ABSTRACT OF THESIS

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Title of Thesis Genetic Studies on Paramecium bursaria.

The two characters of P. bursaria whose inheritance was studied were the ability to maintain symbiotic zoochlorellae in the cytoplasm and mating type.

An attempt was made to detect variation amongst stocks of P. bursaria with respect to their ability to maintain diverse strains of Chlorella. 21 stocks of P. bursaria were freed of their symbiotic algae, and then reinfected with foreign strains of algae. It was hoped that the stocks of P. bursaria would be distinguishable according to the strains of algae they were competent to accept as symbionts. This was found not to be the case, and the investigation was discontinued.

The stocks of P. bursaria which had been collected in Scotland were classified into syngens and mating types. (With a few exceptions, stocks of paramecium conjugate together only if they are of different mating types within the same syngen.) The Scottish stocks were found to comprise three syngens, each containing eight mating types. These three syngens were identified as syngens 4, 6 and 7 on the basis of the mating reactions between their stocks and stocks belonging to known syngens. Intervarietal mating reactions between stocks of syngens 4 and 7, and between stocks of syngen 2 and syngens 4 and 7, were discovered.

The inheritance of mating type after conjugation was studied in syngen 4. The results indicate that the eight mating types are controlled at three gene loci, the A, B and C loci. There are two alleles, one dominant to the other, at each gene locus. The genotypes of the eight mating types are: type I, aa B- C-; type II, aa bb cc; type III, aa B- cc; type IV, aa bb C-; type V, A- bb C-; type VI, A- bb cc; type VII, A- B- cc; type VIII, A- B- C-. (The symbols A-, B- and C- represent the homozygous or heterozygous state of the locus.)

A means was discovered of changing the mating type of a paramecium without segregation amongst the mating type genes. Some stocks of mating type II were collected, individuals of which are able to go through only the preliminary stages of conjugation with individuals of other mating types; the pairs which are formed fall apart prematurely, before any exchange of gamete nuclei can take place. The

Use other side if necessary.

'separating' type II stocks have been called type IIS.

Normal paramecia, after engaging in temporary pair formation with type IIS paramecia, usually grow up into clones of type IIS; i.e., their phenotype has been stably modified in two ways — with respect to their mating type and their ability to complete conjugation. This phenomenon has been called mating type transformation.

The following facts about mating type transformation have been ascertained:

(i) There is no nuclear reorganisation, and hence no segregation amongst the mating type genes, in paramecia separated from temporary pairs.

(ii) Paramecia which are homozygous dominants at the B mating type locus become type II-IIIS after mating type transformation. II-IIIS paramecia form temporary pairs with paramecia of all mating types except II and III.

(iii) Mating type transformation can occur in syngen 2. Transformed stocks of syngen 2 express mating type VIII or VII-VIII (of syngen 2).

(iv) The outcome of mating type transformation depends on the genotype of the paramecium being transformed, not on the phenotype of the paramecium used to transform it.

(v) Newly-transformed paramecia can themselves effect further transformations by temporary pair formation with normal paramecia.

(vi) The transformed phenotypes are very stable. All attempts to cause transformed paramecia to revert to their original phenotype were unavailing.

(vii) Mating type transformation could not be brought about without temporary pair formation, e.g., by exposing normal paramecia to breis of transformed paramecia.

Mating type transformation is discussed as an example of infective heredity, and the changes of mating type that take place after transformation are explained by changes in the expression of the mating type genes.

GENETIC STUDIES ON PARAMECIUM BURSARIA

by

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GENERAL INTRODUCTION

The modern period of genetical work on Ciliates opened with the discovery of varieties and mating types in Paramecium aurelia (Sonneborn, 1937) and Paramecium bursaria (Jennings, 1938). A variety, or as it has been later called, a syngen (Sonneborn, 1957), of a ciliate species corresponds to a biological species of a higher organism; i.e., it contains all those individuals that are potentially capable of interbreeding. Two individuals of the same syngen can however only mate together if they are of different mating types.

The subsequent progress of ciliate genetics has been reviewed by Sonneborn (1947), Beale (1954) and Kimball (1964). No attempt will be made here to provide even a summary of all the work that has been done; but rather it is intended to convey a general impression of some of the advantages of Ciliates as experimental material by describing some features of their morphology and means of reproduction.

The Ciliates occupy an intermediate position between the micro-organisms and 'higher' organisms in a functional, if not phylogenetic, sense; they are at once the most complex of the micro-organisms and the simplest of the higher organisms. Their claim to be micro-organisms rests on their ability to multiply asexually by binary

fission, and on the fact that the ciliate individual is not compartmentalised into a number of separate cells; and the features which align them with the higher organisms are their morphological complexity and ability to undergo stable cellular differentiation.

Much of the genetical work on Ciliates has been concerned with the factors initiating and maintaining these stable cellular differentiations. In particular, the availability of sexual reproduction in Ciliates provides an opportunity of identifying, by genetic analysis, the genes whose differential expression may underlie the differentiation.

From the genetical point of view the most significant of the morphological complexities displayed by Ciliates is their nuclear dimorphism. Each individual typically contains a single massive macronucleus, which divides amitotically, and one or more diploid micronuclei, which divide mitotically. The presence of the macronucleus, but not necessarily of a micronucleus, is essential for the survival of the individual; and from this, and certain other experimentally obtained facts (Sonneborn, 1947), it is inferred that the physiology and phenotype of the individual are largely controlled by the macronucleus.

The macronucleus is generally considered to be polygenomic, but usually no chromosomes can be distinguished

within it, and the exact arrangement of the genetic material remains obscure (for a review of this problem see Grell 1964).

Sexual reproduction in Ciliates is accomplished by the process of conjugation, the details of which make it most convenient for the detection of the operation of cytoplasmic factors in heredity. Conjugation begins with the association together of individuals of different mating types in pairs. In each conjugant the micronucleus undergoes meiosis and, following a series of nuclear degenerations, two gamete nuclei are produced. (The details of the cytogenetics of P. bursaria will be reviewed in a separate section below). Mutual fertilisation then ensues, one of the gamete nuclei from each conjugant passing into its partner and fusing with the gamete nucleus remaining there to form the zygote nucleus. Little or no cytoplasm is exchanged during conjugation.

The zygote nucleus divides mitotically a number of times and the products of these divisions differentiate into the macronuclei and micronuclei of the next generation. The old macronucleus in each conjugant degenerates.

The outcome of these events is that the macronucleus of the progeny develops in a cytoplasmic milieu inherited from the parents; and any cytoplasmic differences between the parents, whether these be the result of the presence of

self-replicating particles in the cytoplasm, or of the activity of the macronuclei of the parents, or of environmental influences, will have a chance of manifesting themselves as a difference in phenotype between the two exconjugants from a pair.

In summary then, the two fields of research for which Ciliates are particularly suitable are the study of clonal differentiation and of the operation of cytoplasmic factors in heredity; in practice, of course, most investigations overlap into both these fields.

The first of the two pieces of work on P. bursaria to be described in this thesis is concerned with the inheritance of the ability to maintain symbiotic zoochlorellae in the cytoplasm; it was undertaken in the hope of identifying a cytoplasmic factor associated with the host genes necessary for the survival of the symbionts. For reasons that will be explained below this objective was not achieved, and the work was discontinued.

The second of the two investigations, into the inheritance of mating type in P. bursaria, has met with more success. By studying the inheritance of mating type after conjugation, mating type genes have been identified. In addition a means of changing the mating type of a paramecium without segregation amongst the mating type genes has been discovered; so it has been possible to use the results from genetic analysis to interpret a case of clonal differentiation.

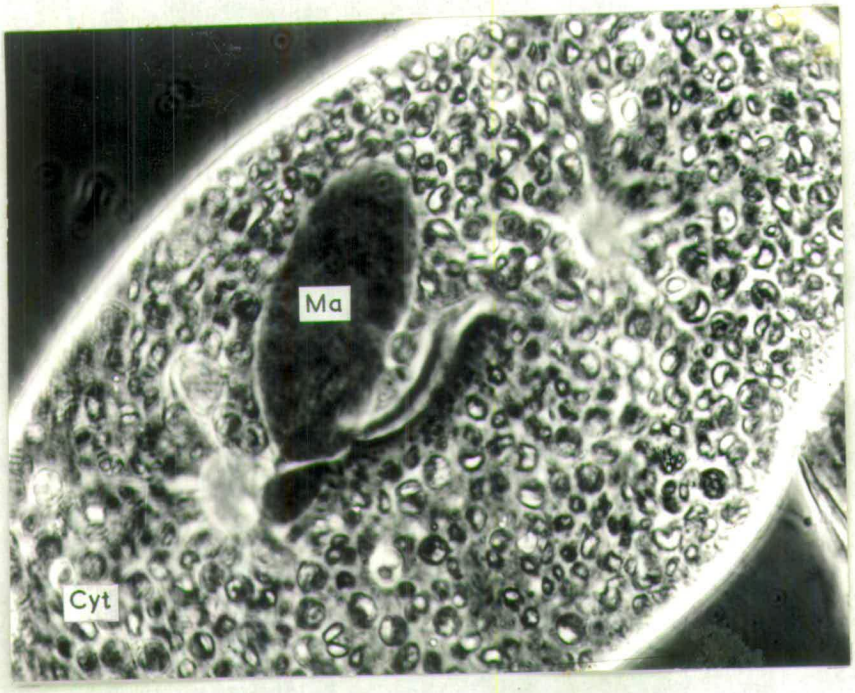


FIGURE 1: An individual of P.bursaria whose cytoplasm (Cyt) contains numerous cells of Chlorella. Ma, macronucleus of paramecium. Phase contrast x 700.

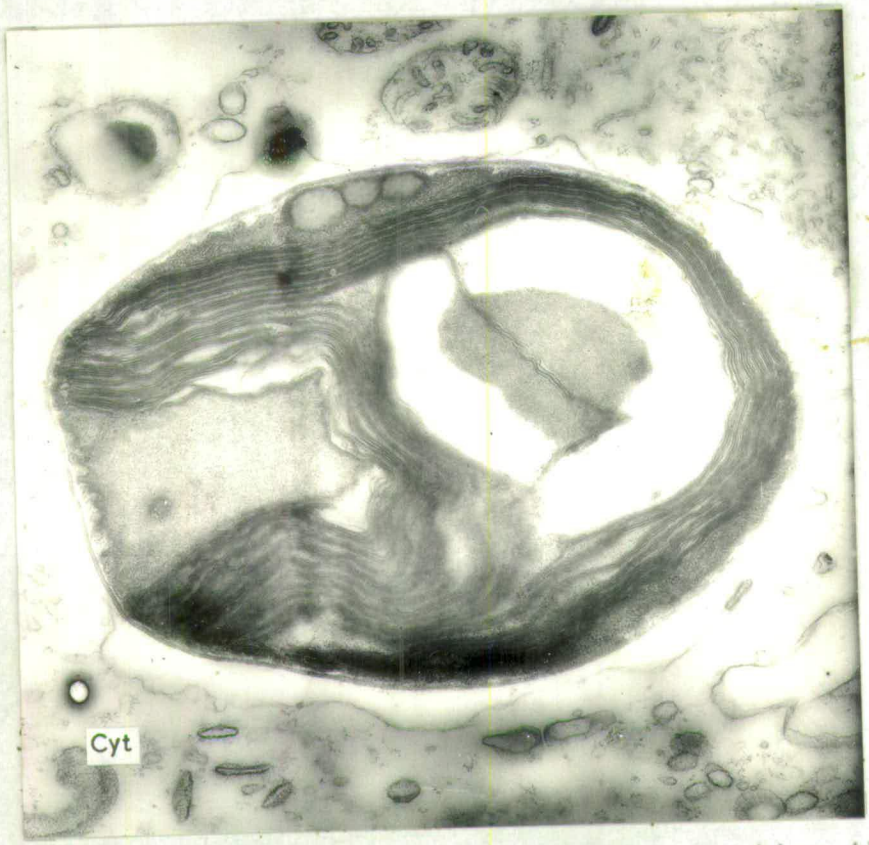


FIGURE 2: Electromicrograph of a section through a single Chlorella lying in a vesicle in the cytoplasm (Cyt) of P.bursaria x 32,000. (This photograph was taken by Dr.A.Jurand in connection with some work that has been described elsewhere; see Jurand & Bomford, 1965).

PART 1

THE MAINTAINANCE OF SYMBIOTIC ALGAE BY P. BURSARIA

INTRODUCTION

Individuals of P. bursaria collected in nature are found to contain symbiotic zoochlorellae in the cytoplasm (fig. 1). The number of algae per paramecium may fluctuate widely, but typically there are several hundred. Electron micrographs of paramecia containing algae reveal that each alga lies in a vacuole in the cytoplasm, surrounded by a membrane (fig. 2).

