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The replication of metagons and mu particles from Paramecium in another cell—Didinium

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INTRODUCTION

The symbionts kappa, mu, lambda, etc., occurring in the cytoplasm of certain strains of *Paramecium aurelia* are particles composed of DNA, RNA, protein and other substances. The host paramecia are termed killers. In several of the cases analysed the maintenance of these particles is dependent upon a specific gene or genes in the nucleus of the host paramecium. In particular, mate-killer stock 540 (syngen 1) of *Paramecium aurelia* contains mu particles which are dependent for their survival on two unlinked dominant paramecium genes—M_1 and M_2. When the genotype changes, after conjugation or autogamy, resulting in the loss of the genes M_1 and M_2, the daughter cells of genotype $m_1 m_1 m_2 m_2$ lose their mu particles and become sensitives. This loss of mu has been shown to be dependent on a gene-initiated intermediate particle, the metagon, which was considered to be non-replicating (Gibson & Beale, 1962). The metagon was shown to consist of RNA and to be capable of transmission from one paramecium to another via the external medium (Gibson & Beale, 1963, 1964). One metagon was found to be sufficient to maintain many mu particles in the absence of the genes M_1 and M_2. When such a cell divided one daughter maintained the mu particles; in the other they were rapidly destroyed.

In view of Sonneborn's recent (unpublished) finding that kappa particles can be introduced into another protozoan—*Didinium nasutum*, and maintained there, the present study was undertaken to determine how both mu particles and metagons from *Paramecium* behave on introduction into *Didinium*, a process readily accomplished owing to the fact that Didinium is a predator of *Paramecium*.

MATERIALS AND METHODS

Growth of Paramecium *and* Didinium

The various stocks of *Paramecium aurelia* which were used have been described previously, as have the methods of culturing paramecia in test-tube and mass culture (Gibson & Beale, 1962, 1964). The following nomenclature will be used to

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denote the various stocks of paramecia. The formula [M_1 M_2; *met*$^+$; *mu*$^+$] signifies stock 540 paramecia with genes M_1 and M_2, bearing metagons and mu particles; [M_1 M_2; *met*$^+$; *mu*$^-$] represents the same stock but with no mu particles; [m_1 m_2; *met*$^+$; *mu*$^+$] represents the cells previously called '11th fission' and '7th fission' animals, possessing metagons and mu particles; [m_1 m_2; *met*$^-$; *mu*$^-$] represents stock 513 and has neither metagons nor mu particles.

The strains of *Didinium nasutum* used were as follows.

		Generation time at 23°C. =
Stock 1	Twin Lakes, Indiana	4 hours
1a	Derived from the above by growth at 31°C. for a long enough time to free it of kappa (Sonneborn, unpublished)	24 hours
2	Obtained from Professor Willis Johnson who obtained it from General Biological Supply House	5 hours
3	Yellowwood Lake, Indiana	4 hours

For normal maintenance of *Didinium*, test-tube cultures were fed every 3 days with enough paramecia to supply food for 2–3 days. A fortunate characteristic of stock 3 is that it encysts when starved and can be regularly excysted by adding paramecia and bacteria. It is usually maintained in the encysted state.

Mass cultures of didinia were prepared by adding 10^6 didinia to each litre of paramecia (2000 animals/ml.) which were grown in mass culture. After 2–3 days at 27°C. only didinia were found, and starting with 50 l. of paramecia it was found possible to isolate 5 g. (wet weight) of didinia.

Preparation of microsomes, ribosomes and RNA from paramecia and didinia

The isolation of microsomes and ribosomes from *Paramecium* has been described previously (Gibson & Beale, 1964). Similar homogenization and centrifugation procedures were used here with *Didinium*, with the following modifications. RNA preparations were made by extracting microsome preparations of didinia by the guanidine hydrochloride technique (Gibson, 1965). The final RNA preparation, precipitated with ethanol, was dissolved in a solution consisting of 0·01 M Tris/HCl, 5×10^{-3} M $MgCl_2$ and Bentonite 1 mg./ml., pH = 7·2 at a final concentration of 1 mg./ml. and dialysed against this solution. 1 mg./ml. RNA samples from *Didinium* microsomes were diluted 1:400 and used as the test solution for presence of metagons.

