presence in the cytoplasm of one or more of the units here called metagons. We have introduced this word to denote a particulate factor which is in some respects intermediate between a gene and the products of a gene's activity. The characteristic 'phenotypic lag' following change of genotype involving replacement of the dominant genes M_1 and M_2 by their recessive alleles can be satisfactorily accounted for by assuming that initially some number of the order of 1000 metagons is present, that no more are produced after removal of the dominant genes, and that those which are present are stable, being passively distributed to daughter cells for at least eighteen fissions (i.e. a minimum of 6 days in the conditions of these experiments). The redistribution of the metagons is not, however, at random: at some fissions there is a tendency for two metagons to pass to the same daughter cell more often than would be expected by chance. The extent of such erratic distribution is not known, especially at earlier fissions.

It is possible that at the earlier stages the metagons are clumped together, and that the clumps eventually break up during the fission after removal of the dominant genes. Such a process would account in a general way for the deviation of the experimental values for the proportions of animals with mu particles from the estimated values calculated on a basis of random distribution of the metagons. Support for this view is also provided by the results in Table 8 (and to some extent Table 7 also), showing that at late stages in the diluting process the proportions of cells containing two or more metagons are higher than would be expected. The apparent increase in numbers of metagons following induced cytoplasmic exchange (see Fig. 3) might be due to a breaking-up of 'clumps' of metagons. However, the erratic distribution of the metagons at certain stages requires further investigation, since mechanisms other than 'clumping' might be responsible. Thus there might be unequal distribution of metagons to anterior and posterior daughter halves during fission; there might be a slow or spasmodic replication of metagons, or again the possibility of a repulsion between metagons needs to be considered. The statistical consequences of some of these possibilities are considered in the appendix, and it is hoped that results of future experiments will enable us to understand the causes of the observed irregularity.

In view of these considerations, accurate estimates of the initial numbers of metagons cannot be made at present. However from the fact that the 50%

points in the two-factor and one-factor curves (see Fig. 1) are separated by approximately one fission, it seems not unreasonable to conclude that the animals of genotype $M_1m_1M_2m_2$ liberate twice as many metagons as animals of genotype $m_1m_1M_2m_2$ or $M_1m_1m_2m_2$, and further that the metagons corresponding to the gene M_1 behave independently of those corresponding to M_2.

Whilst the metagon hypothesis received general support from the statistical analysis of the rates at which animals lacking mu particles appeared at successive fissions following loss of the genes M_1 and M_2, the most informative results were obtained from experiments with animals assumed to contain a single metagon, many mu particles and neither M_1 nor M_2. When such animals underwent conjugation with sensitives—lacking metagons, mu particles and the dominant genes—the mesosomes remained in the cytoplasm of the animal originally containing them, the sensitive conjugants were killed, and only one of the descendants of each mate-killer animal was afterwards found to contain mu particles. However, when a similar cross was made with induced cytoplasmic exchange, the sensitive conjugants were usually not killed and the metagons could pass into the cytoplasm of the animal originally lacking them.

At each fission of an animal containing one metagon, both daughter animals are initially found to contain large numbers of mu particles. During the course of the next inter-fission period all the mu particles are destroyed in the animal not containing the metagon, whilst in the sister animal with the metagon sufficient increase in number of mu particles must occur to make good the loss of half at each fission. Thus the metagons exercise an extremely sensitive control over the mu particles. Nothing is known of the mechanism whereby this control is exerted, but it seems reasonable to conclude that the metagons might act by inhibiting the action of some normally present enzyme capable of destroying the mu particles.