Symbionts showing some resemblances to bacteria are frequently found in the cytoplasm of P. aurelia (Sonneborn, 1959). These symbionts depend for their survival on the presence of specific host genes. Gibson and Beale (1961, 1962) studied the mode of operation of the symbiont-maintaining genes in stocks of syngen I, in which dominant genes at two gene loci, M_1 and M_2, determine the preservation of the symbionts. If, in a heterozygote at one of the M loci, the dominant gene is removed by segregation during sexual reproduction, the symbionts do not disappear immediately; they remain for up to fifteen further fissions in a declining proportion of the individuals of the clone. From this observation Gibson and Beale developed the hypothesis that the dominant M genes act by

controlling the production of a stable particulate element, called the metagon, which remains in the cytoplasm in an active state after the dominant genes have been removed. The metagon has since been shown to possess a series of remarkable properties such as infectibility and the ability to replicate under certain conditions (Gibson and Sonneborn, 1964).

The maintainance of symbiotic algae in P. bursaria was studied in the hope of finding another system for the demonstration of metagons. For this purpose it would first be necessary to identify symbiont-maintaining genes similar to those that exist in P. aurelia.

No stocks of P. bursaria which do not support algae under natural conditions have yet been discovered. This circumstance prevents the direct genetic analysis of the ability to maintain the symbionts, as was possible in P. aurelia (e.g. Gibson and Beale, 1961).

If each different stock of P. bursaria does possess a genotype allowing the replication of its own algae, and not of others, it would of course be possible to detect this by crossing two stocks, and then backcrossing the F1 to the parents. If the backcross generation were then repeatedly crossed to the same parental stock, sooner or later paramecia whose genotype could not support their algae would be produced, and these paramecia could be identified by the loss of their

algae. The success of this procedure would depend on choosing two stocks of P. bursaria in which the relationship between the host genotype and the symbionts is highly specific.

Fortunately there exists a more direct means of detecting specificities between host and symbiont than crossing stocks of P. bursaria two by two at random. The algae may be removed from clones of P. bursaria by growing the paramecia rapidly in the dark, and the resulting 'white' clones of P. bursaria can be cultured independently. (Oehler 1922, Pringsheim 1928, Siegel and Karakashian 1959). If algae are added to the medium in which white paramecia are growing algal cells are ingested and a symbiosis may be reestablished (Siegel and Karakashian 1959, Siegel 1960). This provides an opportunity to investigate the range of different strains of algae with which a stock of paramecia may be infected.

A failure to secure infection with a particular combination could be attributed to either of two causes. It might be the result of the inability of the algae to grow within the paramecia; or alternatively it might be the result of a blockage in the process of infection itself. It is the former possibility which is of interest here, and particularly the situation in which the inability of the algae to grow within the paramecia is determined by the host genes. This situation could be detected by the

results of crosses between the uninfectable stock of paramecium and a stock carrying the algae concerned. The latter would be expected to lose their algae as their genes were replaced by genes from the uninfectable stock.

The previous work on reinfection suggested that incompatibilities between stocks of paramecia and strains of algae may be quite common. Oehler (1922), Siegel and Karakashian (1959) and Karakashian (1963) all record instances in which infection could not be accomplished, although the paramecia concerned could be infected with other strains of algae. In addition Siegel (1960) detected differences between four stocks of P. bursaria with respect to their efficiency of infection by various strains of algae, and the rate of growth of the algae following infection.

This information made it seem probable that, if a large number of different stocks of P. bursaria were examined as to the range of algae with which they might be infected, clearcut differences between stocks would be uncovered. With this end in view a collection of stocks of paramecia and strains of algae, as heterogeneous as possible with respect to geographical origin, was assembled. The results of reinfection experiments performed with this material are described below. At a later stage in the investigation some of the stocks of paramecia were also tested for their ability to form symbiotic associations

with a strain of the green alga Scenedesmus sp. and with a yeast. It has already been reported that a white stock of P. bursaria can be infected with Scenedesmus (Oehler, 1922).

An account of this work has already been published and a copy of the paper is bound at the back of this thesis.

MATERIALS AND METHODS

1. Stocks of P. bursaria

Most of the stocks (a stock represents the asexual progeny of a single individual collected in nature) of P. bursaria used in this investigation were collected by the author at various localities in Scotland, England and Holland; American stocks were kindly supplied by Dr. R.W. Siegel. Table 1 gives a list of the stocks classified according to the syngen of P. bursaria to which they belong. The detailed description of the means employed to assign stocks to their syngen will be presented later in connection with the work on mating types. (Part 2, Section A). Here it is sufficient to recall that each syngen in a ciliate species is genetically isolated from all other syngens. Thus clones of paramecia belonging to different syngens may confidently be expected to be genetically diverse.

The stocks of P. bursaria were cultured in bacterised culture fluid (Sonneborn, 1950).

2. Strains of Algae

Cultures of three free-living strains of Chlorella,

SYNGEN	STOCK DESIGNATION	LOCALITY COLLECTED	
1	3 8	California U.S.A.	
4	TU	Turnhouse	Scotland
	WL	Water of Leith	"
	P	Penicuik	"
	BL16	Blackford Pond	"
	H4a		
	H6c	The Hague	Holland
	H		
6	W	Warwickshire	England
	HW	High Wycombe	"
	Pt	Penicuik	Scotland
7	CO4	Coshieville	Scotland
	BA6	Ballantray	"
	BL	Blackford Pond	"
	LU	L. Lubnaig	"
	M1	Muir Kirk	"
	SM1		
	SM2	St.Mary's Loch	"
	SM9		

TABLE 1. The origins of the stocks of P. bursaria used in the investigations into the maintainance of zoochlorellae.

LA25, Gr-B and IU20 (C. ellipsoidea, Indiana University culture collection) were kindly provided by Dr. R.W. Siegel.

Symbiotic algae were isolated from four stocks of P. bursaria: CO4 and BL (Syngen 7), CAI (Syngen 4) and Sd (Syngen 1). To obtain the algae, paramecia were washed by allowing them to swim through sterile exhausted culture fluid (Sonneborn, 1950). Several individuals were then picked up in a fine pipette and placed on the surface of an agar slant, where they ruptured, liberating the algae. The composition of the medium used to culture algae on 1.5% agar slants was:-

$Ca(NO_3)_2$	0.05	gms
$MgSO_4\ 7H_2O$	0.025	
$Na_2C_9H_5(OH)PO4.\ 5½\ H_2O$	0.025	
KCl	0.0125	
TRIS	0.05	
Trace elements (Huenter et. al. 1950)	0.05 ml.	
Dist. aq.	1 litre	

After the algae had been freed from bacteria by growing them on slants containing 100 units/ml of penicillin it was possible to transfer them to slants prepared from medium supplemented with 0.5% glucose and 0.1% protease peptone, on which faster growth was obtained.

The four cultures of symbiotic algae were purified

by dilution followed by isolation of single colonies before they were used in infection experiments.

Several unsuccessful attempts were also made to isolate and culture the algae from four other stocks of P. bursaria: G and MI (Syngen 7) and W and HW (Syngen 6); but the experiments were not repeated a sufficient number of times to justify any conclusions about the relative adaptability to independent culture of different strains of symbiotic algae.

Algae from these and other stocks of paramecia were prepared for use in infection experiments by disrupting the paramecia by repeated expulsion through a syringe. There could be no certainty that algae so obtained represent a pure strain.

The strain of Scenedesmus sp. was isolated by spinning down the organisms from a sample of water out of a local pond and spreading them on unsupplemented algal medium. The culture was subsequently purified by dilution and reisolation. The original intention behind this operation had been to collect local strains of free-living chlorellae.

3. The Yeast

The yeast used in these experiments was first noticed in the cytoplasm of one of the white stocks of P. bursaria, where it had presumably arrived as a contaminant in the culture. A few of the paramecia were washed by

transfer through sterile exhausted culture fluid, and then placed on the surface of a malt extract agar slant, where they ruptured, liberating the yeast. This formed pink colonies consisting of budding cells of approximately 5 diameter. No further identification of the yeast was attempted.

4. The Removal and Reinstatement of Symbionts

Paramecia lacking symbiotic algae were obtained by culturing stocks as daily isolation lines (Sonneborn, 1950) in the dark at 28°C. When a line was suspected to be free of its algae a single individual was isolated and allowed to divide once. One of the daughter paramecia was examined for algae under the high power of the microscope, and the other allowed to divide again. One of the products of the second division was also examined for algae. If both of the examined individuals were without algae the surviving individual was grown up into a clone in the light. All the chlorella-free cultures established as a result of this procedure were kept in the light, and no 'revertant' individuals were ever observed.

The period of growth in the dark required for the loss of algae averaged about three weeks. No attempt was made to determine the minimum time required in each case, and so no comparative estimates of the tenacity of the association between host and symbiont can be made. That differences do exist in this respect has been shown by Siegel (1960).

In addition some stocks of P. bursaria produced chlorella-free individuals while growing in the light. White stocks derived in this way did not behave any differently in the subsequent reinfection experiments.

The reinfection experiments were performed as follows. Individuals of the white stock to be tested were pipetted into fresh bacterised culture fluid and left there overnight. Thus all the paramecia were in a fully fed condition at the outset of the experiment. Approximately 100 individuals were added to two drops of a suspension of algae or yeast cells in a depression slide. (The algae were suspended in Dryl solution: 0.1M sodium citrate, 20 mls; 0.1M NaH_2PO4, 10 mls; 0.1M Na_2HPO_4, 10 mls; 0.1M $CaCl_2$, 15 mls; dist. aqu. 945 mls). After 24 hours the mixture was transferred to a test-tube together with an equal volume of bacterised culture fluid. The tubes were examined under a binocular microscope six days later. In most cases a mixture of infected, green, individuals and uninfected, white individuals could be seen. If the number of algae per infected individual was low, e.g. in some combinations involving free-living algae, and in all combinations involving Scenedesmus or the yeast, the individuals suspected to be infected were washed into fresh bacterised culture fluid and left for two hours, removed, and washed into fresh fluid, where they were left for a further two hours before being examined under the high power of the microscope. This

procedure ensured that algae or yeasts that had been ingested and were lying in food vacuoles would be expelled before the examination, and would not be mistaken for symbiotic organisms. Control experiments, in which white stocks were allowed to ingest algae, and were then immediately submitted to the washing process, demonstrated that it was effective.

In later experiments a rough estimate was made of the proportion of paramecia infected after six days. This was scored as 'high' if few or not white individuals could be seen, 'low' if the number of green individuals was smaller than about 20, and 'medium' for intermediate conditions. (L, M and H in Table 3).

RESULTS

1. Infection with Chlorella

Nine stocks belonging to syngen 7 were tested for infection by the strains of Chlorella shown in Tables 2 and 3. The only combinations which failed to yield infected individuals are those between P. bursaria stock M1 and algae of strains CA1, CO4, P3 and M1 (Table 2). But, significantly, paramecia of stock M1 could not be reinfected with algae derived from stock M1 itself. It was therefore concluded that the failures were due to the low infectability of the stock M1, rather than to its incompatibility with some strains of Chlorella.

The combinations carried out with stocks of P. bursaria of syngens 4, 1 and 6 are shown in Table 3. The only negative

Stocks of paramecia	Strains of Chlorellae										
	Ex-paramecia									Free-living	
	Syngen 7							4	1		
	CO4	BA6	BL	LU	M1	BA13	P3	CA1	Sd	Gr-B	IU20
CO4	+	+	+	+	+	+	+	+	+	F	F
BA6	+	+	+	+	+	+	+	+	+	F	F
BL	+	+	+	+	+	+	+	+	+	F	F
LU	+	+	+	+	+	+	+	+	+	F	F
MI	-				-	+	-	-	+	F	F

TABLE 2: Infection of five stocks of syngen 7 of P. bursaria with symbiotic strains of Chlorella sp. from syngens 7, 4 and 1 of P. bursaria, and with free-living strains

Symbols used in Tables 2 and 3: +, infected, but proportion of paramecia infected not noted: -, no infection: F, infection, few algae per paramecium: H, M, L, high, medium and low proportion of paramecia infected (for method of scoring see text: blank, combination not tested.

Paramecia		Chlorella								Scenedesmus	Yeast	
		Ex-paramecia					Free-living				Control	Yeast
		CO4	7 BL	4 CA1	1 Sd	6 HW	LA25	Gr-B	IU20			
Syngen	Stock											
7	SM1	H	H	H	H	M	H	F	F	L	-	H
	SM2	H	M	H	L	H	M	F	F	L	-	H
	SM9	H	H	H	H	H	H	F	F	L	-	H
4	TV	H	H	H	L	L	H	F	F		-	H
	WL				H		M			L		
	P	H	H	M	M	H	M	F	F	L	-	H
	BL16	H	H	H	H	H	M	F	F		+	
	H4a	H	H	H	M	H	M	F	F		-	H
	H6c	H	H	H	M	H	M	F	F		-	H
	H	H	H	H	H	H	H	F	F		-	H
6	W	M			L	M	L	-	-			
	HW	H	H	M	L	M	L	-	-	L	-	H
	Pt	H	H	H	L	H	M	-	-		+	
1	3	H	H	M		H	M	F			-	H
	8	M	M	M		M	L	F			-	H

TABLE 3. Infection of stocks of P. bursaria belonging to syngen 7, 4, 1 and 6 with symbiotic strains of Chlorella sp. from P. bursaria of all four syngens, with free-living strains of Chlorella sp., with the green alga Scenedesmus sp., and with a yeast.