Ribosome preparations were made by treating the 105,000 *g* microsomal pellet with 0·1% sodium deoxycholate at 20°C., and finally centrifuging at 105,000 *g* to yield the ribosomes. These were suspended in the same solution as the RNA preparations at a concentration of 5 mg./ml. and diluted 1:2000 before use.

Both preparations could be stored by freezing at −20°C., under which conditions activity was maintained for 6 months.

Method of detection of metagon activity

The presence of metagons in ribosomal or RNA extracts from paramecia and didinia was tested in the following way. With each of the extracts eleventh fission paramecia [m_1 m_2; *met*$^+$; *mu*$^+$] were used as recipients.

Twenty recipient paramecia were mixed with 1 ml. of the ribosomal or RNA extracts (see below for final concentrations) for half an hour at 20°C. Each individual cell was then isolated and allowed to divide three times and the eight paramecia squashed and examined for mu particles under phase contrast. Successful infection was judged to have taken place when most of the eight cells were mu-bearers. Fuller details of the infection process have been described previously (Gibson & Beale, 1964).

RESULTS

The experiments to be described were designed to investigate whether mu and/or metagons could be maintained in didinia after the latter had eaten killer paramecia possessing both these particles. Mu particles are structures which are visible by phase contrast microscopy. Metagon activity is assayed by extraction of ribosomes and by the ability of this extract to support mu particle growth and multiplication in certain tester paramecia.

1. *Introduction into and behaviour of mu particles and metagons in various stocks of* Didinium

Stock 540 mate-killer paramecia—[M_1 M_2; *met*$^+$; *mu*$^+$]—were fed to the stocks of *Didinium* every second day for one week (10^4 animals approximately to 10

Table 1. *Infection of mu particles from* Paramecium *into* Didinium

Stocks of *Paramecium* used as food of *Didinium*	No. of *didinia* seen to contain mμ particles* — *Didinium* stock 1	1a	2	3
Stock 540 (M_1,M_2; *met*$^+$; *mu*$^+$) 1 week, then 513 (sens.) for 6 months	20/20	18/18	0/19	15/16
Stock 540 (M_1M_2; *met*$^+$; *mu*$^-$) 1 week, then 513 (sens.) for 6 months	—	0/15	0/11	0/21
Stock 513 (m_1m_2c *met*$^-$; *mu*$^-$) for 6 months	—	0/30	0/20	0/35
Twenty '7th fission' (m_1m_2; *met*$^+$; *mu*$^+$) cells	15/15	10/10	0/18	21/21

* First number = number with particles, second number = total number cells examined.

didinia at each feeding). The diet was then changed to sensitive paramecia (stock 513)—[m_1 m_2; *met*$^-$; *mu*$^-$]—which possess neither mu particles nor metagons. After 6 months' growth, the didinia were examined for mu particles. RNA extracts from the didinia were also prepared after 6 months and tests made for the presence of metagons by infection into the tester paramecia.

In other experiments, done at the same time, the *Didinia* were fed either on (*a*) sensitive stock [M_1 M_2; *met*$^+$; *mu*$^-$] or (*b*) sensitive stock 513 [m_1 m_2; *met*$^-$; *mu*$^-$ throughout or '7th fission' cells [m_1 m_2; *met*$^+$; *mu*$^+$]. Once again the progeny of the didinia were examined for mu particles and metagons.

The results are shown in Tables 1 and 2. The conclusions from these results are as follows:

Table 2. *Passage of metagons into* Didinium *from* Paramecium *and infection back into* Paramecium (*'11th fission' animals*)

