The mass spectra in both instances were nearly identical to that reported for trans-β-farnesene[2]. Synthesis and mass spectroscopy of trans-β-farnesene revealed that this compound and the aphid odour were identical. Bioassay of trans-β-farnesene was carried out by crushing a sealed glass ampoule containing approximately 10 mg of synthetic trans-β-farnesene in the presence of *M. persicae* on turnip leaves. A large number responded by falling or walking from the plant.

Combined with insecticidal sprays for the control of aphids, the alarm pheromone could enhance their effectiveness. The pheromone will cause those aphids avoiding a direct hit by a spray droplet to walk over the plant and contact the insecticide.

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Note added in proof. At the time this manuscript was submitted for publication Dr W. S. Bowers, USDA, Beltsville, Maryland, informed us that he had established that trans-β-farnesene was also the alarm pheromone for the rose aphid, *Macrosiphum rosae.*

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[1] Kislow, C. J., and Edwards, L. J., *Nature*, **235**, 108 (1972).
[2] Murray, K. E., *Aust. J. Chem.*, **22**, 197 (1969).

Transplantation of Killer Endosymbionts in Paramecium

SEVERAL syngens or breeding groups of *Paramecium aurelia* contain endosymbionts which may result in the development of the killer trait[1]. The symbionts are of different morphological types and it has been suggested that these are non-randomly distributed between syngens[2]. Experiments involving crosses between paramecia with and without symbionts showed that each endosymbiont required a specific nuclear gene for its maintenance. A single gene controlled the presence or absence of endosymbionts as shown by segregation in the F_2 generation. The pattern of loss of symbionts in some of those F_2 clones varies, generally extending over a number of asexual fissions which for different endosymbionts can be from two to over sixty. Infection experiments also demonstrate the requirement for a specific gene. Infection of endosymbionts from homogenates or purified preparations occurs *via* the medium but only into particular cells possessing a specific gene[1,3]. Finally, some endosymbionts growing *in vitro* retain their infectivity into certain paramecia (author's laboratory).

As intersyngenic matings generally do not produce viable progeny, it has not been possible to test for the presence of the maintenance gene in different syngens. Also infection of symbionts from the growth medium back into the source paramecia is not always possible. We tried to transfer endosymbionts between syngens by microinjection. Such intersyngenic transfer, of kappa endosymbionts between syngen 2 and 4 have been recorded[4]. Experiments of this sort may tell us about the distribution of the maintenance genes within the syngens of paramecia, thereby indicating genetical and geographical relationships between symbionts and host paramecia.

Paramecia were grown in grass medium. The transplantation or microinjection technique involves transferring cytoplasmic material from one cell to another with a Fonbrunne micromanipulator (ref. 4 and J. Knowles, personal communication). The presence of endosymbionts was scored by the method of Beale and Jurand[5] or by squashing cells and examining exudates under the phase contrast microscope.

Table 1 Successful Transfer of Symbionts

Symbiont injected†	Recipient cells* which accept symbionts			
1(540) mu‡	1–540(25)§	1–551(39)	1–90(18)	8–138(14)
2(7) kappa	2–7(23)	2–511(54)	4–51(7)	
2(562) alpha	2–562(31)	2–511(18)		
4(51) kappa	4–51(28)	4–116(22)	2–7(3)	
4(A_1) kappa	4–A_1(15)	4–51(23)		
8(138) mu	8–138(21)	8–131(26)	1–540(5)	
8(299) lambda	8–299(8)	8–131(18)		

* None had symbionts.
† Symbionts isolated from packed cells. Paramecia were resuspended in 3 volumes of buffer (0.25 M sucrose, 100 mg ml.$^{-1}$ bovine serum albumin, 0.001 M potassium phosphate, pH=7.0) at 4° C and homogenized in a 'Waring' blender. After centrifugation at 2,000g for 20 min the supernatant contained the symbionts —mu 540, mu 138 [6], alpha 562 and kappa (A_1), (51)[3,7] and lambda 299[8].
‡ Numbers refer to the stock and syngen of *Paramecium*, for example, 1(540) is stock 540 of syngen 1.
§ Actual number of cells injected successfully. Where the symbionts could be injected 100% success was achieved.

Symbionts (kappa, mu, alpha, lambda) were prepared from mass cultures of cells[3,6–8], and transferred to other paramecia. At least twenty cells were injected in each experiment and there was at least 50% survival. The results of various intrasyngenic and intersyngenic transfers of symbionts are shown in Table 1.