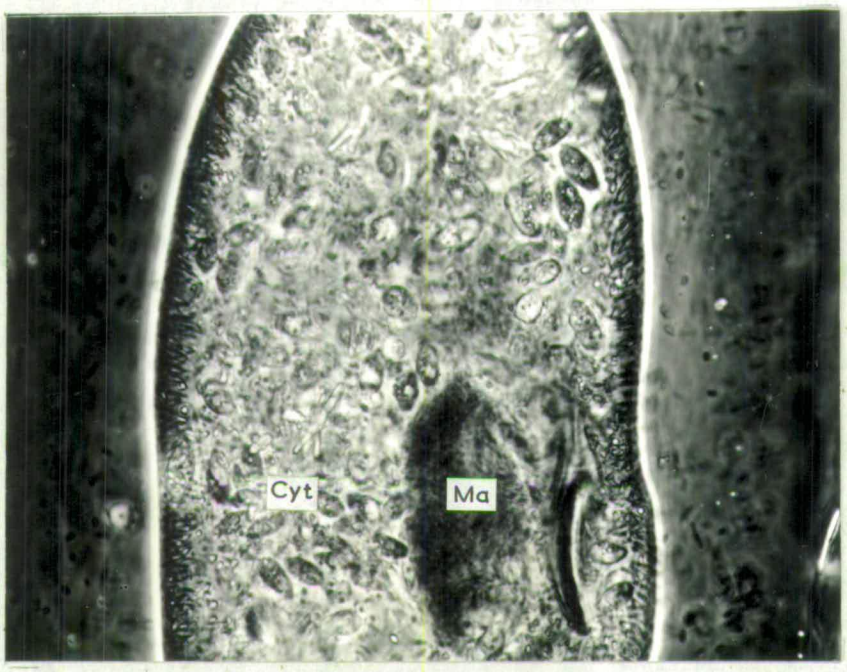


FIGURE 3: An individual of P.bursaria whose cytoplasm (Cyt) contains cells of Scenedesmus. These are spindle shaped, in contrast to the circular chlorella (Fig.1). Ma, nacronucleus of Paramecium. Phase contrast x 700.

combinations are those between the three stocks belonging to syngen 6 - W, HW, and Pt - and the free-living strains of algae Gr-B and IU20. It will be noted that these strains of algae grew relatively badly, producing small populations per cell, in all the other stocks of paramecium which they infected. This fact was noticed for algal strain IU20 by Siegel and Karakashian (1959) with their stocks of P. bursaria.

Attempts were made to remove the Chlorellae from several other stocks of syngen 6, in the hope of obtaining a stock that could be infected with the algae Gr-B and IU20, but paramecia of this syngen grew very badly in the dark, and the attempts failed.

2. Infection with Scenedesmus sp.

As will be seen from Table 3, all the stocks of P. bursaria exposed to Scenedesmus became infected. Up to 400 algae per cell were recorded (Fig. 3). Cultures of paramecia infected with Scenedesmus could survive without the addition of bacterized culture fluid for several months; such cultures contain large numbers of free Scenedesmus cells, which might provide a source of food.

The properties of paramecia infected was always very low, and some of the combinations had to be repeated many times before a single infected individual was recovered.

It was much easier to remove symbiotic Scenedesmus than Chlorella, six days rapid growth in the light being sufficient, compared to two to three weeks in the dark for Chlorella. As an initial population of Scenedesmus of about 400 is eliminated after nine divisions of the paramecium the algae cannot be increasing in numbers, or only very slowly, under these conditions. However Scenedesmus cells in the process of division can be seen in the cytoplasm of infected paramecia which have been growing slowly in tubes. This fact, and the low frequency of infection mentioned above, suggests that Scenedesmus can multiply in the cytoplasm of P. bursaria under appropriate conditions, and that the high populations of algae observed in some paramecia are not merely the results of algal accumulations.

When paramecia containing Scenedesmus are infected with Chlorella a mixed population of algae is at first produced, but later the Scenedesmus is eliminated. Table 4 shows some data from an experiment with P. bursaria stock SM 9 and Chlorellae derived from stock BL; it was known that this alga infects a high proportion of paramecia of stock SM 9. The technique of infection was the same as described above except that exactly 40 paramecia were placed in the control and experimental tubes, which were filled with exhausted culture fluid rather than bacterized culture fluid, and kept in a lighted chamber at 20°C. These modifications were

		Numbers of Algae			
		Control		+ Chlorella	
		Scenedesmus	Chlorella	Scenedesmus	Chlorella
Day after infection	0	50-150	0	50-150	0
	3	50-150	0	50-150	10-20
	6	50-150	0	0	100
	11	50-150	0	0	100

TABLE 4: Super-infection of P. bursaria stock SM9 carrying Scenedesmus with Chlorella. Ten individuals were examined under high power to provide an estimate of the numbers of algae.

introduced to keep the fission rate of the controls as low as possible, so that large numbers of Scenedesmus would be maintained. Ten individuals were removed from each tube at the intervals shown in Table 4 and, after being treated as described above to remove ingested algae, were examined under the high power of the microscope and the numbers of symbiotic algae counted.

At the end of the experiment (day 11) 0.1 ml of fluid was withdrawn from the tubes, and the numbers of paramecia counted. The totals were 32 for the Chlorella-infected culture and 2 for the Scenedesmus-inflected culture. It was concluded that infection with Chlorella had raised the fission rate of the paramecia and that this increase is the cause of the elimination of the Scenedesmus, rather than any effect directly associated with Chlorella.

Paramecia with a full complement of Chlorellae continue to ingest Scenedesmus when this is present in the culture, 20 to 50 cells being seen if the paramecia are examined before the 'washing' process.

3. Infection with yeast

The stocks infected with the yeast are listed in Table 3. In two cases, BL16 and Pt, it will be noticed that the controls were found to be already infected, presumably as a consequence of contamination in the stock

cultures. In all combinations 100% of the paramecia examined (the high power of the microscope was necessary to detect the presence of the yeast) were found to be infected, with between 100 and 300 yeast cells per paramecium. The yeast cells could be seen to be budding. Cultures of yeast-infected paramecia required to be fed regularly with bacterized culture fluid even when the yeast was growing freely in the culture. The yeast could not be eliminated from the paramecia by rapid growth in the dark, but was lost if the paramecia were subsequently infected with Chlorella and left unfed for several weeks. This would account for the absence of yeast endosymbionts in individuals collected from the wild.

DISCUSSION

The cross-infection experiments described above have not provided any evidence that the algae maintained by different stocks of *P. bursaria* depend for their survival on the presence of specific host genes; and so the initial conditions for a 'metagon-type' analysis have not been fulfilled.

The lack of specificity between host and symbiont demands an explanation. It is of course possible that the symbiosis has been in existence for too short a time for specificities to have been developed; but it seems more

likely that time is not the limiting factor, and that specificities have not appeared because they are disadvantageou

All the work that has been done on the physiology of the relationship (Parker, 1926, Pringsheim 1928, Karakashian 1963) has shown that the algae contribute to the nutrition of the paramecia. None the less the latter have retained the habit of heterotrophy, and in the presence of abundant bacterial food can, at least in the laboratory, multiply so fast as to lose their symbionts. If individuals of P. bursaria ever do lose their symbionts in nature they would require to reestablish the symbiosis as quickly as possible afterwards. Stocks of P. bursaria which were too exacting in their demands for specific strains of Chlorella would then be at a disadvantage.

Even if complete loss of Chlorella does not occur in nature, the composition of the algal population in stocks of P. bursaria may change, as more effective symbionts are selected. A crude and artificial example of this process is provided by the replacement of Scenedesmus by Chlorella.

PART 2

MATING TYPES IN PARAMECIUM BURSARIA

This part of the thesis is divided into two sections. Section A deals solely with the classification of the stocks of P. bursaria collected in Scotland, and contains some new information about the syngens and mating types of P. bursaria

Section B is devoted to the inheritance of mating type in syngen 4 of P. bursaria

A. CLASSIFICATION OF THE SCOTTISH STOCKS

INTRODUCTION

Seven syngens of P. bursaria have been described. The details of these are set out in Table 5.

The Scottish stocks were first classified into syngens and mating types by means of the mating reactions between themselves. Three syngens could be distinguished. The mating reactions between these three syngens and marker stocks for as many as possible of the previously-described syngens were then investigated; and on the basis of the information so derived the Scottish syngens have been called syngens 4, 6 and 7.

Jennings and Opitz (1944) discovered some intervarietal mating reactions between stocks belonging to syngens 2 and 4. More examples of intervarietal

Syngen	No. of mating types discovered	Where found	Reference
1	4	N. America	Jennings 1938
		"	Siegel 1958
		China	Chen 1956
		Japan	Siegel 1963
2	8	N. America	Jennings 1938 and 1939
3	4	N. America	Jennings 1938 and 1939
4	2	U.S.S.R.	Jennings and Opitz 1944
5	1	U.S.S.R.	Jennings and Optiz 1944
6	8	Europe	Chen 1946(a) and 1963
7	5	Europe Australia	Chen 1963

TABLE 5: Summary of information about the previously-described syngens of P. bursaria.

mating reactions came to light in the course of the present work.

MATERIALS AND METHODS

1. Stocks of P. bursaria

Over a hundred stocks of P. bursaria were collected from numerous localities throughout Scotland. As these stocks were all found to belong to one of three syngens, each of which was common, there is no need to give the details of the origin of all this material. A selection of localities where stocks of each syngen were collected has already been given in Table 1.

Marker stocks of all four of the mating types of syngen 1 were sent to the author by Dr. R.W. Siegel, of all eight types of syngen 2 by Dr. M. Karakashian, and of one mating type of syngen 6 by Dr. T.T. Chen. The marker stocks for all the other syngens have been lost.

2. Method of classification of the stocks by means of the mating reaction.

This has been fully described by Jennings (1939). The principle of the method is that when paramecia of different stocks are mixed they will, under normal circumstances, only conjugate with one another if the stocks are of different mating types within the same syngen. Conjugation is preceded by a conspicuous mating reaction

which begins the moment the stocks are mixed, and during which paramecia of different mating types agglutinate together in large clumps.

The mating reaction in P. bursaria follows a diurnal rhythm (Jennings, 1939) and only takes place between about nine in the morning until four in the afternoon. The best time to perform tests is therefore about midday. The stocks must be in a semi-starved condition before they can mate.

In some of the intervarietal mating reactions the degree of agglutination between the stocks was very slight, and no pairs were subsequently formed. All the intervarietal mating tests were repeated on three separate occasions, and only those reactions which appeared at least twice in the three trials have been included in Tables 8, 9 and 10.

RESULTS

1. Mating reactions of the Scottish stocks amongst themselves.

Each of the Scottish stocks was found to belong to one of the mating types of one of three syngens; in all three of the syngens there are eight mating types. The combinations of mating types in which conjugation will occur in a syngen with eight mating types are shown in Table 6.

The number of different stocks of each of the eight mating types of the three syngens that were collected are listed in Table 7. For the purposes of Table 7 stocks were judged to be different if they were collected from different localities, or from the same locality at a different place or time. Syngen 4 is represented by more stocks than either syngens 6 or 7 only because a great many collections were made from a single locality where only syngen 4 is found.

Very weak intervarietal mating reactions could be demonstrated between some of the mating types of syngens 4 and 7 (Table 8).

2. Mating reactions of the Scottish syngens with marker stocks of the previously-described syngens.

The marker stocks of the four mating types of syngen 1 reacted with none of the Scottish stocks.

The single marker stock of syngen 6 reacted with seven of the mating types of the Scottish syngen that was subsequently called syngen 6.

The marker stocks of syngen 2 underwent intervarietal mating reactions with the Scottish syngens 4 and 7 (Tables 9 and 10). Some of these reactions were as vigorous as intervarietal mating reactions, and led to the formation of pairs. 144 pairs from six different syngen 2x4 matings,

	I	II	III	IV	V	VI	VII	VIII
I	-	+	+	+	+	+	+	+
II	+	-	+	+	+	+	+	+
III	+	+	-	+	+	+	+	+
IV	+	+	+	-	+	+	+	+
V	+	+	+	+	-	+	+	+
VI	+	+	+	+	+	-	+	+
VII	+	+	+	+	+	+	-	+
VIII	+	+	+	+	+	+	+	-

TABLE 6: Mating reactions in a syngen with eight mating types. (+, mating reaction, pairs formed. - , no reaction).

Syngen	No. of stocks of each mating type							
	I	II	III	IV	V	VI	VII	VIII
4	All > 10							
6	4	5	5	9	2	2	2	2
7	1	2	2	2	2	2	2	4

TABLE 7: Numbers of stocks which were collected of each of the eight mating types of the three Scottish syngens.

Syngen 4	SYNGEN 7							
	I	II	III	IV	V	VI	VII	VIII
I								
II	C				C			
III								
IV								
V								
VI	C				C			
VII								
VIII								

TABLE 8: Intervarietal mating reactions between syngens 4 and 7 (C, weak agglutination, no pairs formed. Blank, no reaction).