Didinium stocks	Stocks of paramecia used as initial food of *Didinium*	Distribution of paramecia obtained by three fissions of '11th fission' animals following infection from *Didinium* extracts: Class 0	1	2	3	4	5	6	7	8	Interpretation: metagons present (+) or absent (−) in final *Didinium* extract
None (control)		33	8	7	7	2	0	0	0	0	−
1	540 (MK) [M_1M_2; *met*$^+$; *mu*$^+$]	18	0	0	0	0	0	1	2	8	+
	540 (sens.) [M_1M_2; *met*$^+$; *mu*$^-$]	26	0	0	0	2	0	3	1	14	+
	513 [m_1m_2; *met*$^-$; *mu*$^-$]	15	2	3	5	1	0	0	0	0	−
	'7th fission' [m_1m_2; *met*$^+$; *mu*$^+$]	32	0	0	0	0	1	1	2	22	+
2	540 (MK) [M_1M_2; *met*$^+$; *mu*$^+$]	15	0	0	0	1	0	3	1	15	+
	540 (sens.) [M_1M_2; *met*$^+$; *mu*$^-$]	19	0	2	1	0	0	0	3	7	+
	513 [m_1m_2; *met*$^-$; *mu*$^-$]	29	3	9	11	1	0	0	0	0	−
	'7th fission' [m_1m_2; *met*$^+$; *mu*$^+$]	31	0	0	0	0	0	1	2	20	+
3	540 MK [M_1M_2; *met*$^+$; *mu*$^+$]	19	0	0	0	0	1	3	3	3	+
	513 (sens.) [m_1m_2; *met*$^-$; *mu*$^-$]	23	4	4	6	3	1	0	0	0	−
	'7th fission' [m_1m_2; *met*$^+$; *mu*$^+$]	15	0	0	0	0	0	0	1	10	+

MK = mate-killer, sens. = sensitive.

The figures in the body of the table give the numbers in each class of the groups of eight paramecia. 'Class 0' indicates a group consisting of 0 animals with particles and 8 without, class 1 indicates 1 with and 7 without. The first line refers to the progeny of '11th fission' paramecia not infected with any Didinium extract. The extracts of Didinium used were composed of RNA.

(*a*) As seen in *Table 1*, *Didinium* stocks 1, 1a and 3 maintained mu particles for 1000 cell generations (6 months), following initial feeding on paramecia containing mu particles. *Didinium* stock 2 however was unable to maintain mu particles under similar feeding conditions. There were no visible mu particles in any of the *Didinium* strains fed exclusively on paramecia lacking mu (i.e. stock 513 or stock 540 sensitives).

(*b*) All four *Didinium* stocks, if fed initially on paramecia containing metagons, whether mate-killers [M_1 M_2; *met*$^+$; *mu*$^+$] or [m_1 m_2; *met*$^+$; *mu*$^+$], or sensitives [M_1 M_2; *met*$^+$; *mu*$^-$], but subsequently fed on paramecia lacking metagons, [m_1 m_2; *met*$^-$; *mu*$^-$], yielded metagon activity in the extracts obtained after 1000

fissions. This result is shown by the increase in numbers of mu-bearing cells derived from '11th fission' paramecia treated with an RNA extract, over the numbers in the controls ('11th fission' animals not treated with any extract), shown in the top line of Table 2. In Table 2 for example, RNA extracts of stock 1 of didinia gave eleven groups of eight cells with six, seven or eight mate-killers out of a total of twenty-nine groups, whereas the controls gave no groups of eight in these classes, out of a total of fifty-seven.

(*c*) The results with the '7th fission' cells show that the maintenance and multiplication of mu particles and metagons in *Didinium* could occur even without the introduction of the genes M_1 and M_2.

Further confirmation of this finding was obtained by placing stock 513 paramecia [m_1 m_2; *met*$^-$; *mu*$^-$] in contact with a RNA extract from *Didinium* known

Table 3. *Passage of metagons from one paramecium (stock 540 killer) into one didinium of each stock*

Didinium stocks	No. of experiments	No. of cells with mu particles	Groups of paramecia obtained by three-fission animals following infection from *Didinium* extracts								
			Class	0	1	2	3	4	5	6	7	8
1	2	15/16		10	0	0	0	0	1	2	0	3
				18	0	0	0	1	0	0	5	7
1a†	2	20/20		9	1	0	0	0	1	3	2	2
				13	0	0	0	0	1	0	6	4
2	1	0/16		31	0	0	1	0	3	4	0	15
3	2	12/12		21	0	0	0	0	0	3	5	9
				15	0	1	0	2	0	1	0	5
None				*29	7	6	8	3	1	0	0	0

The test for metagons in Didinium was the method (*b*) employing RNA extracts.

* These figures are those derived from '11th fission' cells without exposure to extracts.

