DEPENDENCE OF THE KAPPA PARTICLES OF STOCK 7 OF *PARAMECIUM AURELIA* ON A SINGLE GENE^{1, 2}

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KAPPA and several other types of symbiotic particles are found in various stocks of *Paramecium aurelia*; their characteristics have been reviewed extensively by SONNEBORN (1959) and more recently by PREER (1967). Many of these symbiotic particles enable the paramecium containing them to kill paramecia that lack them and to be themselves resistant to the killing activity of animals containing the same type of symbiotic particle. Several of these symbionts are known to be lost when the genotype of the host paramecium changes with respect to certain genes; these are the kappa particles of stock 51 of syngen 4 (SONNEBORN 1943), the mu particles of stock 540 of syngen 1 (GIBSON and BEALE 1961) and of stock 138 of syngen 8 (SIEGEL 1953), and the lambda particles of stock 299 of syngen 4 (SCHNELLER, SONNEBORN and MUELLER 1959). This paper deals with the genic dependence of the kappa particles of stock 7 of syngen 2. The results of this study support the conclusion, slowly emerging in recent years, that each of the various types of symbiotic particles in *Paramecium* is maintained only in the presence of one or two specific alleles of chromosomal genes.

MATERIALS AND METHODS

The naturally occurring stocks of *Paramecium aurelia*, syngen 2, used were stocks 5 (source: Elkridge, Md.), 7 containing kappa (Pinehurst, N.C.), 11 (Johns Hopkins Botanical Gardens, Baltimore, Md.), 54 (Flatrock Creek, Moscow, Ind.), 85 (Blairstown, N.J.), 114 containing sigma (Bloomington, Ind.), B197 (trichocyst mutant of 197 from Germany), 1010 (Cove Lake, Tenn.), and 1011 (Indian Creek, Bloomington, Ind.). *P. aurelia* syngen 3 stocks 12 (Staunton, Va.) and 13 (Baltimore, Md.) were used to test for stock 114 sigma killing (SONNEBORN, MUELLER and SCHNELLER 1959); *P. caudatum* stock C141 was used to test for stock 7 kappa killing. Derived lines of paramecia used (all syngen 2) are designated as follows: d2-4, containing stock 7 kappa and stock B197 mutant trichocysts, d2-5, a temperature-sensitive line containing mutant trichocysts and kappa, d2-7, which contains both kappa and sigma, and d2-9, which contains mutant trichocysts and cannot support kappa. The paramecia of all these lines, naturally occurring and derived, are homozygous at all loci. The following gene symbols will be used: *tr* for the recessive gene causing the mutant trichocysts of stock B197 (PREER 1959), *t* for the recessive gene causing temperature sensitivity (PREER 1957), *k* for the gene that fails to support stock 7 kappa, and *K* for its allele that supports kappa.

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The culture medium was an extract of ground desiccated Scottish grass (M. McIntyre Co., Edinburgh, Scotland) 1.5 g/liter, buffered with 1 g $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ /liter, autoclaved, and inoculated the day before use with *Aerobacter aerogenes*. Symbiont-containing lines were fed for 0.5 fission/day (method of PREER 1948a) in order that both sexual reactivity and high symbiont populations be maintained; populations of stock 7 kappa are greatly reduced or lost when killer paramecia are cultured in such a way as to divide rapidly. Symbiont-containing F_1 lines were fed for approximately 0.5 fission/day; autogamy was induced in animals isolated from lines of F_1 animals which had divided approximately 18 times. All F_2 were obtained from the F_1 by autogamy, a process that renders all loci homozygous, so that the expected segregation ratio for any pair of alleles is 1:1.

An American Optical Co. "Bright Medium" oil immersion phase objective was used to detect kappa and sigma particles in the cytoplasm of paramecia, to detect the toxic, highly refractile R bodies in some of the kappa particles, and to distinguish mutant from wild-type trichocysts. Counts of total kappa content were made with regular oil-immersion optics on animals fixed in hot Schaudinn's fixative by the method of PREER (1950) and stained with basic fuchsin according to DIPPELL and CHAO's modification of DeLAMATER's technique (in SONNENBORN 1950).