Each endosymbiont can be successfully injected into at least one other stock within the same syngen. Successful intersyngenic transfers were observed for mu from stock 138 of syngen 8 to stock 540 syngen 1 (and vice versa), and kappa (51) of syngen 4 to syngen 2. However, mu symbionts did not grow in syngens 2 and 4, nor did kappa in syngens 1 and 8. In all cases control injections were carried out using homogenates or other cell fractions including mitochondrial and post ribosomal fractions from paramecia without endosymbionts. Post-ribosomal fractions from cells with endosymbionts were also unsuccessful in bringing about the establishment of endosymbionts. The possibility that a gene for maintenance is being transferred during microinjection has been ruled out by the following experiment.

Cytoplasm from paramecia without symbionts but which possess the gene was injected into some cells lacking the gene, for example stock 299 into stock 138. Microinjection of stock 299 lambda symbionts into the stock 138 survivors never resulted in the establishment of endosymbionts. Incidentally, successful microinjection of endosymbionts into paramecia which previously contained the same symbionts has been recorded (Table 3). Several experiments have been carried out where endosymbionts were transferred from one paramecium to another with different endosymbionts (Table 2).

The maintenance of a particular endosymbiont depends on environmental conditions and on the presence or absence of a nuclear gene. Successful injection implies the presence of a specific symbiont-supporting gene in the recipient. Cells which do not grow the symbionts following injection would then lack the gene. The exchange of kappa particles between syngens 2 and 4 and mu between 1 and 8 would mean that the gene in the recipient cell can function with close relatives of the resident endosymbionts or else that there is a second gene in the recipient similar to that of the donor cell. I therefore examined the effect of the nuclear gene on symbiont maintenance. Different symbionts were injected into stocks which have recently lost the

maintenance gene as determined by breeding experiments and results are shown in Table 3.

It is clear that in some cases endosymbionts can be transferred to cells without the gene and that they persist through many (>100) fissions. These cells were tested by mating to paramecia with the endosymbionts and were shown to lack the gene, as judged by the appearance of 1:1 segregation of clones which maintain or lose endosymbionts in the F_2 generation. Evidence for the gene which does not allow maintenance of mu 540 in stock 513 has been recorded elsewhere[9], and the presence of a gene which does not allow alpha maintenance (here in stock 576) has also been recorded[2].

The presence of the gene which could not maintain kappa 51 was shown by making a cross between stock 51 (with kappa) and stock 116. Following autogamy of the hybrid, an F_2 was obtained comprising 25 clones capable of maintaining kappa and 30 unable to do so, indicating a 1:1 ratio as would be expected for segregation of a pair of alleles. In some experiments there was no gene difference between stocks 131 and 138 in terms of maintenance of mu 138[1]. By contrast, I have found a gene difference between the two stocks. Stock 131 also possesses a gene which does not allow lambda 299 to grow in the cytoplasm.

The most surprising result, however, is that with the exception of mu 138 and mu 540 which seem to be interchangeable, the resident symbionts only can be maintained in cells which lack the specific maintenance gene. For example, it is possible to transfer mu 540, lambda 299 or alpha 562 to paramecia which have recently lost the same endosymbiont following replacement of the particular maintenance gene during breeding. This result is similar to the long term maintenance of endosymbionts in the absence of the gene as described elsewhere[10].

However, successful injection of alpha, lambda, and mu only takes place in such cells if they are not "older" than twenty, thirty and twenty fissions respectively following gene replacement. Cells at later fissions following replacement of the gene do not accept the endosymbiont which was previously present. This result suggests either that the genes for maintenance are still active for a certain period, then become inactive or that genes which prevent the growth of symbionts become active after a delay. The maintenance gene in the latter case is inactive and the alternative allele produces an inhibitor which functions to prevent the survival of the endosymbionts. To explain the infection of stocks 131 and 116 which will possess this active gene and also the fact that successful injections can only occur over twenty fissions, we must also postulate that the gene is only active under certain conditions.

In summary, symbionts can be microinjected successfully between paramecia independently of the presence or absence of the major gene, stock and even syngen. Such results may seem to suggest that the growth and replication of the endosymbionts do not depend on specific genetic factors or, alternatively, that a similar gene is present in different syngens and protozoa.