† The experiments with stocks 1, 1a and 3 were carried out twice and both results are included here separately.

Class number indicates number of cells out of eight bearing mu particles.

to contain metagons. These paramecia, which had never contained the gene $M_1 M_2$, were then eaten by didinia free of metagons. After subsequent maintenance on animals of type [m_1 m_2; *met*$^-$; *mu*$^-$] extracts of the didinia were made and tested for metagons. The results showed that metagons were indeed present in these didinia: out of sixty-nine '11th fission' paramecia treated, thirty of them gave rise to groups of eight all with mu, and thirty to groups of eight with no mu particles.

(*d*) When *Didinium* was fed throughout on stock 513 paramecia [m_1 m_2; *met*$^-$; *mu*$^-$] no metagon activity was detected in the Didinium extracts finally prepared (e.g. Stock 1 *Didinium*, line 3).

A similar set of experiments was performed with the modification that only a single paramecium containing metagons was used for the initial feeding of the didinia. These experiments were carried out twice and the results are shown in Table 3. The results were similar to those with feeding on many metagon-containing

paramecia (killers or sensitives), i.e. extracts of didinia again gave rise to groups of eight cells, composed of mainly mu bearers (see column 4 for stock 1 of didinia). Furthermore, mu was also maintained in all stocks of didinium except stock 2 (see Table 1). The sets of metagons and mu particles in a single cell were thus sufficient to initiate the introduction and maintenance of metagons and mu.

2. *Effect of feeding* Didinium *on ribonuclease-treated paramecia*

Since it was known from previous work that mu particles in *Paramecium* were destroyed in a cell not containing at least one metagon (Gibson & Beale, 1962), it was necessary to find out if in *Didinium* also, mu particles were maintained only in

Table 4. *Observations on presence of mu particles in didinia fed on ribonuclease-treated paramecia*

Stock of paramecia	No. of fissions after ribonuclease treatment before paramecia were fed to didinia	Mu particles present in paramecia before fed to didinia	No. of didinia containing mu particles after 150 fissions	
			Stock 1	Stock 3
Stock 540 MK (M_1M_2; met^+; mu^+)	(a) 0	Yes	0/50	0/15
	(b) 1	Disappearing	0/10	—
	(c) 2	No	0/18	0/21
	(d) 4	No	0/16	—
'7th fission' animals (genotype m_1m_2; met^+; mu^+)	(e) 0	Yes	0/38	0/21
	(f) 4	No	0/21	0/18

MK = mate-killer.

the presence of metagons. Notwithstanding the results of the last section showing that metagons were present in didinia which contained mu particles, it would still be conceivable that didinia could maintain mu particles without metagons. This was investigated as follows:

(*a*) Killer paramecia of stock 540 [M_1 M_2; met^+; mu^+] were treated with ribonuclease (0·5 mg./ml. for 12 hours at 20°C.). This treatment is known to destroy or inactivate the metagons in all the survivors (i.e. 10%) but to leave the mu particles intact until after one division of the treated cell. The mu particles are then rapidly destroyed in the daughter cells (Gibson & Beale, 1963). Two groups of ten enzyme-treated killer paramecia cells—[M_1 M_2; met^+; mu^+], before dividing, were fed separately to didinia of stocks 1 and 3, which previously neither possessed

visible mu particles nor gave extracts with metagon activity. The didinia were then fed with sensitive paramecia [m_1 m_2; *met*$^-$; *mu*$^-$] for 150 fissions. Samples were then examined for mu particles, and tested for metagon activity by infection into '11th fission' animals. The results are shown in Table 4, line (a), and Table 5, lines (a) and (e). From Table 4 it is seen that mu particles of paramecia (either $M_1M_1M_2M_2$ or $m_1m_1m_2m_2$) treated with enzyme for 12 hours, are not maintained in *Didinium*. Furthermore, that metagons are not present in didinia fed with ribonuclease-treated paramecia is seen in Table 5.