There appears to be some disagreement between our results on the effect of a reintroduction of a dominant gene (M_2) and those previously obtained by Sonneborn (1943, 1945) and Chao (1953), and discussed by Sonneborn (1959). We have found that when the dominant gene (M_2) is introduced, at any time up to at least fifteen fissions after its removal, into an animal which still contains mu particles (supported by at least one metagon), re-establishment of the capacity to maintain mu particles can always be achieved. There is then a delay of only one fission between reintroduction of M_2 and its manifestation. The earlier workers found a much longer delay (eight fissions), but this was for the doubling in number of kappa particles due to change of genotype from Kk to KK, not the ability to permit the maintenance of kappa particles at all. They also failed to obtain maintenance of kappa particles if the gene K was reintroduced into kk clones when they were more than five fissions old.

Concerning the nature of the metagons the most reasonable assumption would seem to be that they are disrupted elements of the macronucleus containing a dominant gene (M_1 or M_2) which is still physiologically active but unable to replicate. The high degree of stability of the metagons is in conformance with

this view, as is also the finding that animals containing two dominant genes (M_1 and M_2) liberate approximately twice as many metagons as animals containing only one gene. Further, it has recently been reported by Woodward, Gelber & Swift (1961) that, based on photometric measurements of DNA, the macronucleus of *P. aurelia* contains about 860 haploid sets of chromosomes. This would imply that the heterozygote M_2m_2 contained 430 M_2 genes. Such a number is roughly of the same order as the number of metagons estimated to occur, but all these calculations are liable to errors.

Some support for the view that it is possible for genes to continue their physiological functioning whilst no longer able to replicate is supplied by the phenomenon of abortive transduction known in bacterial genetics. Stocker, Zinder & Lederberg (1953) found evidence in *Salmonella* that fragments of genetic material bearing a gene for motility could be introduced into a recipient bacterium lacking such a gene, but that integration of the fragment into the linkage group of the recipient did not always occur. The fragment might then be transmitted at each cell division to only one of the daughter cells, but was nevertheless able to confer motility on that cell, and this process could continue for a number of fissions. A similar 'unilinear' transmission of motility in *Salmonella* was also found to take place with some spontaneous motile variants (Lederberg, 1956; Quadling & Stocker, 1957). Again, Ozeki (1956) obtained abortive transduction of wild-type alleles to purine-requiring mutants of *Salmonella typhimurium*. When grown on the appropriate medium, minute colonies were found containing only a single cell with the wild-type allele, which again was transmitted without replication to one daughter cell, and this process could continue for at least eighteen cell divisions.

Notwithstanding these analogies, there is no direct evidence favouring the view that the metagons in *P. aurelia* are non-replicating 'genes' liberated into the cytoplasm at times of macronuclear reorganization (conjugation or autogamy). In some respects, indeed, our results point to a different kind of interpretation. Thus we have found that a single metagon is able to support as many mu particles as are ordinarily present with a full set of a thousand or so metagons, though there are indications that animals containing only a small number of metagons are under certain conditions weaker in mate-killing activity—taking a longer time to kill—than animals containing many metagons. This would imply, if the metagons were chromosomal fragments bearing genes, that a single dose of a dominant allele (M_1 or M_2) would prevail over a thousand or so recessive alleles (m_1 or m_2) in the macronucleus. Such a degree of dominance would seem to be very improbable. In this connexion it should be recalled that Sonneborn (1954) found that the gene K, when present in the micronucleus but absent in the macronucleus (being replaced there by the recessive allele k), was unable to maintain the kappa particles. Hence, in some respects at least the metagons seem to have a greater physiological activity than genes.

It should also be remembered that there is no correlation between the metagons and the visible macronuclear fragments which presumably contain the genes of the disrupted macronucleus after conjugation or autogamy. There are about sixty

macronuclear fragments per animal at first, as compared with the thousand or so metagons, and the macronuclear fragments are markedly reduced in number by starvation, whereas the number of metagons is unaffected by any environmental variations so far studied. It is certain that when single metagons are being distributed to daughter cells at the fifteenth and later fissions after loss of the genes M_1 and M_2, there are no visible macronuclear fragments present.