SYNGEN 4

SYNGEN 2		I	II	III	IV	V	VI	VII	VIII
I	45		C+	C+			C+	C+	
	Hem		C+	C+			C+	C+	
II	20		C+	C+			C+	C+	
III	16		C+	C+	c		C+	C+	
	B20		C+	C+	c		C+	C+	
	B32		C+	C+	C	c	C+	C+	
IV	67		C+	C+			C+	C+	
	B23		C+	C+	C	C	C+	C+	
	B30		C+	C+	C		C+	C+	
V	42								
VI	65								
VII	93								
	B29		C		C	c	C		
VIII	86								

TABLE 9: Intervarietal mating reactions between syngens 2 and 4. (c, weak agglutination. C, strong agglutination. +, pairs formed. blank, no reaction).

SYNGEN 7

SYNGEN 2		I	II	III	IV	V	VI	VII	VIII
I	45 Hem								
II	20	c							
III	16		c						
	B20		C+		c			C	C
	B32		C+		C			C	C
IV	67		C+		C			C	C+
	B23		C+		C			C	C+
	B30		C		C			C	C
V	42		C+		C+			C+	C+
VI	65								
VII	93		C+		C			C	C
	B29		C		C			C	C
VIII	86	c							

TABLE 10. Intervarietal mating reactions between syngens 2 and 7. (c, weak agglutination. C, strong agglutination. +, pairs formed. blank, no reaction).

and 60 pairs from two different syngen 2x7 matings gave rise to exconjugants that died without dividing.

DISCUSSION

1. The identity of the three Scottish syngens.

One of the Scottish syngens can confidently be called syngen 6 by reason of its mating reactions with the single marker stock of that syngen.

The remaining two Scottish syngens cannot be identified directly. The mating reactions of these two syngens with the marker stocks shows that neither of them is the same as syngen 1, 2 or 6; they could however be syngens 3, 4, 5 or 7.

Syngen 3 comprises stocks collected in a very restricted region of the United States (Jennings 1938, 1939) and so is probably not identical with any of the Scottish syngens.

The single mating type of syngen 5 was represented by a few unmatable clones (Jennings and Opitz, 1944). These could very well have been sexually immature clones of another syngen, as the period of sexual immaturity in P. bursaria may last for several years (Jennings, 1945). The status of syngen 5 is therefore dubious and it may be omitted from consideration.

Syngen 4 was established from European stocks, and one of its two mating types reacted with four of the mating types of syngen 2 (Jennings and Opitz, 1944). From Tables 9 and 10 it may be seen that, considering strong reactions alone, in each case four of the mating types of the Scottish syngen react with four of the mating types of syngen 2. Therefore on the grounds of geographical distribution and intervarietal mating reactions it is almost certain that one of the two Scottish syngens is syngen 4; and one of them has been so named. The choice was arbitrary. The fact that the original syngen 4 contained only two mating types is unimportant as it was established on the basis of very few stocks.

The only question that now remains is whether the last Scottish syngen should be called syngen 7. Chen (1963) does not mention that his syngen 7 showed any intervarietal mating reactions with syngen 2, but as he speaks of "a possible seventh syngen" there must be some doubt as to whether he carried out all the necessary tests. As the last Scottish syngen was definitely different from syngens 1 to 6 it was decided to call it syngen 7 rather than to create a new syngen.

It is noteworthy that, if the identity of two of the Scottish syngens with syngens 4 and 7 is accepted, all the syngens of P. bursaria with the exception of syngen 5 have now been shown to contain four or eight mating types. The reason why the number of mating types in the syngens of P. bursaria would be expected to follow the series $(2)^n$ will

become clear after the account of the genetics of the mating types in syngen 4 in section B.

Lastly the question of whether all the mating types of the three Scottish syngens have been discovered may be considered. The data of Table 7 show that all but one of the mating types so far identified are represented by more than one stock. As the next expected number of mating types above eight in a syngen is sixteen, it seems probable that all the mating types have been identified, although it is conceivable that another series of eight mating types could exist at very low frequency in one or more of the syngens.

2. The intervarietal mating reactions.

Intervarietal mating reactions have been discovered between the syngens of a number of species of Paramecium (for review, see Sonneborn, 1957). Such reactions are often weaker than intravarietal reactions and, if pairs are formed, the progeny are always inviable in the F2 generation if not in the F1. Therefore, as pointed out by Sonneborn, intervarietal mating reactions do not break down the genetic isolation between syngens.

It is interesting that some of the mating reactions between syngens 2 and syngens 4 and 7 are vigorous (Tables 9 and 10), whereas those between syngens 4 and 7 themselves are very weak (Table 8). This is perhaps connected to the fact

that syngens 4 and 7 are sympatric, both both geographically isolated from syngen 2.

The intervarietal mating reactions provide some information about the mode of action of the mating type genes. This matter will be taken up again in section B.

B. THE INHERITANCE OF MATING TYPE IN SYNGEN 4 OF PARAMECIUM BURSARIA

INTRODUCTION

1. The background to the present work.

Because a knowledge of the inheritance of mating type is essential for genetical work on Ciliates, the genetics of the mating types have been studied in a large number of species (see Kimball, 1964 for references). The systems of mating type inheritance in different species, and in different syngens of the same species, have been revealed to be very diverse, but they may be divided into two categories - the caryonidal and the genetic.

In the caryonidal system, characteristic of some of the syngens of P. aurelia (Beale, 1954) and syngen I of Tetrahymena pyriformis (Nanney and Caughey, 1953), the genotype of an individual specifies the range of different mating types that may potentially be expressed. The actual mating type of an individual is determined after conjugation by a differentiation of the developing macronuclear anlagen. Since, in the species mentioned above, there are two macronuclear anlagen in each exconjugant, and these become differentiated independently of one another, a single exconjugant clone may

give rise to two caryonides (or clones whose macronuclei are derived from the same macronuclear anlage) of different mating types. The range of types that can be developed after conjugation may differ amongst stocks, and crosses between such stocks show that these differences are controlled by mendelian genes. (Butzel,1955; Caughey and Tefankjian, 1955).

The genetic system of mating type inheritance operates in syngen 1 of P. bursaria (Siegel and Larison, 1960; Siegel, 1963), syngen 8 of I. pyriformis (Orias, 1963), and in Euplotes vannus (Heckmann, 1963) and E. crassus (Heckmann, 1964). In all these cases the mating type of an exconjugant is determined directly and uniquely by its genotype. Thus all the descendents of an exconjugant will be of the same mating type and, if the two gamete nuclei of a conjugant are derived from a single product of meiosis, the two clones developed from the conjugants of a pair will also have the same mating type.

The genetic system of mating type determination can be illustrated in more detail by describing the results of Siegel and Larison (1960) on syngen 1 of P. bursaria. They found the four mating types of this syngen to be controlled at two unlinked gene loci, the A and B loci; there being two alleles, one dominant and one recessive, at each locus. The mating type of an individual is thus determined by the genes it carries in the following manner:- mating I, A- B-: type II,

aa B-; type III, aa bb: type IV, A- bb. (The symbols A- and B- here represent the genotypes AA or Aa, and BB or Bb, respectively.

The first task of an investigation into the inheritance of mating type in new material is to determine whether the genetic or caryonidal system is operating. The results of the crosses that have been performed between stocks of the eight mating types of syngen 4 will indicate that in this syngen the system is genetic; and that, in principle, it is similar to the situation in syngen 1 of P. bursaria. There are three gene loci, the A, B and C loci, with two alleles, one dominant and one recessive, at each locus. The genotypes of the eight mating types are:- A- B- C-, type VIII: A- B- cc, type VII: A- bb C-, type V: aa B- C-, type I ; A- bb cc, type VI: aa B- cc, type III: aa bb C-, type IV, aa bb cc, type II.

The interesting and novel part of the work on syngen 4 is the discovery of a means of changing the mating type of an individual by means other than segregation amongst the mating type genes at conjugation. Some unusual clones of mating type II were collected. When these clones are mated to clones of other mating types the pairs which are formed fall apart prematurely before gamete nuclei have been exchanged. The 'separating' type II clones will be referred to as type IIS.

After forming a temporary pair with a type IIS individual

a normal paramecium frequently grows up into a clone which has become type IIS itself: i.e., it has been transformed in two ways; with respect to its mating type and to its ability to undergo normal conjugation. This phenomenon will be called mating type transformation.

Non-conjugating clones, which agglutinate with clones of other mating types and may form temporary pairs with them, have previously been reported in Paramecium caudatum (Gilman, 1941), P. bursaria (Jennings, 1944; Chen, 1946b; Ehret, 1948) and P. aurelia (Sonneborn, 1942; Metz and Foley, 1949). None of these authors seem to have noticed whether the non-conjugating phenotype could be transmitted to normal paramecia.

The rest of the results to be described below are concerned with the experimental investigation of mating type transformation.

2. The cytogenetics of P. bursaria

(i) The prezygotic and postzygotic nuclear divisions.

The nuclear divisions that take place during and after conjugation in P. bursaria have been studied by Hamburger, (1904, cited in Chen, 1946d), Chen (1940a, b; 1946a, b), Wichterman (1953) and Ehret and Powers (1955). All these authors are agreed on the details of the prezygotic divisions. The micronucleus of each conjugant divides three times. One product of each of the first two divisions degenerates. One of the two resulting nuclei becomes the stationary gamete nucleus, the other the migratory gamete nucleus. The migratory

nucleus of each conjugant then passes into its partner and fuses with the stationary nucleus remaining there to form the zygote nucleus.

The first two prezygotic divisions are believed to be meiotic, the third mitotic. The cytological evidence for this is not altogether conclusive, as Ciliates are notoriously intractable material for chromosome studies. However at the onset of the prophase of the first prezygotic division the micronucleus becomes extended in the so-called 'crescent', characteristic of all species of Paramecium, and it is during this stage that homologous chromosomes are believed to pair (Jones, 1956).

Siegel (1963) has pointed out that the mating types in syngen 1 of P. bursaria are inherited as if the two gametes nuclei of a conjugant are isogenic, and hence originate by mitotic division from one of the products of meiosis. The frequencies of the mating types amongst the progeny from crosses are in accordance with mendelian ratios (Siegel and Larison, 1960) which means that the mating types are directly controlled by nuclear genes. Furthermore in the vast majority of cases the two exconjugant clones from the same pair are of the same mating type, and so must have the same genotype. This could only have come about if the two gamete nuclei in each conjugant were isogenic.

Two different accounts have been given of the postzygotic

nuclear divisions. Chen (1950a) and Wichterman (1953) maintain that the zygote nucleus divides three times, one product of the first division degenerating. Of the resulting four nuclei, two become macronuclei and two micronuclei. On the other hand Hamburger (1904, cited in Chen, 1946d) and Ehret and Powers (1955) claim that in their material only two postzygotic nuclear divisions take place, yielding four products directly. It seems most reasonable to assume that both these accounts are accurate, and that different stocks of P. bursaria may vary in this respect. Similar differences in the details of the postzygotic nuclear divisions have been reported amongst stocks of a single syngen of P. aurelia (Jones, 1956). They do not affect the genetic consequences of conjugation.

No cytological observations on the process of conjugation in the stocks of syngen 4 used for this work have been made. Individuals of all of the stocks were however examined under a phase contrast microscope to check that they possessed micronuclei.

(ii) Polyploidy in P. bursaria.

Chen (1940c, d) reported differences in chromosome number between stocks of the same syngen of P. bursaria. The diploid numbers ranged from 80 to several hundred. Chen proposed that the chromosome number may be raised by anomalies at conjugation leading to the fusion of more than two gamete

nuclei in one conjugant, and lowered as a result of conjugations involving amicronucleate individuals. As pointed out by Kimball (1943), polyploidy would be expected to alter the outcome of crosses, but no results which require the assumption that the parental stocks were polyploid have yet been obtained from genetic work on P. bursaria.

(iii) Autogamy in P. bursaria

In P. aurelia the micronuclei of unpaired individuals regularly pass through meiosis, the two gamete nuclei so formed fusing with each other (for details see Beale, 1954). This process of self fertilisation is known as autogamy.

Erdmann (1927) observed uniparental nuclear reorganisation in her stock of P. bursaria. She called this endomixis. This term is now reserved for nuclear reorganisations which involve the replacement of the macronucleus with a division product of the micronucleus without the occurrence of meiosis (Beale, 1954, p.35). From Erdmann's account it is impossible to decide whether the process she witnessed was endomixis proper or autogamy.

Woodruff (1931) could not detect any uniparental nuclear reorganisation amongst the individuals of his stock of P. bursaria by cytological means.

Siegel (1963) has noted that if autogamy were to take place in clones heterozygous at a mating type locus, individuals of different mating types would appear in the clone (the two homozygotes), and intraclonal conjugation would follow. He has never observed this; nor has it been detected in any

stock of syngen 4.

It may be concluded that autogamy is certainly not of general occurence in P. bursaria.

MATERIALS AND METHODS

1. Genetics of the mating types

The stocks of syngen 4 of P. bursaria used in the crosses were all collected from two habitats: Blackford Pond, Edinburgh and a small loch near Penicuik, Midlothian. Stocks from these two localities are designated by the initial letters 'BL' and 'P' respectively.