(*b*) Other experiments were carried out with '7th fission' killer cells of genotype [m_1 m_2; *met*$^+$; *mu*$^+$], in place of stock 540 as in (*a*) above. These paramecia were

Table 5. *Test for presence of metagons in didinia fed on ribonuclease-treated paramecia* (*stock* 540)

Didinium stocks	No. of fissions after ribonuclease treatment before paramecia fed to didinia	Groups of paramecia obtained by three fissions of '11th fission' animals following infection from *Didinium* extract								
		Class	0	1	2	3	4	5	6	7	8
1	(a) 0		15	4	5	3	0	0	0	0	0
	(b) 1		21	5	4	7	1	0	0	0	0
	(c) 2		28	0	0	0	0	2	1	6	11
	(d) 4		30	0	0	0	0	0	0	2	21
3	(e) 0		24	6	4	7	3	1	0	0	0
	(f) 1		18	4	3	4	2	1	0	0	0
	(g) 2		36	0	0	0	1	3	1	2	22
None	(h)		*32	5	8	12	2	1	1	0	0

* These results were obtained after three fissions of '11th fission' cells without infection from *Didinium* extracts.

Class = number of mate-killers in each group of eight cells.

treated with ribonuclease for 12 hours and then immediately fed to *Didinium* stock 3. The latter cells were then maintained on stock 513 sensitives and scored for presence of mu particles as before. The results are also shown in Table 4, line (e), and confirm the findings previously described. Thus, in *Didinium*, as in *Paramecium* mu particles seem to be maintained only if metagons are present.

3. *The re-synthesis of metagons in enzyme-treated Paramecia*

Using the technique previously described, involving ribonuclease treatment of mate-killer cells [M_1 M_2; *met*$^+$; *mu*$^+$], further experiments were done to study re-synthesis of metagons in Paramecium and their introduction into Didinium. It has been shown previously (Gibson & Beale, 1963) that although metagons are destroyed by ribonuclease treatment, if the genes M_1 and M_2 are present, re-synthesis of metagons occurs after the 2nd fission. After treatment of mate-killer paramecia of either genotype [M_1 M_2; *met*$^+$; *mu*$^+$] or [m_1 m_2; *met*$^+$; *mu*$^+$] with

ribonuclease, the survivors were allowed to pass through one, two and four fissions before being fed to the didinia. Subsequently the didinia were fed on sensitive paramecia (stock 513) for 150 fissions then examined for mu and metagons. The results showing the behaviour of the mu particles are in Table 4, lines (b) ,(c) and (d), and the metagons in Table 5, lines b, c, d, f and g.

The results in Table 4, lines (b), (c), (d) and (f), show that mu particles, when lost following enzyme treatment of either type of killer do not return.

The results obtained when $M_1 M_1 M_2 M_2$ cells were fed to Didinium (stocks 1 and 3) (see Table 5, column 3) at certain stages after enzyme treatment of the paramecia, indicated that metagons could be detected in the progeny of didinia, but only when the paramecia used as food had passed through at least two fissions after enzyme treatment. This is concluded from the increased numbers of mu-bearing cells derived from '11th fission' cells after infection from didinia, see Table 5. Immediately after enzyme treatment and after only one fission, paramecia do not transfer metagon activity to didinia. These results substantiate the hypothesis that metagons are reformed sometime following the completion of the 2nd fission after enzyme treatment.

4. *The effect of feeding* Didinium *with metagons on ribonuclease-treated paramecia*

Since in the last section it has been shown that didinia would not maintain mu particles from ribonuclease-treated paramecia, experiments were carried out to see if the prior infection of metagons from killer paramecia [M_1 M_2; *met*$^+$; *mu*$^+$] would allow the mu particles from the enzyme-treated cells to be maintained. Two animals of stock 1 of *Didinium* were fed on one sensitive stock 540 cell [M_1 M_2; *met*$^+$; *mu*$^-$] and subsequently on sensitive stock 513 (m_1 m_2; *met*$^-$; *mu*$^-$]. Sixty '7th fission' paramecia were treated with ribonuclease as in the previous section and fed to the above stock of Didinium. The latter were then fed on sensitive cells [m_1 m_2; *met*$^-$; *mu*$^-$] for 200 fissions and extracts made to test for metagons. Thirty didinia were also examined by phase-contrast microscopy for mu. It was found that all the didinia examined now had mu particles and also the extracts tested with '11th fission' paramecia were positive for metagons. It is concluded that the prior introduction of metagons into a *Didinium* now allowed the mu from the ribonuclease-treated paramecia to be maintained in *Didinium*.