RESULTS

Stock B197, which has mutant trichocysts caused by a single recessive gene, the *tr* gene (PREER 1959), was crossed with stock 7 killers to obtain animals containing both kappa and the mutant trichocysts. This derived line, d2-4, with its marker *tr* gene, was then crossed with several different syngen 2 stocks. Kappa-containing F_1 lines from these crosses were put through autogamy to obtain the F_2 ; conjugation and autogamy were verified by following the disappearance and reappearance of the mutant trichocysts.

A cross between d2-4 and stock 1010 gave rise to F_2 lines of all the four types which would be expected if stock 1010 contained a gene that would not support kappa: lines with normal trichocysts and kappa (tr^+ , κ), lines with normal trichocysts and no kappa (tr^+ , $-$), lines with mutant trichocysts and kappa (tr , κ), and lines with mutant trichocysts and no kappa (tr , $-$). These results

TABLE 1

Crosses showing the dependence of stock 7 kappa on a single gene

Phenotypes of F_2 lines	Crosses*			
	1	2	3a	3b
(tr^+ , κ)	41	24	21	8
(tr^+ , $-$)	44	35	23	11
(tr , κ)	2	23	18	18
(tr , $-$)	3	25	26	12
Total F_2 lines	90	107	88	49
Total (tr^+) lines	85	59	44	19
Total (tr) lines	5	48	44	30
Total (κ) lines	43	47	39	26
Total ($-$) lines	47	60	49	23

* Cross 1: d2-4 (tr , κ) $\times$ 1010 (tr^+ , $-$); cross 2: 1010 (tr^+ , $-$) $\times$ (tr , κ) line from cross 1; cross 3a: d2-4 (tr , κ) $\times$ (tr^+ , $-$) line from cross 1; cross 3b: d2-4 (tr , κ) $\times$ (tr^+ , $-$) line from cross 3a.

In no case is there a statistically significant deviation from 1:1 for segregation either for κ or tr , with the exception of tr in cross 1, where $P < .001$ (from chi-square). The results are in good agreement with independent segregation of tr and κ .

are shown as cross 1, Table 1; the numbers of (κ) and (—) F_2 lines resulting from the cross were approximately equal. (Aberrant segregation of the *tr* gene, seen in this cross, was observed frequently in crosses between *tr/tr* lines and stock 1010 and will be dealt with in another paper.) Lines in whose animals no kappa could be observed microscopically were tested for ability to kill *P. caudatum* stock C141 animals; no killing was observed, so that loss of kappa from these lines was confirmed. Crosses between d2-4 and other syngen 2 stocks (stocks 5, 11, 54, 85 and 1011) did not result in F_2 lines that lost kappa, results which indicate that these lines do not have genes injurious to kappa maintenance. The fact that loss of kappa was not observed after autogamy induction in a selfing stock 7 line indicates that the results of the d2-4 $\times$ 1010 cross were not due to technical difficulties in maintaining kappa populations in animals undergoing autogamy.

To further confirm the presence of a non-kappa-supporting gene (henceforth called the *k* gene) in stock 1010 and in the F_2 lines which lost kappa a (*tr*, κ) F_2 line was crossed to stock 1010 (cross 2, Table 1) and a (*tr*⁺, —) F_2 line to d2-4 (cross 3a, Table 1). The F_2 from these crosses, the d2-4 $\times$ 1010 cross, and a second cross to d2-4 (cross 3b, Table 1), all derived from the F_1 by autogamy, showed numbers of kappa-containing and kappa-free lines which fit a 1:1 segregation pattern for the *k* gene; the results of these crosses also indicate that the *k* and *tr* genes are not linked. The results of a series of five crosses to stock 1010 of (*tr*, κ) lines from the preceding cross, of which cross 2, Table 1, is the first, showed no statistically significant deviation from this 1:1 segregation. These results indicate that no genes of stock 1010 other than the *k* gene contribute to the loss of kappa.