In fact none of our results are inconsistent with a quite different hypothesis, namely, that the metagons are normal gene products which pass from the nucleus into the cytoplasm during vegetative growth of the ciliates. Such a process would be quite in conformance with the facts at present available, but there is no direct supporting evidence. In a future publication we shall describe some studies on the nature of the chemical material within the metagons.

SUMMARY

1. Replacement of the dominant genes M_1 and M_2 in *Paramecium aurelia*, stock 540 (syngen/variety 1), results in loss of ability to maintain mu particles and manifestation of mate-killing after a delay of eight to fifteen fissions in most cells. The change, when it does occur, is relatively abrupt, extending over less than the space of one inter-fission period.

2. The delay between change of genotype and loss of mu particles is interpreted as being due to presence in the initial cytoplasm of some thousand 'metagons', which are non-replicating gene derivatives having the physiological activity of the corresponding genes. During successive fissions of paramecia deprived of M_1 and M_2 the metagons are passively distributed amongst the progeny, until virtually all animals lack them.

3. On reaching a stage at which some individuals of genotype $m_1 m_1 m_2 m_2$ contain only a single metagon, the paramecia still contain large numbers of mu particles and are mate-killers. Fission of such animals gives rise to one daughter again with mu particles, and another in which the latter are destroyed during the next inter-fission period.

4. By induced cytoplasmic exchange between conjugants, metagons can be transferred from one animal to another via the cytoplasm. Where such transference is into an animal not originally containing mu particles, that animal is converted into a condition in which it favours the maintenance of mu particles and transmits the latter to one or more of its offspring.

5. Distribution of metagons amongst progeny of dividing paramecia is not random, due possibly to clumping of the metagons. Induced cytoplasmic exchange seems to break up the clumps.

6. Reintroduction of a dominant gene (M_2) into a cell recently deprived of the same gene, succeeds—even after fifteen fissions—in re-establishing the ability to support growth of mu particles, provided that the recipient cell contains at least one metagon and one or more mu particles. There is a regular lag of only one fission between introduction of such a dominant gene and its phenotypic manifestation.

7. Mathematical formulae are developed for calculating the expected initial number of metagons, the proportions of animals lacking mu particles at each fission following loss of the dominant genes, and the proportions of cells containing 0, 1, 2 ..., etc. metagons per cell at any stage. The consequences of one of the possible types of irregular distribution of metagons in dividing paramecia are also considered mathematically.

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APPENDIX: MATHEMATICAL STUDIES OF MESOSOME DISTRIBUTION

By E. C. R. Reeve

We shall discuss here some attempts to characterize mathematically the distribution of metagons, with respect to both the frequencies of 'empty' cells in successive generations following loss of the M_2 gene, and the distribution of occupied and empty cells among the progeny when further fissions are induced in individual cells of a given generation. In what follows, we shall refer entirely to the numbers of metagons in a cell, an 'empty' cell being a cell with no such particles and an 'occupied' cell carrying one or more.

The simplest hypothesis is that an $m_1 m_1 m_2 m_2$ daughter of an $m_1 m_1 M_2 m_2$ paramecium contains N metagons, which are distributed at random, without any loss, among its progeny at each fission.

HYPOTHESIS 1: RANDOM DISTRIBUTION OF N METAGONS AT FISSION

A single paramecium, after n successive fissions, will give rise to 2^n progeny, and a given metagon in the parent cell clearly has a chance $(\frac{1}{2})'$ of entering a particular one of these cells at the nth fission. So the probability that after n fissions a cell contains r metagons is

$$F_n(r) = \binom{N}{r}\left(\frac{1}{2^n}\right)^r\left(1-\frac{1}{2^n}\right)^{N-r}. \tag{1}$$