5. *The possible role of the* Didinium *genome in maintenance of paramecium metagons*

In Section 1 it was shown that *Didinium* fed permanently on sensitive paramecia of stock 513 [m_1 m_2; *met*$^-$; *mu*$^-$] did not develop metagon activity. This was evidence that *Didinium* did not possess the gene M of *Paramecium*. Another experiment was carried out to test whether *Didinium* can form metagons due to the presence of a gene M_1 or M_2 in the *Didinium* genome. 10^3 didinia of stocks 1 and 3 possessing mu particles and metagons following feeding on killer paramecia [M_1 M_2; *met*$^+$; *mu*$^+$] were treated with an 0·5 mg./ml. ribonuclease solution at 20°C. for 12 hours. It should be recalled that *paramecia* following RNAse treatment

can, if either of the genes M_1 or M_2 are present in the nucleus, resynthesize metagons but not mu particles. The survivors (25%) of the enzyme-treated didinia were then washed through bentonite solution, allowed to divide once and a sample taken and examined for mu particles. The other cells were allowed to undergo 150 cell generations, extracts were then prepared to test for metagons and the didinia were finally examined again for mu particles. In all cases it was found that no metagon activity was present in the extracts, nor were any mu particles maintained in the didinia. These were found to disappear at the first fission following enzyme treatment of the *Didinia*.

These results are interpreted to mean that the enzyme treatment destroyed or inactivated the metagons in *Didinium* just as in *Paramecium*. There would seem also to be no further source of metagons from the *Didinium* genome itself. Since the didinia had been fed on a *Paramecium* source with M_1 and M_2 genes the result would also suggest that these genes, when introduced into *Didinium*, do not continue to function, confirming the results described in Section 3.

6. *The behaviour of metagons and mu particles in* Didinium *at encystment*

Stock 3 of *Didinium* was found to encyst when the supply of paramecia was exhausted. It was also able to excyst readily when paramecia and bacteria were introduced into the medium again.

Stock 3 didinia possessing metagons and mu particles were induced to encyst, and subsequently to excyst by feeding on sensitive stock 513 paramecia. The didinia were then grown a further 160 cell generations on stock 513, then examined for mu particles and a sample of 10^8 didinia examined for metagons, as described above. It was found that 5 days' encystment resulted in the elimination of mu particles and metagons. Shorter periods of encystment were not studied.

7. *Behaviour of metagons when transferred from* Didinium *back into* Paramecium

In order to determine if the metagons continued to increase in numbers when transferred back to *Paramecium* from *Didinium*, ribosomal extracts from *Didinium* (stock 3) which had been fed with paramecia of killer stock 540 [$M_1\,M_2$; met^+; mu^+] for 3 months were placed in the medium in which 100 '11th fission' paramecia were swimming. After half an hour twenty survivors were isolated, then allowed to grow and divide six times. Thirty-two of the sixty-four cells from each survivor were then squashed and examined for mu particles. The other thirty-two were allowed to divide once more and half of the cells again scored for mu particles. This process was repeated for three fissions. '11th fission' cells were also examined without exposure to extracts, and following exposure to extracts from didinia fed on sensitive paramecia [$m_1\,m_2$; met^+; mu^+]. The results in Table 6 show the distribution of mate-killers in the pedigreed daughter cells from three infected paramecia.

The treatment of '11th fission' cells with extracts from didinia previously fed with killer cells gave rise to an increased number of mu-bearing cells, by comparison with the number obtained when paramecia were exposed to extracts from didinia fed on sensitives (column 4). This would appear to exclude the possibility that the

extracts from any didinia were inducing the pre-existing metagons to divide in the '11th fission' tester paramecia.

These results also showed that the '11th fission' cells infected with metagons gave rise eventually to small numbers of cells with mu particles, suggesting that the metagons segregated out during the subsequent asexual fissions of '11th fission' cells. The metagon therefore lost the replicative property when transferred from *Didinium* to *Paramecium*.