A three-factor cross was made between stock 1010 and a second derived line, d2-5, which in addition to containing kappa and mutant trichocysts is rendered temperature-sensitive by a single recessive gene, the *t* gene (PREER 1957). The resulting independent 1:1 segregation of all three genes is shown in Table 2.

K/K animals of d2-4 and *K/k* animals, the F_1 from a cross between d2-4 and stock 1010, were cultured under the same conditions (27°C, 0.5 fission/day) in order to compare their total kappa content and the percentage of these kappa particles that contained the R bodies shown to be the cause of killing activity in stock 7 (PREER and PREER 1964; PREER, HUFNAGEL and PREER 1966). Counts of total kappa content (Table 3) are somewhat lower than counts reported earlier (PREER 1948b). The cause is unknown. The counts indicate that the heterozygous

TABLE 2

F_2 from a cross between d2-5 (*tr/tr*, *t/t*, *K/K*) and stock 1010 (*tr*⁺/*tr*⁺, *t*⁺/*t*⁺, *k/k*)

	Normal trichocysts		Mutant trichocysts		Total
	Temperature sensitive	Temperature resistant	Temperature sensitive	Temperature resistant	
Kappa maintained	9	10	14	13	46
Kappa lost	12	16	16	9	53
Total	21	26	30	22	99

Chi-square tests show that the results are in good agreement for 1:1 segregation of all three genes, and for independent segregation of all three.

TABLE 3

Kappa content of K/K and K/k paramecia

	Genotype	
	<i>K/K</i>	<i>K/k</i>
Number of animals examined	15	15
Total number of kappa counted	3499	1801
Mean number of kappa per animal	233.3	120.1
Variance	2341.4	2346.6
Joint variance	2379.3	
	$t = 6.35$ with 28 df, $P < 0.01$	
$\frac{1}{2}$ mean number of kappa per animal	116.6	
Adjusted variance	585.5	
Comparison of <i>K/k</i> mean with $\frac{1}{2}$ <i>K/K</i> mean	$t = 0.02$ with 28 df, $P > 0.50$	

K/k animals contain significantly fewer kappa particles than do *K/K* animals; the mean kappa population of the *K/k* paramecia (120.1 particles per animal) does not differ significantly from half the mean kappa population of *K/K* paramecia (116.6 particles per animal). Counts of kappa particles with and without R bodies were made on animals crushed and examined with a phase-contrast microscope. The mean percentage of kappa particles containing R bodies for a group of 15 *K/K* animals was 18.3%; that for a group of 15 *K/k* animals was 20.2%. These ratios were tested for agreement using a 2×2 contingency table; they are in good agreement.

A line of animals which contained both stock 7 kappa and stock 114 sigma was obtained from a cross between d2-4 and stock 114 in which cytoplasmic exchange took place. This derived line, d2-7, was crossed with another derived line, d2-9 (*tr/tr k/k*) to see whether the *k* gene has any deleterious effect on sigma. No such effect could be detected, as can be seen from the results of the cross, shown in Table 4.

DISCUSSION

Four of the nine known killer paramecium symbionts have previously been shown to be dependent on one or more of the genes of the host paramecium for

TABLE 4

Failure of the k gene to affect sigma: F₂ from a cross between d2-9 (tr/tr, k/k) and d2-7 (tr⁺/tr⁺, K/K, containing kappa and sigma)

	Normal trichocysts		Mutant trichocysts		Total
	Sigma maintained	Sigma lost	Sigma maintained	Sigma lost	
Kappa maintained	18	0	16	0	34
Kappa lost	31	0	19	0	50
Total	49	0	35	0	84

Chi-square tests show the results to be in agreement with 1:1 segregation for both *k* and *tr*, and with independent segregation of the two genes.