The frequency of empty cells after n fissions, which we can alone observe, is

$$F_n(0) = \left(1-\frac{1}{2^n}\right)^N. \tag{2}$$

Suppose that, in tests made after s, $s+1$, ..., $s+k+1$ fissions, the proportions of empty cells are observed to be a_s, a_{s+1}, ..., a_{s+k-1}, and we wish to estimate N by comparing these proportions with their expected values given by equation (2). An approximate estimate, good enough for many purposes, may be quickly obtained by equating the product of the observed proportions with the product of the expected proportions for the generations tested (limiting these to values of a_s which are not too small). The resulting equation is

$$N = \sum \log a_n / \sum \log\{1-(\tfrac{1}{2})^n\}, \tag{3}$$

where n runs from s to $s+k-1$. In the present case, this formula may be simplified further. Since s is not less than 8, we have, with sufficient accuracy,

$$N = -\frac{2^{s-1}}{1-(\tfrac{1}{2})^k} \sum \log a_n, \tag{4}$$

where 'log' stands for the natural logarithm, and k is the number of generations tested.

Applying equation (4) to the data from generations 8 to 15 gives estimates for N of 917 and 1124 for the one-factor and two-factor cases, respectively.

It is preferable, though more laborious, to use the maximum-likelihood estimate. If $T_n = A_n + B_n$ cells are tested at the nth generation, and A_n are found to be empty, the likelihood function is

$$L = N \sum A_n \log p_n + \sum B_n \log (1 - p_n^N),$$

where $p_n = 1 - (\frac{1}{2})^n$ and summation is over the generations n tested.

The maximum-likelihood estimate $\hat{N}$ of N is the one which satisfies the equation

$$\sum T_n \log p_n = \sum \frac{B_n \log p_n}{1 - p_n^N}, \tag{5}$$

and the variance of $\hat{N}$ is

$$\mathrm{Var}\,(\hat{N}) = 1 / \sum p_n^{\hat{N}} B_n \left(\frac{\log p_n}{1 - p_n^{\hat{N}}}\right)^2. \tag{6}$$

To solve equation (5), we compute the L.H.S. and the numerators for each n of the R.H.S. A few trial values of N will allow the correct value $\hat{N}$ to be determined by graphical interpolation. The variance of $\hat{N}$ can then be computed from (6).

The maximum-likelihood estimates based on generations 8–15, and the χ^2 values for goodness of fit, are:

	N	χ^2	d.f.	P
One-factor case:	1090 ± 70	40·8	7	0·001
Two-factor case:	1480 ± 92	82·9	7	0·001

These estimates, together with equation (2), give the theoretical curves shown in Fig. 1 of the paper. The values of χ^2 bear out the impression given by Fig. 1 that both sets of observations show significant deviations from the theoretical curves, the observed frequencies of empty cells being too high at the beginning and too low at the end of the period studied.

It will be noted that the maximum-likelihood estimates are both appreciably higher than those estimated from equation (4). This arises from the fact that there are systematic deviations of the theoretical from the actual trend, which are not given equal weights by the two methods.

Before discussing any other hypotheses as to the type of metagon distribution, we shall consider the mathematics of the case in which individual paramecia from a given generation are put through three further fissions and the resulting progeny are scored for the presence of metagons. This makes possible a further check on the hypothesis of random distribution.

In the general case, a paramecium is taken from the nth generation and put through s further fissions so as to give $k = 2^s$ progeny. Suppose the parent cell contains r metagons; then, by a well-known theorem (see Feller, 1950, p. 69), the probability that m of its k progeny are empty is

$$p_{m,r} = \binom{k}{m} \sum_{\nu=0}^{k-m} (-1)^\nu \binom{k-m}{\nu} \left(1 - \frac{m+\nu}{k}\right)^r. \tag{7}$$

Multiplying $p_{m,r}$ by the probability that a cell of the nth generation contains r particles, and summing over r, we obtain the total probability of getting m empty in groups of k cells produced in this way. It is

$$P_{m,k,n} = \sum_{r=0}^{N} \{p_{m,r} F_n(r)\}$$

$$= \binom{k}{m} \sum_{\nu=0}^{k-m} (-1)^\nu \binom{k-m}{\nu} \left(1 - \frac{m+\nu}{2^n k}\right)^N \tag{8}$$