Crosses were carried out at room remperature by mixing a few drops of clones of different mating types, and removing the pairs that had formed into bacterised culture fluid in the evening after the unpaired paramecia had ceased to clump. As in the other syngens of P. bursaria the pairs remain united for about 30 hours, and the exconjugants do not divide for at least 36 hours after their separation (Jennings, 1939). These facts provided an opportunity to check that proper conjugation was taking place. The isolated pairs were examined the next morning, and any that had separated discarded. Two days later the two exconjugants, having separated, were isolated, and matings in which the separated individuals had already divided were again discarded. The exconjugants were examined after three more days and those that had by that time

passed through two divisions were scored as viable. Unless otherwise stated, both the exconjugant clones from a pair were grown to maturity.

This was accomplished by culturing the exconjugant clones in test tubes at room temperature about six inches from two 80 watt daylight fluorescent strip lights. The test tubes were kept about half full, and every two days most of the fluid was poured out and replaced with fresh bacterised culture fluid. It is probable that faster growth would have been obtained by growing the clones as daily isolation lines in depression slides in a heated incubator, but the risk of accidental mixing of the clones under these conditions is much greater. In the tubes the exconjugant clones entered sexual maturity any time after about four weeks from the day of mating, which agrees with the estimate of about 50 to 100 fissions needed to reach sexual maturity in syngen 1 (Siegel and Larison, 1960). The onset of sexual maturity was detected by removing samples from the first six clones of the cross at weekly intervals, allowing them to starve down, and testing their reactivity with all eight mating types. When the samples reacted satisfactorily the rest of the clones were left to starve down in their tubes and tested.

2. Mating type transformation.

The original stocks of type IIS were collected from Blackford Pond. Individuals of type IIS comprise about 50% of the population in this habitat.

Mating type transformation was brought about by allowing

individuals of IIS or other transformed stocks to form temporary pairs with individuals of normal stocks. Since it was necessary to be able to recognise the normal paramecium after it had separated from the temporary pair, the transformed clones were marked by the removal of their algae. The pairs formed in mixtures of transformed and normal paramecia always consisted of one green and one white individual.

Alga-free stocks of P. bursaria are difficult to maintain in mating condition. The procedure used to elicit a mating reaction from these stocks was as follows. In the evening a few drops of a culture of the normal stocks were mixed with a few drops of a white transformed stock, and a few drops of bacterised culture fluid were added. The following morning a vigorous mating reaction was usually observed, and pairs were isolated into exhausted culture fluid. The pairs remained united for about six hours, and after they had separated the green individuals were transferred into bacterised culture fluid and grown up into test-tube cultures which were tested for mating type.

The experimental investigation into mating type transformation involved the following procedures:-

Cytological Observations: Paramecia separated from temporary pairs were caused to adhere to a coverslip covered with albumen by the method described by Wichterman (1953), and then

stained with the Feulgen reagent in the usual way.

Enzyme treatment of transformed paramecia: DNAase and RNAase were dissolved in Dryl solution at a concentration of twice that required for the treatment. An equal volume of the enzyme solution was then added to culture fluid containing several hundred paramecia.

Acridine Orange treatment: The dye (acridine orange 792, Hopkins and Williams Ltd.,) was dissolved in bacterised culture fluid, which was then used to feed tube cultures of paramecia. The pH of the cultures was between 7.2 and 7.6.

Ultra-violet and X-ray irradiation: For UV irradiation small petri dishes were filled to a depth of about 1 mm with dense cultures of paramecia containing about 400 individuals per ml. The source of UV light was an Hanovia (772/64) 15 watt discharge tube.

For X-ray irradiation a few drops of dense cultures of paramecia were placed in depression slides. The source of X-rays was a Newton Victor Raymax 140 Industrial X-ray unit.

Preparation of breis of type IIS paramecia: Two litre cultures of sexually-reactive type IIS paramecia were concentrated in an oil-testing centrifuge, and the paramecia were then disrupted either by freeze-thawing or by repeated expulsion through a syringe. 0.5 mgs/ml of bentonite, an

inhibitor of RNAase (see Gibson and Beale, 1963) were added to the brei. Aliquots of the brei were then added to tube cultures of sexually reactive normal paramecia.

RESULTS

1. The genetics of the mating types.

(i) The results of the crosses.

Tables 11 and 12 show the numbers of synclones (i.e. the two exconjugant clones from the same pair) that were of each mating type from fifteen crosses between stocks of syngen 4. The exceptional synclones listed at the right of Tables 11 and 12 are those in which the two members were not of the same mating type.

In Table 13 an interpretation of these results according to the 3-locus scheme mentioned in the Introduction is presented. The eight mating types are supposed to be controlled at three unlinked gene loci, A, B and C, with two alleles, one dominant and one recessive, at each locus. The following genotypes have been assigned to the mating types:

MATING TYPE	I	II	III	IV
GENOTYPE	aa B- C-	aa bb cc	aa B- cc	aa bb C-
	V	VI	VII	VIII
	A- bb C-	A- bb cc	A- B- cc	A- B- C-

The crosses of Table 11 are between stocks of mating type II (aa bb cc) and stocks of each of the seven other mating

Cross No.	Designations and mating types of parents	No. of progeny synclones of each mating type								Exceptional Synclones	Total	Viability %
		I	II	III	IV	V	VI	VII	VIII			
1(a)		2		14	15						31	43
(b)	(PGxPK)^{X}(I) x PL(II)	2		33	34						67	46
(c)		4			5						9	-
2	PJ (III) "		9	10							19	26
3	(PGxPK)(IV) "				64					1	65	90
4	(PGxPK) (V) "				18	18					36	52
5	BL34 (VI) "		31				38			1	70	64
6	PS (VII) "			11				13		1	25	42
7(a)			8				19		1		28	39
(b)	BL17 (VIII) "		6				4		2		12	20
(c)		9	14				36		12		71	49
8	PG (VIII) x BL31(II)	2		8	11	8		6	1		36	34

TABLE 11: The results of crosses between stocks of mating type II (genotype aa bb cc) of syngen 4 P.bursaria and the other seven mating types.

X (PGxPK) signifies on F1 clone from cross No.12, Table 12.

Cross No.	Designations and Mating Types of Parents	No. of Progeny Synclones of Each Mating Type								Exceptional Synclones	Total	Via-bili %
		I	II	III	IV	V	VI	VII	VII			
9	(PGxPK)(IV) x PJ (III)	37			27						64	67
10	BL34(VI) x PJ (III)		12	12			15	14		5	58	34
11	PS(VII) x PK (V)	15							40		55	38
12	PG(VIII) x PK (V)	2			7	23			19		51	73
13	PY(IV) x PK (V)				4	1					5	7
14	(PGxPK)(I) x (PGxPK) (IV)	6			5						11	11
15	BL34(VI) x BL44 (IV)		1		1	1	1				4	5

TABLE 12: More results of crosses between stocks of syngen 4, P.bursaria

Cross No.	Genotypes of parents	Loci Segregating	Expected ratios of progeny classes	χ^2	P
1	aa Bb Cc (I) x aa bb cc (II)	B,C	1 I : 1 II : 1 III : 1 IV	86.09	.001
2	aa Bb cc (III) x "	B	1 II : 1 III	0.03	.75-.90
3	aa bb CC (IV) x "	-	All IV	-	-
4	Aa bb CC (V) x "	A	1 IV : 1 V	0.00	1
5	Aa bb cc (VI) x "	A	1 II : 1 VI	0.71	.25-.50
6	Aa BB cc (VII) x "	A	1 III : 1 VII	0.17	.50-.75
7	Aa Bb Cc (VIII) x "	A,B,C	All 8 types in equality	58.60	.001
8	Aa Bb Cc (VIII) x "	A,B,C		26.75	.001
9	aa bb CC (IV) x aa Bb cc (III)	B	1 I : 1 IV	1.60	.10-.25
10	Aa bb cc (VI) x "	A,B	1 II : 1 III : 1 VI : 1 VII	0.51	.90-.95
11	Aa BB cc (VII) x Aa bb CC (V)	A	1 I : 3 VIII	0.15	.50-.75
12	Aa Bb Cc (VIII) x "	A,B	1 I : 1 IV : 3 V : 3 VIII	3.83	.25-.50
13	aa bb C- (IV) x "	A	1 IV : 1 V	-	-
14	aa bb CC (IV) x aa Bb Cc (I)	B	1 I : 1 IV	-	-
15	Aa bb cc (VI) x aa bb Cc (IV)	A,C	1 II : 1 IV : 1 V : 1 VI	-	-

TABLE 13: The interpretation of the results of the crosses of tables 11 and 12 according to the 3-locus scheme.

types; and the crosses of Table 12 are miscellaneous.

The expected ratios of progeny classes given in column 4 of Table 13 were worked out on the assumption that the three gene loci are unlinked. The chi-square values listed in the next column of Table 13 were calculated using expected values based on these ratios. They indicate that in three of the crosses (1, 7 and 8) the observed results differ highly significantly from the expected. In these three crosses alone segregation is taking place at both the B and C loci of one parent simultaneously (column 3 of Table 13). This suggests that the deviation from expectancy is due to linkage between the B and C loci. This matter will be dealt with more fully in the final discussion.

(ii) Exceptional synclones.

The details of the synclones the two members of which did not have the same mating type are set out in Table 14. The possible explanations for these exceptions will be discussed below.

The type II exconjugant clone from cross 6 was unusual in a further way in that, although it entered into vigorous mating reactions with clones of other mating types, no pairs were formed in the mixtures.

(iii) Viability of the exconjugants.

For some crosses a record was kept of the number of synclones only one member of which survived - Table 15. The

Cross No.	Genotypes of Parents	Mating types of two members of Exceptional Synclones	No. of Synclones of each type
3	aa bb CC (IV) x aa bb cc (II)	II - IV	1
5	Aa bb cc (VI) x "	II - VI	1
6	Aa BB cc (VII) x "	II - VII	1
10	aa Bb cc (III) x Aa bb cc (VI)	II - III	4
		VI - VII	1

TABLE 14: Details of exceptional synclones from the crosses of Tables 11 and 12.

Cross No.	No. of Synclones of which both, one, or neither member clones survived Upper figures observed - lower expected Both	One	Neither	χ^2	P
1(a)	32 21	14 36	26 15	27.34	<.001
5	98 91	41 69	37 23	12.86	<.01
6	30 19	11 36	31 17	35.26	<.001
7	76 62	37 65	31 17	34.01	<.001
8	37 22	23 53	48 33	26.75	<.001
10	64 79	115 85	8 23	23.23	<.001

TABLE 15: Survival of exconjugant clones.

expected values of Table 15 were calculated on the assumption that the probability of death was constant for all exconjugant clones, in which case the proportions of the three classes of synclones should be determined by the binomial distribution.

All of the observed values differ significantly from the expected. In cross 10 there is an excess of synclones one member of which survived, and a deficiency of synclones of which both or neither members survived. The exact opposite is true of all the other crosses.

The situation in cross 10 is most easily explained on the assumption that there has been a high frequency of uniparental death caused by an incompatibility between the genes of one parental stock and the cytoplasm of the other. Unfortunately it is not known if the surviving clone of the one-survivor synclones was always derived from the same parental stock as the parents were not marked in any way.

In the rest of the crosses it can be assumed that the major cause of death is the unfitness of the new genotype, which is the same in each member of a synclone.

(iv) Adolescence

This term was used by Jennings (1942) to describe the stage in the life history of exconjugant colones in syngen 1 of P. bursaria between sexual immaturity and full maturity, when they will mate with two of the mating types, but fail

to mate with the other two.

Although no systematic study has been made of this phenomenon in syngen 4 a few adolescent clones were encountered whilst growing exconjugant clones to maturity - Table 16. These results will be of some interest when discussing the mode of action of the mating type genes.

2. Mating type transformation

(i) Initial observations.

Temporary pairs between normal and type IIS paramecia remain together for about six hours; and at any time during this period the two members of the pair can be forced apart mechanically without damage. Normal pairs cannot be separated after about half an hour from the commencement of pairing.

The frequency of mating type transformation of normal paramecia after temporary pairing is usually very high. Thus in experiments in which six paramecia of each of twenty different stocks were isolated after temporary pairing 97 out of the total of 120 became transformed.

No normal stocks insusceptible to transformation have yet been encountered.

(ii) Cytology of transformation

Chen (1946b) studied the cytology of paramecia separated from temporary pairs in syngen 1, and discovered that their micronuclei became enlarged during pairing and

Adult Mating Type	Genotype	Adolescent Mating Reactions								Locus not Expressed
		I	II	III	IV	V	VI	VII	VIII	
I	aa Bb Cc	-	+	-	+	+	+	+	+	C
II	aa bb cc	+	-	+	-	+	+	+	+	C
	"	+	-	+	+	+	-	+	+	A
VI	Aa bb cc	+	+	+	+	-	-	+	+	C
VII	Aa Bb cc	+	+	-	+	+	+	-	+	A
	"	+	+	+	+	+	+	-	-	C
VIII	Aa Bb Cc	-	+	+	+	+	+	+	-	A

TABLE 16: Mating reactions of adolescent clones (+, strong agglutination, pairs formed; -, no reaction.