Symbionts have been introduced into another protozoan, *Didinium*[10,11]. The symbionts can also grow *in vitro* in the absence of the genes with a similar growth rate as *in vivo*, showing that the locus does not control the growth rate[12]. Opposite conclusions were reached in infection experiments

Table 2 Injection of Symbionts into Paramecia with Other Symbionts Present

Recipient cells*	Symbionts injected: Mu (540)	Mu (138)	Lambda (299)	Kappa (51)	Alpha (562)	Kappa 7	Kappa A_1
Mu 540	– (10)†	√ (9)	√ (31)	√ (26)	√ (20)	√ (9)	√ (15)
Mu 138	√ (15)	– (7)	0 (15)	√ (18)	√ (6)	X (12)	√ (9)
Kappa 51	√ (8)	√ (15)	√ √ (6)	– (9)	√ √ (8)	√ (7)	√ √ (10)
Lambda 299	√ (21)	√ (18)	– (2)	√ (8)	√ (5)	X (15)	X (11)
Alpha 562	√ (10)	√ (21)	√ √ (15)	√ √ (15)	– (19)	√ √ (8)	√ (12)

– =Impossible to tell if maintained as same symbiont injected. However, injection carried out as a control and symbionts were maintained in all cases.
√ √ =Both symbionts retained.
√ =Resident symbiont only retained.
X=Injected symbiont only retained.
0=Neither particle retained.
* At least twenty cells were injected and the results represent all the survivors in each injection.
† Actual number of survivors which maintain or reject injected symbiont.

Table 3 Injection of Symbionts into Paramecia which Have Recently Lost Symbionts

Recipient cells*	Symbionts injected: Mu (540)	Mu (138)	Lambda (299)	Kappa (51)	Alpha (562)	Kappa 7	Kappa A_1
540/513	√ (25)†	√ (29)	0 (34)	0 (32)	0 (17)	0 (16)	0 (15)
138/131	√ (29)	√ (18)	0 (29)	0 (18)	0 (19)	0 (15)	0 (11)
51/116	0 (10)	0 (17)	0 (7)	√ (17)	0 (25)	0 (13)	0 (8)
299/131	0 (15)	0 (15)	√ (19)	0 (16)	0 (23)	0 (12)	0 (17)
562/576	0 (30)	0 (20)	0 (15)	0 (20)	√ (15)	0 (9)	0 (4)

* Cells which lost symbionts in the 20 asexual fissions before injection. These were produced by mating cells with endosymbionts to those without and passing F_1 clones through autogamy. Where one gene is involved in maintaining the symbionts this breeding programme results in clones of cells which lose their symbionts. At least 20 cells were injected.
† Number of cells injected which survive and which show maintenance or absence of symbionts.
√ =Symbiont injected in 100% of cases.
0=Symbiont rejected in 100% of cases.

and genetic experiments where a specific gene was deemed to be necessary for the growth and reproduction of the symbiont[1,7].

My results are consistent with the presence of a genetic locus which is involved with the maintenance of endosymbionts. It is not clear, however, if the activity of the locus involves, (1) protecting the symbiont from destruction during the early fissions after conjugation or infection, or (2) producing a destructive agent which prevents the maintenance of the symbionts. Also the results presented here make it necessary to postulate that the gene may not always be active. The apparent specificity of a locus for one endosymbiont is still consistent with either mechanism.

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[1] Sonneborn, T. M., *Adv. Virus. Res.* (1959).
[2] Beale, G. H., Jurand, A., and Preer, J. R., *J. Cell Sci.*, **5**, 65 (1969).
[3] Preer, L. N., *J. Protozool.*, **16**, 570 (1969).
[4] Koijumi, S., and Preer, J. R., *J. Protozool., Supp. 13*, 27 (1966).
[5] Beale, G. H., and Jurand, A., *J. Cell Sci.*, **1**, 31 (1966).
[6] Baker, R., *Heredity*, **25**, 657 (1970).
[7] Preer, J. R., in *Methods in Cell Physiology*, **4**, 241 (Academic Press, New York, 1970).
[8] Soldo, A. T., Van Wagtendonk, W. J., and Godoy, G. A., *Biochim. Biophys. Acta*, **204**, 324 (1971).
[9] Gibson, I., and Beale, G. H., *Genet. Res.*, 3, 24 (1962).
[10] Byrne, B. J., *Genet. Res.*, **11**, 97 (1967).
[11] Gibson, I., *Genet. Res.*, **6**, 398 (1965).
[12] Williams, J., *J. Gen. Microbiol.*, **61**, 253 (1971).

Cyclophosphamide: Induction of Superovulation in Rats

THE number of ova released at ovulation in rats varies within a narrow limit, usually between 10 and 15. Factors which maintain this relative constancy are not known. Several agents capable of inhibiting ovulation are known[1,2], but apart from gonadotrophin injections there appears to be no way to increase the normal ovulation rate[7]. An agent capable of doing this might be useful in improving breeding potential of laboratory or farm animals, and provide a tool for the study of the neuroendocrine basis of superovulation. (Superovulation is defined as an increase in the ovulation rate beyond that found in the controls.) Exposure of mice[3], rats[4] and hamsters[5] to X-rays has been found to increase ovulation rate and/or litter size. As radiomimetic agents[6] duplicate several effects of X-rays, they might be expected to induce superovulation in rats. Only cyclophosphamide (CPA) amongst those we tested had this effect.