If $Q_{m,k} = P_{k-m,k}$ is the probability that m cells are occupied, then

$$\left.\begin{aligned}
Q_{0,k} &= \{1 - (\tfrac{1}{2})^n\}^N, \\
Q_{1,k} &= k\left[\left\{1 - \frac{k-1}{2^n k}\right\}^N - \{1 - (\tfrac{1}{2})^n\}^N\right], \\
Q_{2,k} &= \binom{k}{2}\left[\left\{1 - \frac{k-2}{2^n k}\right\}^N - \binom{2}{1}\left\{1 - \frac{k-1}{2^n k}\right\}^N + \{1 - (\tfrac{1}{2})^n\}^N\right],
\end{aligned}\right\} \tag{9}$$

etc. $Q_{0,k}$, it should be noted, is the probability that a cell picked at random after the nth fission is empty. Estimates based on equations (9) can be compared with the observed numbers in Tables 7 and 8. A good deal of labour would be required to obtain any kind of 'best' estimates; and we have used the approximate expectations obtained by equating $Q_{0,k}$ to the observed frequencies of empty cells at generations 15 and 11 in Table 1 (93% and 61%). This means that N is assumed to be 2378 for Table 7 and 1012 for Table 8. The relative frequencies of octets of cells with 1, 2, etc. occupied are then multiplied by the total such octets observed, to give the expected numbers in Tables 7 and 8.

Comparison of observed and expected numbers in the two tests shows that at both stages far more octets with more than one occupied cell were observed than was expected on the random-distribution hypothesis. This was particularly striking in the test on generation 11, when sixty-six octets contained at least three occupied cells, compared with the expected number of four.

Clearly, at the very least, some modification of the random-distribution hypothesis is necessary. Among the theoretical possibilities which might be considered are:

(a) unequal distribution of metagons between the two daughters at each fission;

(b) gradual increase in the numbers of metagons, due to a low frequency of division of the particles;

(c) a tendency of the metagons to clump together in groups, with a small probability of a clump separating again;

(d) a tendency for the metagons to repel each other, so that they tend to be more evenly distributed over the cell than they would be if distributed at random.

Some preliminary results with hypothesis (a) will be discussed below, and it is hoped to publish further studies on these hypotheses later.

E

HYPOTHESIS 2: NON-RANDOM DISTRIBUTION OF METAGONS AT FISSION

It is now assumed that a cell always divides at fission into two daughters, say X and Y, which differ in their chances p and q of inheriting a metagon from the mother cell. We suppose that p and q remain constant from cell to cell, and that $p + q = 1$, so that no metagons are lost. The chance of a particular cell produced by the nth fission inheriting a given metagon then depends on the number of X- and Y-type ancestors in the cell's lineage. If it has descended through s X and $(n - s)$ Y ancestors, this probability is

$$L_s = p^s q^{n-s}.$$

If there are N metagons before the first fission, then the probability that a cell picked at random after n fissions contains r of them is

$$F_n(r) = (\tfrac{1}{2})^n \binom{N}{r} \sum_{s=0}^{n} \binom{n}{s} L_s^r (1 - L_s)^{N-r}, \qquad (10)$$

which reduces to (1) when $p = q$.

The frequency of empty cells after the nth fission is

$$F_n(0) = (\tfrac{1}{2})^n \sum_{s=0}^{n} \binom{n}{s} (1 - L_s)^N. \qquad (11)$$

To apply this equation, it seems necessary to try out a number of values of N and p, calculate some measure of goodness of fit, and then estimate the best-fitting values of the two parameters by graphical interpolation. This requires a great deal of computation, and only some preliminary tests have been made so far. These were sufficient to suggest that the hypothesis does not enable us to obtain a satisfactory fit to the trends of Fig. 1. More detailed tests will be described in a later paper.