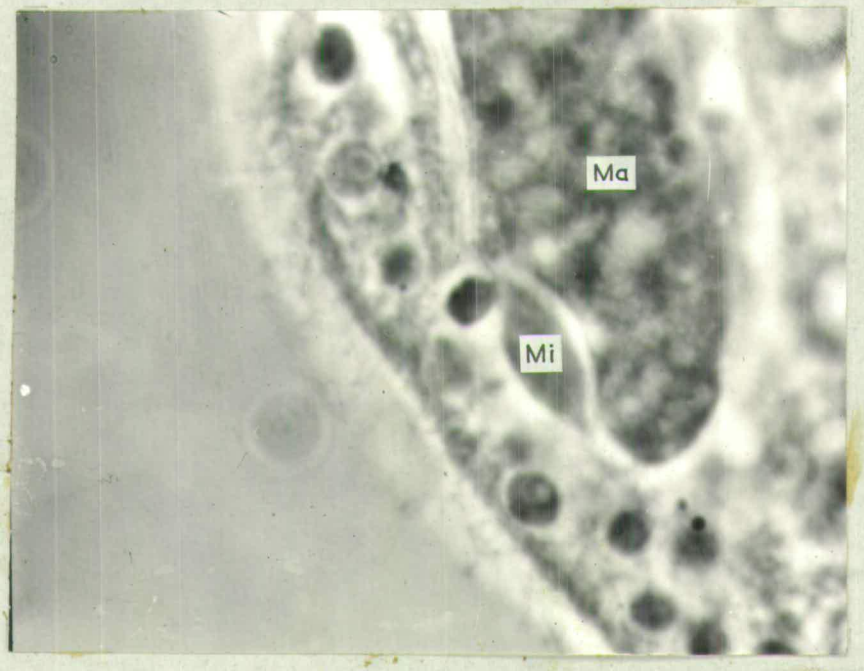


FIGURE 4: Macronucleus (Ma) and micronucleus (Mi) of P.bursaria in normal state. Feulgen stained, x 1400.

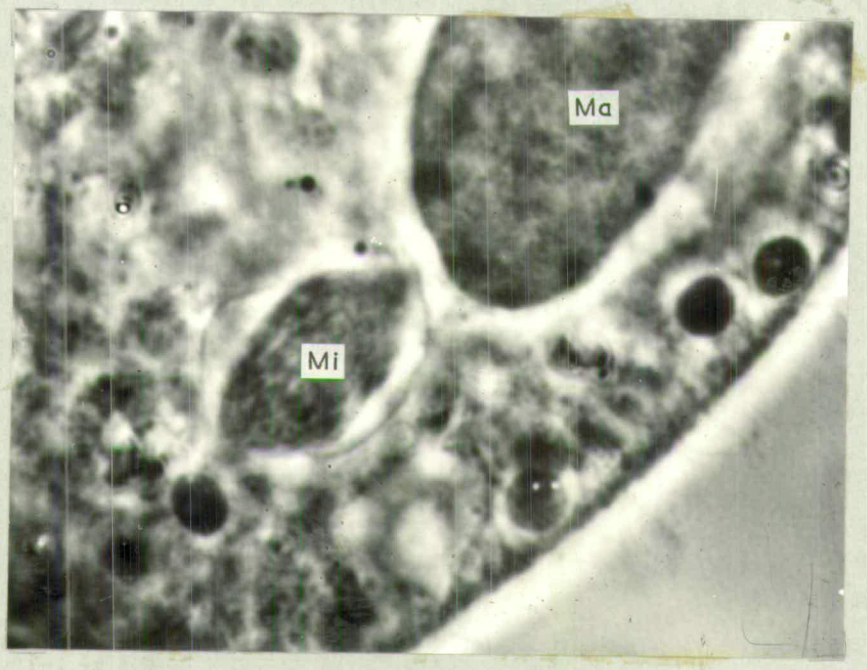


FIGURE 5: Macronucleus (Ma) and micronucleus (Mi) of P.bursaria 10 hours after temporary pair formation. Feulgen, stained, x 1400

then later returned to their normal size. The formation of gamete nuclei followed by self-fertilisation (autogamy) did not occur.

In order to discover if the same nuclear effects take place in syngen 4, paramecia separated from temporary pairs between stock BL13, mating type I, and BL5, type IIS, were stained 10 and 25 hours after the onset of mating. The paramecia were maintained in exhausted culture fluid prior to staining. The controls consisted of unpaired paramecia from the mating mixture.

The diameter of the micronucleus and the length of the macronucleus were then measured in ten paramecia of each sort with a micrometer eyepiece (Tables 17 and 18). Since only relative measurements were required the eyepiece was not calibrated.

Stock BL5, type IIS, turned out to be amicronucleate. This is not a universal characteristic of IIS stocks.

Table 17 clearly shows that temporary pairing has been followed by a swelling of the micronucleus which, under conditions of starvation, has not started to regress 25 hours after the onset of mating. It should especially be noted that there is no overlap between the micronuclear diameters recorded for the paired and unpaired paramecia.

Typical normal and enlarged micronuclei are illustrated in Figs. 4 and 5. The membrane of the enlarged

Hours After Separation	BL13 (I)		BL5 (IIS)
	Paired	Unpaired	
10	10-15 (12.0)	5-8 (6.2)	Amicronucleate
25	10-20 (13.8)	5-7 (6.0)	Amicronucleate

TABLE 17: The diameter of micronuclei (in arbitrary units) of paramecia separated from temp-:orary pairs ('paired') and of controls ('unpaired'). Ten nuclei were measured in each case; and the smallest and greatest measurements are given together with the average.

Hours After Separation	BL13(I)		BL5(IIS)	
	Paired	Unpaired	Paired	Unpaired
10	49.5	53.6	57.5	61.6
25	50.6	51.9	57.4	58.2

TABLE 18: The average lengths of the macronuclei of paramecia separated from temporary pairs and of controls. Ten nuclei were measured in each case.

Average s.e. = $\pm$ 1.7

micronucleus has the appearance of having lifted away from the chromatin.

The data of Table 18 show that the average length of the macronucleus is smaller in paramecia from temporary pairs than in unpaired paramecia for all the four possible comparisons that can be made. The average difference between the paired and unpaired classes is just significant ($p = < .05, > .02$). These data have also produced an unlooked-for result. The macronucleus of stock BL5 (IIS) is longer than that of stock BL13 (I). This difference is highly significant ($p = < .001$).

Six separated individuals of stock BL13 from this experiment were grown up into clones and tested for mating type. Three were type I, and three IIS. Thus although all the paired individuals of stock BL13 underwent micronuclear swelling, not all of them became transformed.

Some individuals from one of the transformed BL13 clones were stained and their micronuclei measured. They were the same size as those of the controls of Table 17, demonstrating that the micronuclear swelling that occurs after temporary pairing is not permanent.

The results so far presented in this section have not completely eliminated the possibility that paramecia from temporary pairs could go through autogamy, because the return of the micronucleus to its original size has not been followed. However if paramecia separated from temporary pairs are

transferred to bacterised culture fluid, rather than exhausted culture fluid, they divide without an appreciable fission lag; and the products of the first fission, examined under a phase contrast microscope, have the normal nuclear complement, without any signs of a degenerating macronucleus.

(iii) The influence of the genotype on the outcome of transformation.

Transformation in syngen 4.

The first individuals which were subjected to mating type transformation all changed to type IIS. Normal mating type II paramecia have the genotype aa bb cc. Therefore in clones which are expressing the phenotype IIS, and which were originally some other mating type, the expression of the dominant gene or genes at one or more the mating type loci is being suppressed. If a clone were a heterozygote at a mating type locus it would be easy to explain mating type transformation, in a formal way, by the repression of the dominant allele and consequent expression of the recessive allele. But what will happen if the individual to be transformed is an homozygous dominant at one or more of the mating type loci?

This question is partially answered by the data of Table 19, which shows the phenotypes expressed by all the transformed clones whose genotype at the mating type loci

Designation of clone	Mating type of clone	Genotype of clone	Type produced after transformation
(PGxPK)*	I	aa Bb Cc	IIS
(PSxPL)	III	aa Bb cc	IIS
(BL34xBL44)	IV	aa bb Cc	IIS
(PGxPK)	IV	aa bb CC	IIS
PK	V	Aa bb CC	IIS
(BL34xBL44)	VI	Aa bb cc	IIS
(PSxPL)	VII	Aa Bb cc	IIS
PS	VII	Aa BB cc	II-IIIS
BL17	VIII	Aa Bb Cc	IIS

TABLE 19: Transformation of clones of known genotype at the mating type loci.

* (PGxPK) designates an F1 clone from the cross PGxPK.

Mating type of stocks	No. of stocks tested	No. transforming to type IIS	No. transforming to type II-IIIS	Genotype
I	7	5	2	aa B- C-
III	8	7	1	aa B- cc
IV	4	4	-	aa bb C-
V	1	1	-	A- bb C-
VI	5	5	-	A- bb cc
VII	4	4	-	A- B- cc
VIII	10	7	3	A- B- C-

TABLE 20: Transformation of stocks whose exact genotype at the mating type loci has not been determined.

were known. The following facts are worthy of note:-

(a) Two clones, (PG x PK) IV and PKV, which were homozygous dominants at the C locus, transformed to type IIS.

(b) The single clone that was a homozygous dominant at the B locus, PS, transformed to a new phenotype, II-IIIS. Clones of this phenotype react and form temporary pairs with clones of all mating types except II and III.

(c) No clones known to be homozygous dominants at the A locus are yet available. Reasons for believing that clones with this genotype transform to type IIS will be given below.

Some experiments have been performed to confirm the hypothesis that all individuals which are homozygous dominants at the B locus, and only such individuals, will transform to type II-IIIS.

Stock PS, which transforms to type II-IIIS (Table 19), had been crossed to stock PL (aa bb cc) - cross 6, Table 11. The type III and type VII progeny from this cross, which must be heterozygotes at the B locus, should transform to type IIS. Three type VII and three type III clones, each from a different synclone, were transformed to check that this was the case.

A large number of individuals of different stocks of known mating type, but unknown genotype, were transformed - Table 20. It is to be expected that only stocks of mating types I, III, VII or VIII should change to type II-IIIS, as

these are the four mating types that have the genotype Bb or BB. The data of Table 20 are in agreement with this.

Stocks of types I and III, transforming to types IIS and II-IIIS, have been crossed to stock BL3[illegible] (aa bb cc). Only one member of each synclone was grown to maturity. The results of these crosses (Table 21) indicate that stocks transforming to IIS have the genotype Bb, and those transforming to type II-IIIS the genotype BB; except that in cross 5 (stock BL 58) a single type II exconjugant clone appears. This clone is probably the result of some anomaly at conjugation such as self-fertilization rather than cross-fertilization. All the crosses of Table 22 are being repeated on a larger scale, both members of each synclone being grown to maturity.

It may have been noticed that only two of the four expected progeny classes from crosses I and 2 of Table 21 appear. This is another instance of the presumed linkage between the B and C loci, and will be discussed below.

Stock BL4O (III) and stock BL1O (I), which the results of Table 21 show to be heterozygotes at the B locus, have been crossed. One quarter of the progeny, which are not yet mature, should have the genotype BB, and hence should transform to type II-IIIS.

Transformation in syngen 2.

Intervarietal mating reactions occur between four of the mating types of syngen 2 and mating type II of syngen

Cross No.	Designation and mating type of clone		Type to which clone Transforms	Mating type of exconjugant clones* I	II	III	IV	V	VI	VII	VIII	Probable Genotype of Type I or III Parent
1	BL10)		IIS	11	4							aa Bb Cc
2	BL13)	I	IIS	7	12							aa Bb Cc
3	BL18)		II-IIIS	6	7	7						aa BB Cc
4	BL40)	III	IIS		9	6						aa Bb cc
5	BL58)		II-IIIS		1	11						aa BB cc

TABLE 21: Crosses between stocks of mating types I and III, transforming to types IIS and II-IIIS, to stock BL31, mating type II (aa bb cc)

* In these crosses only one member of each synclone was grown to maturity.

Stock	Original Mating Type	Reactions with syngen 2								Reactions with syngen 4							
		I	II	III	IV	V	VI	VII	VIII	I	II	III	IV	V	VI	VII	VIII
45	I	+	+	+	+	+	+	+	-	+	-	-	+	+	-	-	+
Hern	I	+	+	+	+	+	+	+	-	+	-	-	+	+	-	-	+
20	II	+	+	+	+	+	+	+	-	+	-	-	+	+	-	-	+
16	III	+	+	+	+	+	+	-	-	+	-	-	+	+	-	-	+
67	IV	+	+	+	+	+	+	-	-	+	-	-	+	+	-	-	+

TABLE 22: Mating reactions of transformed stocks of syngen 2 with syngen 2 and 4 (+, strong agglutination, temporary pairs formed, -, no reaction).