Adult sexually mature rats (Wistar strain) were maintained under controlled environmental conditions of light (from 0600 h to 2000 h) and temperature (22° C). Vaginal smears were taken daily and rats which had at least one and frequently more consecutive 4 day oestrous cycles were included in the study. One group of five rats received a single oral dose of CPA (50 mg kg^{-1}) in 0.2 ml. of 0.5% 'Tween 80' in saline, the other received vehicle alone. The progression of oestrous cycles following CPA treatment remained unaffected and all treated animals showed cornified smears as in controls on the expected day. When rats were killed on this day, about 100 h after CPA administration, the number of tubal ova recovered from the CPA treated rats was significantly higher than from the control rats (Table 1). The uterine and ovarian weights of the treated rats were comparable to those of controls (Table 1). Except for a slight loss in the body weight (4.2%) no other side effects of the treatment were observed. When this experiment was repeated, the mean ovulation rate in the treated rats was 17.2 ± 1.3 ($n = 5$) which was again significantly higher than that in the control rats (10.5 ± 0.5; $n = 23$). When the drug was administered on other days of the cycle, metoestrus, dioestrus or pro-oestrus, it failed to increase ovulation rate and the length of the treated cycle remained unaffected. Increasing the dose to 100 mg kg^{-1} also failed to cause any further increase in the number of ova shed. Histological examination of serial sections of ovaries of rats treated with CPA and killed on the morning of oestrus showed increased numbers of freshly formed corpora lutea as expected. No luteinized follicles with entrapped ova could be found.

Table 1 Effects of Oral Administration of CPA on Ovulation Rate in Rats

Group	*n*	Ovulation rate	Ovarian wt (mg)	Uterine wt (mg)
Control	5	12.2 ± 0.37 (11–13)	73.3 ± 5.7	519 ± 33
CPA treated	5	17.6 ± 1.29* (15–22)	81.7 ± 3.0	457 ± 34

* $P < 0.01$ when compared with control.
CPA (50 mg kg^{-1}) administered on day of oestrus. All animals had tubal ova. Values, mean ± s.e. Figures in parentheses denote range.

To determine whether ova shed in the CPA treated rats were physiologically normal and capable of fertilization and implantation, the experiment was repeated but females were housed with fertile males on the night of first pro-oestrus following CPA treatment. Mating was confirmed on the following morning by finding spermatozoa in the vaginal smear (day 1 of pregnancy). At autopsy on the ninth or fourteenth day of pregnancy, the numbers of implantation sites and corpora lutea were found to be significantly higher than in controls (Table 2). A gross examination of embryos revealed no abnormalities. Embryo loss, that is the disparity between the number of corpora lutea and implantation sites, was significant in the CPA treated rats ($P < 0.05$) but not in the control animals, and it seemed to increase with advancing gestation. This may be due to crowding of embryos in the uterus with an increase in competition for nutrients. Increased embryo mortality is also known to occur in animals superovulated with gonadotrophin treatment[7].

Table 2 Effects of CPA on Number of Corpora Lutea and Implantation Sites in Rats

Parameter	Autopsy on day 9 Control	Autopsy on day 9 Treated	Autopsy on day 14 Control	Autopsy on day 14 Treated
No. of rats	9	9	15	10
No. corpora lutea	13.1 ± 0.7	18.4 ± 1.0*	12.2 ± 0.4	18.6 ± 1.4*
No. implantation sites	12.0 ± 0.4†	16.7 ± 1.0‡	11.4 ± 0.4†	15.0 ± 1.0‡

* $P < 0.01$ when compared with the contemporary control group.
† Not significantly different ($P > 0.05$) from the number of corpora lutea in the same group.
‡ Significantly different from the number of corpora lutea in the same group.
Oral administration of CPA, 50 mg kg^{-1}. Values, mean ± s.e.

The data show that a single oral administration of CPA at an appropriate stage of the oestrous cycle markedly increases ovulation rate. This may result either from an increased gonadotrophin secretion from the pituitary, permitting maturation of increased number of follicles, and/or from decreased rate of atresia of ovarian follicles. We believe this is the first report of a chemical substance unrelated to gonadotrophins capable of increasing ovulation rate.