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Mate-killer (*mu*) particles in *Paramecium aurelia*: further mathematical models for metagon distribution

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Gibson & Beale (1962, 1963) proposed the metagon hypothesis to explain the delay in elimination of the *mu* particles from mate-killer *Paramecia* after loss of the *M* gene(s). This delay is attributed to the presence of a large number *N* of metagons (particulate products of the *M* genes) in the mate-killer cell, which must be diluted out during subsequent fissions following loss of the *M* genes, before any cells without metagons are produced. Only these cells destroy their *mu* particles, and such 'empty' cells first appear after about the eighth fission.

On the *assumption* (1) of passive random distribution of the *N* metagons among daughter cells, a cell of the *r*th fission-generation has a probability $P_{0,r} = [1-(\frac{1}{2})^r]^N$ of being 'empty', but this simple hypothesis gives a poor fit to the observed frequencies (Reeve, 1962). Its inadequacy is brought out clearly by equating $P_{0,r}$ to the observed frequency of empty cells so as to estimate *N* separately for each generation from 8 to 15, when the frequency rises from 5 to 93% (Table 1). The number of metagons required rises steadily from about 800 to nearly 3000 during this period.

Table 1. *Estimates of* N *for each generation on simple dilution hypothesis*

Generation:	8	9	10	11	12	13	14	15
M_2 lost:	770	910	1170	1010	1400	1930	2660	2380
$M_1 + M_2$ lost:	820	1130	1270	2210	1830	2360	3250	2730

The regular nature of these trends suggested that a simple modification of the random dilution hypothesis might explain the discrepancy, and several mathematical models have been tested. One such model (*hypothesis* 2)—that the metagons, apart from being distributed at random, have a small probability *p* of dividing after each cell fission—gives a very good fit to the data with $p = 0{\cdot}2$ and *N* about 300 (Reeve & Ross, 1962). But this does not, of course, prove that the division model is the correct one, and we shall consider some other hypotheses below. These are:

(3) *The clump hypothesis.* An early suggestion was that the metagons might initially be aggregated in clumps, which gradually split up so as to increase the total number of units being distributed. We have tested the simplest situations of this kind—the systems starting with *N* clumps of two and of three metagons, respectively. Each clump is assumed to have a probability *p* of separating into its elements during any interfission period.

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(4) *The decay hypothesis.* The metagons might be gradually destroyed or lost by the cells, in a manner analogous to radioactive decay. We assume the N metagons are distributed at random but each has a chance $p = 1-q$ of being destroyed during any interfission period.

(5) *The unequal distribution hypothesis.* Since the cell divides into an anterior and a posterior half which receive unequal shares of the cell inclusions of the parent, the two daughter cells may have unequal chances of inheriting any metagon. We assume these chances are always p and $1-p$. Formulae for the frequency distributions of metagons among cells of each generation, under this hypothesis, were given by Reeve (1962).

Hypotheses 1–4 can be handled mathematically by modifications of a single set of generating functions. Let $F_1, F_2 \ldots F_r$ be the generating functions for cells produced by the first, second ... rth fission. Then F_1 and the substitutions which convert it into F_2, F_r into F_{r+1}, etc., are as given in Table 2.

The fraction of cells of the rth generation expected to contain t metagons is the coefficient of x^t in the expansion of $(F_r)^N$, for *hypotheses* 1, 2 and 4, while for *hypothesis* 3 the coefficient of $x^s y^{t-s}$ in $(F_r)^N$ gives the fraction of cells of the rth generation with s clumps and $t-s$ single metagons.

Table 2. *Generating functions for various hypotheses*

Hypothesis	F_1	Substitution to convert F_r into F_{r+1}
1. Random dilution	$\frac{1}{2}+\frac{1}{2}x$	$x = \frac{1}{2}+\frac{1}{2}x$
2. Division	$\frac{1}{2}+\frac{1}{2}qx+\frac{1}{2}px^2$	$x = \frac{1}{2}+\frac{1}{2}qx+\frac{1}{2}px^2$
3*a*. Clumps of 2	$\frac{1}{2}+\frac{1}{2}qx+\frac{1}{2}py^2$	$x = \frac{1}{2}+\frac{1}{2}qx+\frac{1}{2}py^2$ $y = \frac{1}{2}+\frac{1}{2}y$
3*b*. Clumps of 3	$\frac{1}{2}+\frac{1}{2}qx+\frac{1}{2}py^3$	$x = \frac{1}{2}+\frac{1}{2}qx+\frac{1}{2}py^3$ $y = \frac{1}{2}+\frac{1}{2}y$
4. Decay	$1-\frac{1}{2}q \ +\frac{1}{2}qx$	$x = 1-\frac{1}{2}q \ +\frac{1}{2}qx$