Table 6. *Distribution of mu particle-bearing paramecia after infection with extracts from didinium into '11th fission' paramecia* (m_1 m_2; met^+; mu^+)

No. of additional fissions of '11th fission paramecia	No. of cells with mu particles from three '11th fission' cells (a), (b) and (c) infected with didinia extracts when the latter had been fed on killers			No. of cells with mu particles without exposure to any extract	No. of cells with mu after exposure to extract from *didinia* fed on 513 sensitives
	(a)	(b)	(c)		
6	6/32	10/32	7/32	3/32	1/32
7	2/32	11/32	7/32	4/32	1/32
8	1/32	3/32	4/32	2/32	1/32
9	2/64	1/64	0/64	2/64	3/64

Only the '11th fission' cells giving rise to mu particle-bearing cells are shown here. 62% of the cells infected gave rise entirely to sensitive cells and were not considered further. After six fissions thirty-two cells were examined for mu particles and thirty-two cells allowed to undergo one further fission, thirty-two were again taken and thirty-two allowed to divide once.

DISCUSSION

The discovery by Sonneborn (unpublished) that kappa particles can be maintained in *Didinium*, has been successfully exploited with the mate-killers of syngen 1 of *Paramecium aurelia*. It is shown in the experiments reported here that the mu particles of these killers can be maintained over long periods in cultures of *Didinium* (previously lacking mu), even when such predators are allowed to feed on only a single mate-killer, containing a few thousand mu particles, and subsequently on paramecia lacking mu particles. One strain of *Didinium*, however, was never able to maintain the mu particles.

It was also shown that metagons derived from paramecia could be introduced into *Didinium* and maintained there, and this applied to all of the *Didinium* stocks tested, including the one which was unable to maintain the mu particles. The metagon is an infectious RNA particle which is presumed to be derived from the

genes M_1 and M_2 of *Paramecium* and is indispensable for the maintenance of mu particles in *Paramecium*. It is metabolically stable and can function in the absence of the aforementioned genes. It is unknown why the mu particles were destroyed in *Didinium* stock 2 despite the presence of metagons.

That the presence of metagons is essential for the maintenance of mu particles in *Didinium* (as previously known for *Paramecium*), was clearly shown by the results given in Section 2, describing the effect of feeding ribonuclease-treated paramecia, (M_1M_2) containing mu particles, but lacking metagons, to didinia. Didinia treated in this way did not maintain the mu particles initially introduced from the paramecia.

A further result was shown in Section 3 (Table 5). When didinia devoid of metagons were fed with the progeny of killer paramecia of genotype $M_1M_1M_2M_2$ previously treated with ribonuclease, the introduction of metagons (as judged by their presence in the extracts of the progeny) was only achieved when the paramecia used as food had completed the second fission after enzyme treatment. Cells at earlier stages did not supply the didinia with metagons. This shows that metagons reappear in paramecia containing the genes M_1 and M_2 after destruction of metagons by enzyme treatment, confirming previous results from experiments involving cytoplasmic exchange between paramecia (Gibson & Beale, 1963).

A method of eliminating metagons and mu particles from *Didinium* was disclosed in Section 5. In strain 3 encystment occurred and when the didinia excysted the metagons and mu particles were no longer present.

The main problem posed by these results is to explain the presence in *Didinium* of metagons for 1000 cell generations after being introduced. Various possibilities may be considered. If the *Paramecium* genes M_1 and M_2 were also maintained in *Didinium* they might replicate and cause synthesis of metagons, even while the didinia were feeding on sensitive paramecia. The experiments described in Section 1 with '7th fission' killer cells were carried out to test this possibility. Here mu particles and metagons were found to be maintained in *Didinium* after introduction from killer paramecia of genotype $m_1m_1m_2m_2$, i.e. there could be no intake of nuclei containing genes M_1 or M_2. It is still possible, but unlikely, that a few macronuclear fragments containing the gene M_2 might be taken in by the didinia, since the '7th fission' paramecia had been recently derived by autogamy of animals of genotype $m_1m_1M_2m_2$, and it is known that macronuclear fragments persist for some fissions after autogamy. At the '7th fission' stage, at which the $m_1m_1m_2m_2$ paramecia were fed to the didinia, only 50% of the paramecia contain a single fragment and the remainder have none (Gibson & Beale, 1963). Thus the possibility of transferring a macronuclear fragment containing the gene M_2 from *Paramecium* to *Didinium* was very slight.