4 (Table 9). These four types of syngen 2 can also form temporary pairs with type IIS, which permits the study of the phenomenon of mating type transformation to be extended to syngen 11.

The mating reactions of transformed stocks of syngen 2 with their own syngen and with syngen 4 are shown in Table 22. All the pairs formed in these reactions were temporary. The transformed syngen 2 stocks have changed their pattern of reactivity with their own syngen, and have become one of two types which may be called VIIIS (2) and VII-VIIIS (2). The pattern of intervarietal reactions with syngen 4 has also been changed, reactions occurring with types I, IV, V and VIII. This pattern of reactivity is exactly complementary to that displayed by the untransformed stocks of syngen 2, which react with types II, III, VI and VII (Table 9); and it is also entirely new in that none of the untransformed stocks of syngen 2 show a similar pattern of reaction.

The untransformed stock 67 of syngen 2 reacts with four of the mating types of syngen 7 (Table 10). Transformed stock 67 clones lose this ability.

Mating types V to VIII of syngen 2, with the exception of stock B29, do not react with mating type II of syngen 4 (Table 9). B29 does not form pairs in its reaction. Therefore these four types of syngen 2 cannot be transformed by type IIS. The stocks of mating types V, VI and VII could

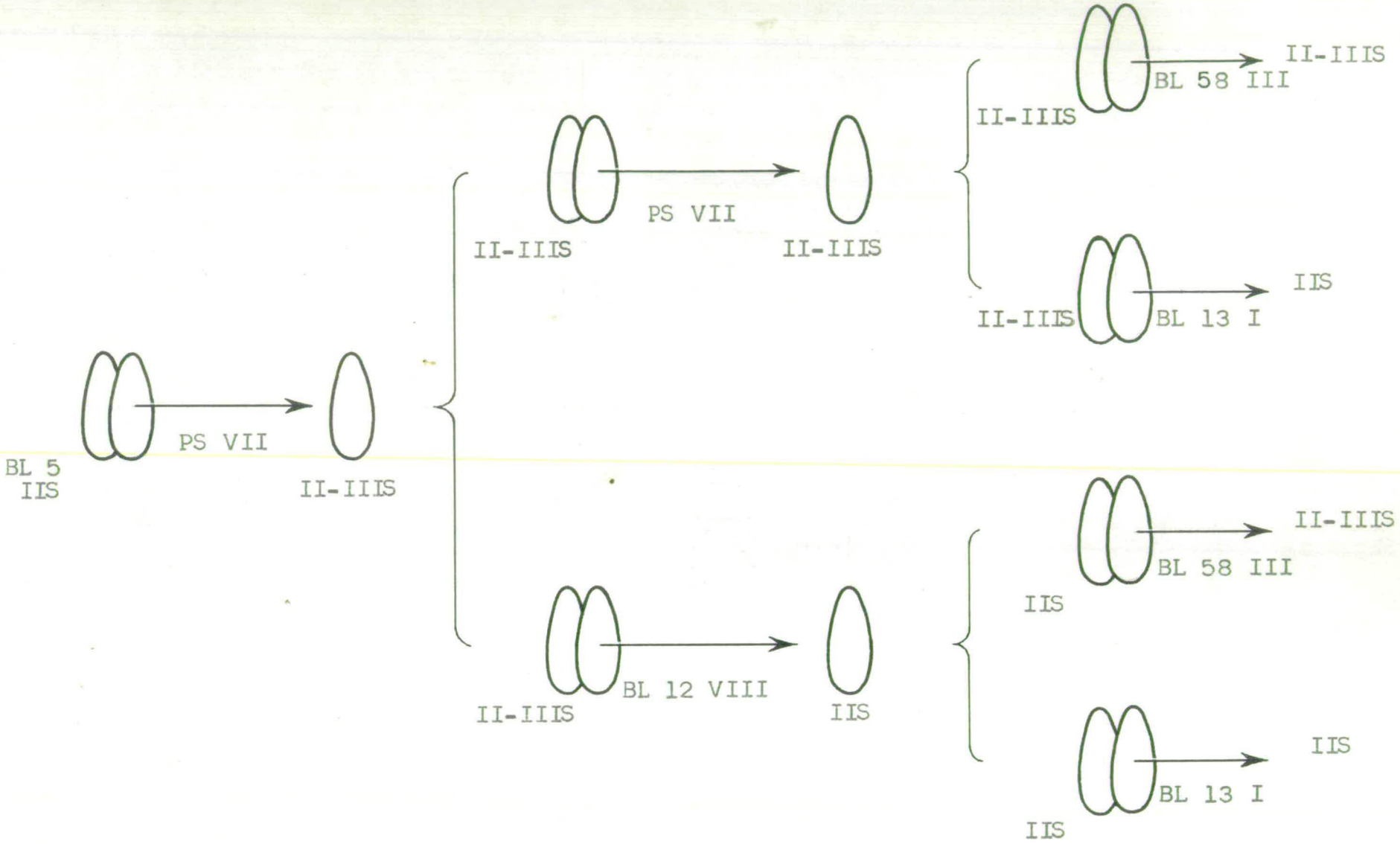


Fig.6: Experiment to show that the phenotype expressed after transformation, IIS or II-IIIS, does not depend on the phenotype of the paramecium used to effect transformation. (For explanation, see text).

be transformed by an VIIIS (2) stock. This has not yet been done.

(iv) The influence of the type of the stock used to bring about transformation on the outcome of transformation.

The experiment which is illustrated in Fig. 6 demonstrates that the phenotype of a transformed individual does not depend on the phenotype of the paramecium that was used to transform it. In this experiment a IIS clone was used to transform the stock PS to type II-IIIS. This II-IIIS clone was in turn 'mated' to stocks PS and BL12 (type VIII), the second of which was known to have been transformed to IIS by a IIS stock. PS and BL12 changed to types II-IIIS and IIS respectively. Finally these two newly-transformed clones were each allowed to transform two other stocks into types IIS and II-IIIS.

This experiment also proves that the ability to effect further transformations is possessed by newly-transformed clones.

It will be remembered from section (iii) that syngen 4 stocks can transform syngen 2 stocks. Whether the reverse is true has not yet been tested.

(v) Stability of transformation

Attempts were made to cause IIS or II-IIIS paramecia to revert to their original phenotype by a number of means. The rationale of these experiments was briefly that, if mating type transformation were the result of the transfer of a

self-replicating infective agent during temporary pair transformation, the loss or destruction of this agent in transformed paramecia should lead to a reversal of transformation.

Prolonged and rapid growth.

Three IIS clones and two II-IIIS clones, all of different origin, were grown at the maximum fission rate of about two fissions a day as daily isolation lines at 28°C, until they had passed through about 75 divisions. They all remained IIS or II-IIIS.

Treatment with chemical and physical agents.

The details of the nature, strength and duration of these treatments, which were given to stocks BL5(IIS) and transformed PS (II-IIIS), are set out in Table 23 below:-

Nature of Treatment	Concentration or Dose	Duration of Treatment
DNAase	0.5 mgs/ml	18 hours
RNAase	"	"
Acridine orange	0.5 µgms/ml	10 days
UV light	30 cms from 15 watt source	30 mins
X rays	100,000 r	

TABLE 23: Details of chemical and physical treatments given to stocks BL5 (IIS) and transformed PS (II-IIIS). See also 'Materials and Methods'

In order to detect if any changes of mating type had taken place amongst the trested paramecia they were grown up into test-tube cultures which were allowed to come into mating condition and observed for selfing (intra-clonal conjugation). This was never seen and the cultures, when tested for mating type, always reacted as IIS or II-IIIS.

A few notes about each of the treatments must be added.

DNAase and RNAase: Isolated treated paramecia showed a fission lag as compared to the controls, which had been treated with Dryl solution without any enzyme, but there was no mortality. If the concentration of RNAase was raised to 0.66 mgs/ml 100% of the paramecia died.

Acridine orange: 0.5 μ gms/ml was found to be the highest concentration of dye at which P. bursaria could continue to grow at a rate of one fission per day. The treated paramecia did not lose their symbiotic algae. Unfortunately no observations were made on their micronuclei.

UV light and X-rays: Isolated irradiated paramecia showed a fission lag but afterwards divided at the normal rate. The algae in the X-irradiated paramecia disappeared after a few days. This effect of X-irradiation has previously been noted in syngen I by Wichterman (1948). The white clones (of syngen 4) could be reinfected with algae; and they were still capable of transforming normal clones by

temporary pair formation.

(vi) Attempts to bring about mating type transformation without temporary pairing

Fluid from IIS cultures.

The addition of filtered fluid from IIS cultures to normal cultures did not cause the latter to self.

Breis of IIS paramecia.

The addition of breis of IIS paramecia (for details of preparation of breis see 'Materials and Methods') was equally ineffective. Since samples of the brei were added to dense tube cultures of normal paramecia, and the transformed phenotype, if established in a single paramecium, should gradually spread throughout the culture, a very low frequency of transformation would have been detectable.

Agglutination without pair formation.

30 paramecia from each of two different normal stocks were isolated with a micropipette into separate, very small drops of fluid, together with a single sexually-reactive type IIS paramecium. Under these conditions the two paramecia adhere to one another by the ciliary union of the mating reaction, but are unable to orientate themselves into a pair. None of the normal paramecia become transformed.

DISCUSSION

It will be helpful at the outset to give an indication of the arrangement of this discussion. The first part of section I is concerned with the application of the three-locus

hypothesis to the results of the crosses between stocks of syngen 4; and particularly with the question of the linkage between the B and C loci. The second part of section I contains an account of the peculiar features of mating type inheritance in syngen 1 of P. bursaria, which are important because they suggest that the mating type genes may not be completely stable in expression. In section 2 the physical basis of the mating reaction is discussed; and the concept of mating type substances introduced. The direct evidence for the existence of these substances will be reviewed, and also the indirect evidence that is provided by the phenomena of intervarietal reactions and adolescence. Then, in section 3, the different means whereby the mating type genes could control the synthesis of the mating type substances will be considered with reference to the known properties of the mating type genes.

Having reached some conclusions about the possible relationships between the mating type substances and mating type genes it will be possible to attempt an explanation of mating type transformation in section 4: and also to undertake a general review of mating type determination in Ciliates in section 5. Finally, section 6 contains some concluding remarks about those aspects of mating type determination in Ciliates that are of general biological significance.

I. The genetics of the mating types in P. bursaria

(i) The three-locus scheme in syngen 4.

Linkage between the B and C loci was proposed on the grounds that the results from crosses, 1, 7 and 8 of Table 11 did not agree with the expectations based on free assortment between these two loci. It will now be shown how the data from these crosses, and from crosses 1 and 2 of Table 21, can be explained by assuming that the alleles at the B and C loci are in coupling or in repulsion; and to assist the explanation the non-recombinant and recombinant progeny classes to be expected from the four relevant kinds of segregation have been set out in Table 24.

Cross 1, Table 11: Mating type 1 parent with alleles in repulsion, progeny predominantly types III and IV. In cross I(c) there is an excess of type 1 recombinants, and a deficiency of type III non-recombinants. A test for heterogeniety amongst crosses I(a), (b) and (c) gives χ^2 = 32.4 with 4 d. f., P = < .001. Most of the heterogeniety is obviously contributed by cross I(c), which leads to the suspicion that this cross was the result of an experimental error.

Cross 7, Table 11: A heterogeneity test on the three repetitions of this cross gives χ^2 = 15.34 P = < .01, > 001. It is disturbing that the only two crosses to have been repeated should both have yielded

Mating Type of Parent	State of Alleles at B and C loci	Genotype of Parent	Non-recombinant Gamete nuclei		Recombinants	
I	Coupling	$\frac{a}{a}\ \frac{B\ C}{b\ c}$	a B C	I	a B c	III
			a b c	II	a b C	IV
	Repulsion	$\frac{a}{a}\ \frac{B\ c}{b\ C}$	a B c	III	a B C	I
			a b C	IV	a b c	II
VIII	Coupling	$\frac{A}{a}\ \frac{B\ C}{b\ c}$	a B C	I	a B c	III
			a b c	II	a b C	IV
			A b c	VI	A b C	V
			A B C	VIII	A B c	VII
	Repulsion	$\frac{A}{a}\ \frac{B\ c}{b\ C}$	a B c	III	a B C	I
			a b C	IV	a b c	II
			A b C	V	A b c	VI
			A B c	VII	A B C	VII

TABLE 24: Non-recombinant and recombinant progeny classes to be expected from type I and VII parents when the alleles at the B and C loci are in coupling or repulsion.

heterogeneous results. Some of the other crosses, where the situation is not complicated by linkage, are now being carried out again.

Progeny of types I, II, VI and VIII appear in cross 7. These are the four non-recombinant classes to be expected from a type VIII parent which is heterozygous at the A locus, and in which the alleles at the B and C loci are in coupling.

However it is obvious without statistical treatment that the four progeny classes of cross 7 do not conform to a 1:1:1:1 ratio. The best explanation for this is selective mortality. The mortality rate in crosses 7(a) and (b), where the deficiency of progeny of types I and VIII is particularly marked, was higher than in cross 7(c) - column 6, Table 11. The increased mortality could have differentially affected progeny of types I and VIII. This would account for the heterogeniety amongst the crosses.