Maximum likelihood estimates of N and p have been obtained for all five hypotheses using the Rothamsted Digital Computer, and Table 3 summarizes the results of applying them to the data of fissions 8–15 in the one-factor case (loss of M_2). Very similar levels of agreement were obtained with the two-factor case.

Table 3. *Tests of hypotheses for loss of gene* M_2

	Simple dilution	Division	Clumps of: Two	Clumps of: Three	Decay	Unequal distribution
N	1090	286	725	570	1090	2250
p*	0	0·18	0·09	0·08	0·0	0·66
χ^2	41	4·1	29	23·5	41	6·4
D.F.	7	6	6	6	6	6
Prob.	$<0·001$	0·6	$<0·001$	$<0·001$	$<0·001$	0·4

* p = chance of division, separation of clump, decay or inheritance.

As was shown previously, simple dilution gives a poor fit but the metagon division hypothesis fits the data very well. On the other hand, we can now exclude the clump

hypothesis, starting with clumps of either two or three, since it fits little better than simple dilution. The decay model fits best when $p = 0$, making it identical with the random dilution model, and even a small rate of decay makes the fit much worse; so this model can also be ruled out—a conclusion which is obvious from the trends in Table 1.

The computer unexpectedly produced a new model by showing that a 'negative' rate of decay, using the decay model generating functions, gives a good fit to the data ($p = -0{\cdot}2$, $N = 180$, $\chi^2 = 5{\cdot}0$). A negative value of p converts the decay model into a kind of creation model in which the total number of metagons is increased by $-100p\%$ each generation. However, this purely mathematical scheme does not have any simple mechanical basis which could provide a model for the actual distribution of the metagons, as a few trial values of N and p will show. Thus when $N = 1$ new metagons always go into empty cells, and when $p = -1$ all cells of every generation possess N metagons. The model does not in any way simulate the effects of a leaky gene, and we shall regard it as a mathematical curiosity which need not be considered further. It gives a good fit because of its algebraic similarity to the division model.

Finally, the unequal distribution hypothesis also turns out to give a very good fit to the data. So, forgetting the 'creation' model, we have two very different hypotheses which will account for the experimental results. In the first, there are initially about 300 metagons, and each has a 20% chance of dividing in any interfission period. In the second, there is a constant number of about 2300 metagons, and one daughter at fission always has a 66% chance, the other a 34% chance of inheriting each.

A further test has been applied to these two models. Gibson & Beale (1962, Table 8) give the numbers of mate-killers among the eight third-fission progeny of each of 166 eleventh-fission individuals which still contain *mu* particles. The expectations with our two models and the simple dilution model are compared with the observed distribution in Table 4.

Table 4. *Classification of third-generation progeny of eleventh-fission* Paramecia *containing* mu-*particles: test of various hypotheses*

Class: + : −	Number observed	Number expected assuming the following models		
		Simple dilution	Division	Unequal distribution
1 : 7	54	132	63	90
2 : 6	46	30	50	41
3 : 5	45	4	29	19
4 : 4	8	0	16	9
5 : 3	6	0	6	4
6 : 2	4	0	2	2
7 : 1	3	0	0	1

\+ indicates mate-killer cell.

None of the models fits very well, perhaps because of the high frequency in the 3 : 5 class, which raises a suspicion of experimental error here; but the metagon division hypothesis certainly gives a much better fit than the others, and so is the hypothesis to be preferred among those examined so far. This conclusion must be considered tentative until further data, making more critical tests possible, become available. It should also be remembered that only a limited variety of models have so far been examined, and the regular type of modification of simple dilution built into each of them may not be a biologically realistic one.

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