Experiments which finally eliminated this possibility are reported in Section 1(*c*). Here RNA from *Didinium* possessing metagon activity was introduced into paramecia of genotype $m_1m_1m_2m_2$, which had never previously contained either M genes or metagons, and these paramecia were then used as food for didinia. No M gene from the *Paramecium* source could have accompanied the metagons and

given rise to the metagons subsequently formed in the progeny of the didinia. The lack of effect of m_1 and m_2 genes in bringing about metagon production in *Didinium* is also shown in Section 1.

The results described in Section 5 also show that metagons are not maintained in *Didinium* with the aid of *M* genes derived from *Paramecium*, since following ribonuclease treatment no metagon activity was found in the progeny of the treated cells.

Another possible source of metagons in *Didinium* might be the *Didinium* genome itself. It is conceivable that *Didinium* contains a gene having function similar to that of M_1 and M_2 in *Paramecium*. This possibility is however not borne out by the experiments described in Section 5, which showed that after elimination of metagons with ribonuclease no further activity could be detected.

Further critical evidence indicating the absence in *Didinium* of an *M* gene, either indigenous or introduced, will be presented in a subsequent paper describing nucleic acid hybridization (Gibson, unpublished, 1965).

At present the favoured interpretation of the persistence of metagons in growing cultures of *Didinium* is that after metagons from *Paramecium* have been introduced into *Didinium*, the metagons increase by self-replication. On being transferred from *Paramecium* to *Didinium*, however, several properties of the metagon remain unchanged. It still controls the maintenance of mu particles and remains an infectious RNA particle. Moreover, on being 'returned' from *Didinium* to *Paramecium* the metagons once more show no obvious ability to replicate: they begin to segregate out in the dividing paramecia (Section 7). The metagons therefore differ in their ability to increase in numbers, in the absence of genes M_1 and M_2, in the two protozoan hosts. In *Didinium* each of the metagons must duplicate at least once every 4 hours in order to permit their persistence in every *Didinium* for 1000 fissions. In *Paramecium* there is evidence based on a statistical analysis of metagons distribution in dividing $m_1 m_1 m_2 m_2$ cells, of a possible duplication of the metagons only once every 40 hours, in the absence of genes M_1 and M_2 (Reeve & Ross, 1963), but in this case, other explanations of the analysed data, not involving replication of metagons, are possible.

In general, our current view is that in *Didinium* there is clear evidence for replication of metagons, whilst in *Paramecium* the metagon is most probably non-replicating. In *Paramecium* the metagon seems to be a product of the activity of genes M_1 or M_2 as shown by the reappearance of metagons (after loss) only when one of these genes are present. In a later paper (Gibson, 1965) further results bearing on these problems will be described.

Since at least one enzyme in *Didinium*, a dipeptidase, is known to originate from *Paramecium* (Doyle & Patterson, 1939), it is of interest to show that nucleic acids from one cell can be maintained in another. The metagon RNA, however, is particularly interesting since it is now interpreted as a gene product of *Paramecium* which replicates in *Didinium*.

The significance of these findings has been briefly discussed elsewhere (Gibson & Sonneborn, 1964).

SUMMARY

1. The maintenance of mu particles and metagons from *Paramecium aurelia* (stock 540, syngen 1) in another ciliate protozoan *Didinium nasutum* has been shown to occur.

2. It has been shown that mu particles could not be supported in *Didinium* without metagons. One particular strain of *Didinium* has, however, never been able to support mu even when metagons were present.

3. The continued production of metagons in *Didinium* was shown to take place even when the *Didinium* was fed on killer *Paramecium* of genotype $m_1m_1m_2m_2$.

4. After destruction of the metagons in *Didinium* by ribonuclease there was no subsequent reappearance of metagon activity.

5. Metagons and mu particles were eliminated from *Didinium* during encystment.

6. It is considered that the increase of metagons in *Didinium* is due to self-replication. In *Paramecium* the metagons replicate only slowly or not at all.

7. It is concluded that the metagon is a gene product in *Paramecium* which takes on at least one other property, replication, when introduced into *Didinium*.

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