their maintenance; these are stock 51 kappa (SONNEBORN 1943), stock 540 mu (GIBSON and BEALE 1961), stock 138 mu (SIEGEL 1953), and stock 299 lambda (SCHNELLER, SONNEBORN and MUELLER 1959). Three of these, stock 51 kappa, stock 138 mu, and stock 299 lambda, are lost when the single gene on which they depend is replaced by an allele which does not support them; stock 540 mu is not lost unless two different genes are both replaced by alleles which will not support the symbiont. There is preliminary evidence that stock 214 kappa is also gene-dependent (SCHNELLER, SONNEBORN and MUELLER 1959). Therefore it is not surprising to find that stock 7 kappa is gene-dependent, and it seems probable that the other paramecium symbionts are also dependent on paramecium genes. The predominant pattern seems to be that of dependence on one gene; this may be the case only because the stocks to which killers have been crossed differed from them at only one locus insofar as symbiont support was concerned. If these killer stocks were crossed with a greater number of other paramecium stocks, different genes which excluded or otherwise affected the symbionts might be found. In the case of stock 51 kappa, the S_1 and S_2 genes that increase the probability of kappa loss were found in stock 29 (SONNEBORN 1947; BALBINDER 1956), after the k gene that excludes kappa and that is present in stock 29 had been first found in stock 32 (SONNEBORN 1943).

The symbiont-supporting genes seem to be specific for their symbionts. We have found that the syngen 2 k gene does not exclude sigma; previously SCHNELLER, SONNEBORN and MUELLER (1959) found that stock 239 lambda was not excluded by the k gene that excludes stock 51 (syngen 4) kappa, and that stock 214 (syngen 8) kappa was not excluded by the gene that excludes stock 299 lambda. This group also found preliminary evidence for more than one gene in stock 299 which excluded stock 214 kappa; these would have to be genes which had no deleterious effects on stock 299 lambda. The only paramecium symbionts known to be dependent on the same gene are those which are known or assumed to be derived from each other by mutation; these were derived ultimately from stock 51 kappa and include the nonkilling symbiont pi (DIPPEL 1948 and 1950). One mutant of stock 7 kappa, 7 m1 kappa (PEER 1948a), is known; because of technical difficulties we have not thus far been able to determine whether this mutant is excluded by the syngen 2 k gene.

The dose effect of the syngen 2 K gene on the size of kappa populations is similar to that demonstrated by CHAO (1954, a, b) for the syngen 4 K gene. Unfortunately the question of whether the K allele results in the production of a substance necessary for kappa or the k allele results in a destructive one is not resolved by these data. GIBSON and BEALE (1962), however, hypothesized that the mu particles in stock 540 are maintained by a stable RNA intermediate, the metagon, produced by the M genes. Studies of the kinetics of particle loss which led to the metagon hypothesis have not been possible with stock 7 kappa, which is rapidly diluted in both K/K and k/k animals under the conditions used here to induce autogamy and is lost within a few fissions after animals become k/k . The fact that the percentage of kappa particles containing the toxic R bodies is not affected by the number of K genes indicates that the development of the R body

within the kappa particle is independent of the dose effect which the *K* gene has on either the reproduction of kappa particles or the maintenance of the whole kappa population.

SUMMARY

The kappa particles found in stock 7, syngen 2, are dependent on a single gene, *K*; the allele *k*, that will not support kappa, is found in stock 1010. *Paramecia* homozygous for *K* contain approximately twice as many kappa particles as do heterozygous *K/k* *paramecia* when animals of these two genotypes are cultured under the same conditions. The percentage of kappa particles which contain the toxic R bodies, however, is the same in *K/K* and *K/k* *paramecia* cultured in the same way. — The *k* gene does not cause the loss of sigma, the symbiont found in stock 114 of syngen 2. Sigma can coexist with stock 7 kappa in the same cytoplasm indefinitely. — The genes *k*, *tr* (mutant trichocysts), and *t* (temperature sensitivity) assort independently.

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