The other possible explanation is variable expression of the mating type genes due to the presence of epistatic modifying factors; it would require further crosses to deomonstrate the operation of such factors.

Cross 8, Table 11: The type VIII parent must have been a heterozygote at the A locus, with the alleles at the B and C loci in repulsion.

The recombinant type VIII clones from this cross has

been back-crossed to the type II parent. The progeny are not yet mature.

Crosses 1 and 2, Table 21: In these two crosses the alleles at the B and C loci were in coupling.

The fact that results compatible with the arrangement of the alleles at the B and C loci both in coupling and in repulsion have been obtained is strong evidence for linkage between the two loci. Data from the back-crossing of recombinants will be needed to confirm this.

Because of the provisional nature of the linkage hypothesis only a rough estimate will be made of the degree of linkage needed to account for the data. In crosses 1(a) (b) and 8 of Table 11, and crosses 1 and 2 of Table 21 there are 5 recombinants amongst 168 non-recombinants, which give a cross-over frequency of between 2 and 3 percent.

Exceptional synclones

The exceptional synclones of Table 14 could have arisen in four different ways:-

(a) Failure of conjugation. No nuclear reorganisation in either conjugant.

This could account for those synclones in which the two members are of the same mating type as the parents, i.e. those from crosses 3, 5 and 6. But since precautions which were taken to ensure that only pairs which had remained united, and exconjugants which showed a fission lag, were retained, it is unlikely that nuclear reorganisation did not occur.

(b) One-directional fertilisation. One exconjugant is fertilised properly, but the other becomes a haploid. This event could have produced exceptional synclones of all the observed types. However if it were the cause of the exceptional synclones of cross 10 it is rather unlikely that four of the exceptional synclones would be the same, i.e. with one member type II and the other type III. If unidirectional fertilisation occurred randomly with respect to the genotypes of the gamete nuclei the proportions of the different types of synclones would be:- 2II-II: I III-III: I VI-VI: I II-III: I II-VI: I VI-VII: I III-VII. The first three types would be indistinguishable from synclones produced by normal fertilisation, leaving the last four each with a probability of 1/4. Therefore the chances of obtaining four synclones of type II-III and one of type VI-VII in a sample of five are $5/(4)^5$.

(c) Cytogamy. Self-fertilisation in each conjugant. All of the exceptional synclones could have arisen through cytogamy, except for the type VI-VII synclone from cross 10, where nuclear exchange must have occurred in one direction at least to yield the type VII exconjugant. For cross 10 cytogamy should produce the following proportions of synclones:- 2 II-II: I II-VI: I II-III. The probability of obtaining four exceptional synclones of type II-III in a sample of four would be $(\frac{1}{2})^4$.

(d) Non-penetrance of dominant mating type alleles. Fertilisation is normal in the exceptional synclones, but one

or the other of the dominant mating type alleles is non-penetrant in one of the exconjugants.

This phenomenon could be the cause of all the exceptional synclones, but is especially attractive as an explanation for those of cross 10. In this cross it must be assumed that it is the dominant allele at the B locus which is non-penetrant in one of the exconjugants; this would yield the two observed types of exceptional synclone, II-III and VI-VII. It was in cross 10 that nucleo-cytoplasmic incompatibility between the conjugants was inferred from the observation of a high frequency of uni-parental death (Results, section 1 (i)).

(ii) The two-locus scheme in syngen 1.

The purpose of this section is to give an account of some instances of instability of expression of the mating type genes in syngen 1 of P. bursaria. The similarity between the genetics of the mating types in syngens 1 and 4 makes it reasonable to suppose that the mating type genes of the latter syngen could potentially be affected in the same way, although this has not yet been observed.

Jennings (1941) reported 'self-differentiation', or the appearance of individuals of another mating type in previously pure clones, in his stocks of syngens 1, 2 and 3. The phenomenon was rather rare, occurring with a frequency of about once per 2000 culture days (1 culture day is one

culture observed for one day). However, by using observations collected over a number of years, Jennings was able to make the following generalisations:-

(a) Any one stock always differentiated to one and the same derived mating type, even if differentiation took place independently on more than one occasion.

(b) Clones of the derived mating type could redifferentiate, but only back to the original mating type. Therefore any one clone alternated between only two mating types.

(c) Clones could alternate between all possible combinations of two mating types.

Jennings provides extensive evidence for generalisation (a), but rather limited evidence for (b) and (c). Thus he only observed reverse differentiation on two occasions; but the fact that reverse differentiation was possible at all rules out autogamy, and hence segregation amongst the mating type genes, as a cause of self-differentiation.

Jennings (1942) used a number of his self-differentiated clones of syngen 1 in breeding experiments. As pointed out by Siegel and Larison (1960), the results from these crosses are compatible with the idea that self-differentiation involves changes in the <u>micronuclear</u> genes. A more extensive re-interpretation of Jennings' results is presented in Table 25. The stocks are numbered 32, 33 etc., and the postscripts 'a'

Cross No.	Parental Clones	Mating types of progeny synclones I A- B-	II aa B-	III aa bb	IV A- bb	Interpretation of Cross on two-locus hypothesis
1	38(a) IV x 38b (III)	1	2	13	15	Aa bb x aa bb
2	35(a) II x 35b (III)		9	14		aa Bb x aa bb
3	" x 33b (I)*	16	17	5	1	aa Bb x Aa Bb
4	" x 32b (IV)*	24			25	aa Bb x AA bb
5	36 (III) x "			2	36	aa bb x AA bb

TABLE 25: Data on inheritance of mating type in syngen 1 P.bursaria abstracted from Jennings (1942), and interpreted according to the two-locus scheme of Siegel and Lanion (1960).

* Clones 33a and 32a were both type II (aa B-)

and 'b' refer to clones of the original and derived mating types respectively. It may be seen that it is possible to account for each of the crosses by assuming that the micronuclear genotypes of the parental clones correspond to their expressed mating types, except that in crosses 1 and 5 'forbidden' progeny classes appear; types I and II in cross 1 and type III in cross 5. The possible causes for the appearance of these clones will not be discussed here.

There are two further points to be made about Jennings' results. First, if it is accepted that the micronuclear genes change during self-differentiation, it must also be assumed that the change can take place in both directions (i.e. from a state corresponding in expression to the dominant allele to a state corresponding to the recessive allele and vice versa); and also that on occasions both loci can be affected simultaneously. Thus stock 32 differentiated from type II (aa B-) to type IV (A- bb). Secondly, the fact that the micronuclear genotype of an individual always corresponds to its expressed mating type means that, during self-differentiation, coordinated changes must be taking place in the mating type genes ofthe micronucleus and macronucleus. (Since the macronuclear genes control the phenotype of the individual, these must also be affected during self-differentiation). This implies that

some mechanism of physiological interaction between the nuclei must exist; or that the initial changes in the micronucleus are followed by a destruction of the macronucleus, which is regenerated anew from the modified micronucleus - endomixis.

Altogether the assumptions needed to account for these results of Jennings' are rather extraordinary. If his observations are accurate, and if the above interpretation of them is correct, it seems that the micronuclear genes can undergo stable heritable changes which are distinguished from what are conventionally known as mutations in that they are relatively frequent and affect certain alleles selectively (as indicated by the fact that clones always differentiated to the same derived mating type).

Lee (1949) claimed to have induced permanent changes of mating type in about 50% of X-irradiated individuals of a number of stocks of syngen 1. Paramecia from a single stock all changed to the same new mating type. Lee did not breed from his irradiated individuals, so it is unknown what was happending to the micronuclear genes. It is possible that Lee was inducing self-differentiation at a greatly increased frequency. The experiments need repeating.

Siegel (1963) has analyzed two cases of the instability of the expression of the mating type alleles in micronucleus of syngen 1. He was able by a special technique to prepare a clone of genotype AA BB (clones of

this sort do not normally occur as they would be the progeny of a A- B- x A- B- mating). In a cross between the AA BB clone and a type III clone (aa bb), two progeny clones of type IV appeared, which were shown to have the micronuclear genotype Aa bb. Siegel interprets this to mean that the dominant allele at the B locus derived from the AA BB parent has been changed or lost.

Later Siegel (1965) has discovered that the dominant allele at the B locus may be changed to a condition where it behaves as a recessive only amongst the progeny of crosses to certain particular type III clones, and has postulated the existence of a "mutator factor" for the dominant allele of the B locus in these stocks.

So far Siegel has detected modifications only at the B locus, and the changes have all been in one direction; dominant alleles 'mutate' to the recessive condition. Although the nature of the changes remain unknown, they are distinguished from what are usually known as mutations by their relatively high frequency (there are between I and 2% of exceptional clones in Siegel's 1965 results), and by the fact that they occur only amongst the progeny of certain crosses.

Siegel's observations may be of some relevance to the interpretation of the phenomenon of mating type transformation in syngen 4, which also involves changes in the expression of dominant mating type genes. This matter will be taken up again in section 4 (ii) of this disucssion.

2. The physical basis of the mating reaction in Ciliates.

Metz (1954) has proposed that the mating reaction in Ciliates is brought about by the presence of specific mating type substances on the cilia of each mating type. The substance or substances on the cilia of an individual of one mating type are supposed to be complementary to the substance or substances on individuals of different mating types, in a manner analogous to the complementarity between antigen and antibody.

Similar hypotheses of sextype-specific chemical groupings on the cell surface have been put forward to account for the cellular adhesion leading to mating in a heterothallic yeast Hensenula wingei (Brock, 1959), the bacterium Escherichia coli (Sneath and Lederberg, 1961), and Chlamydomonas (Wiese and Jones, 1963). In each of these organisms it was proved or inferred that the sex-specific substance of one sex type was a glycoprotein.

Metz (1954) pointed out that the number of mating types in different syngens of P. bursaria follows the series $(2)^n$. He suggested that there would be one pair of complementary substances in a syngen with two mating types, two pairs of substances in a syngen with four mating types, and so on. Thus in a syngen with eight mating types every type would carry one substance from each of three pairs. If the pairs of substances are designated as x_1, x_2, y_1, y_2, z_1 z_2,

the eight alternative combinations of substances may be written:- $x_2\ y_2\ z_2$, $x_2\ y_2\ z_1$, $x_2\ y_1\ z_2$, $x_1\ y_2\ z_2$, $x_2\ y_1\ z_1$, $x_1\ y_2\ z_1$, $x_1\ y_1\ z_2$, $x_1\ y_1\ z_1$. Each mating type differs from all the others by at least one substance.

It is noteworthy that Metz arrived at this interpretation of the mating reaction in P. bursaria without any knowledge of the genetics of the mating types. As soon as Siegel and Larison (1960) worked out the genetics of the mating types in syngen 1 they realized the possible correspondence between the mating type substances and mating type genes, i.e. the two alleles at a locus could control the synthesis of a pair of mating type substances. Extending this idea to syngen 4, the relation between the mating type genes and substances would be:-

Genes	A	a	B	b	C	c
Substances	x_1	x_2	y_1	y_2	z_1	z_2

A number of attempts have been made to identify and isolate mating type substances in Ciliates. None of them have entirely been successful.

Metz (1954), using the two mating types of P. aurelia and Paramecium calkinsi, discovered that if sexually reactive paramecia were killed with formalin they could still agglutinate with living paramecia of the opposite mating type. He treated formalin-killed paramecia with a variety

of agents and noted that those which are known to affect proteins caused a loss of the ability to agglutinate; one of the mating types of P. calkinsi was exposed to periodate, a test for carbohydrate surface groupings (see Brock, 1959), without affecting its reactivity. This is the only attempt so far to identify a carbohydrate component in the mating type substances of Ciliates. Metz concluded that the mating type substances have a protein component. He was not able to prepare an extract of paramecia of one mating type that would specifically block the mating reaction of paramecia of the opposite mating type; nor was he able to obtain an antiserum that would specifically inhibit the mating reaction of paramecia of the same type.

Cohen and Siegel (1963) detached cilia from sexually reactive individuals of syngen 1 of Paramecium bursaria and showed that the free cilia would adhere to individuals of different mating types. If the cilia were pre-treated with trypsin they no longer reacted. By heat treatment within a critical range it was possible to eliminate the reaction of the cilia with one of the other mating types, whilst preserving the reactions with the remaining two types. This effect could be convincingly explained by the differential destruction of one of the two mating type substances assumed to be present on the cilia of each type. By comparing the reactions of cilia from individuals of different genotypes after heat treatment it was inferred that the substance

of one of the complementary pairs are more heat sensitive than those of the second pair.

Vivier (1960), working with Paramecium caudatum, observed that some of his stocks had lost the ability to agglutinate with stocks of the opposite mating type. He was able to restore this ability by exposing the non-conjugating stocks to breis prepared from reactive cultures of the same mating type; the brei was still effective if it was filtered, but lost the power to induce reactivity if it was heated above 60°C. From this Vivier concluded that the active substance in the brei was a soluble protein, which he believed to be the mating type substance itself, which is adsorbed on to the cilia of the non-conjugating stocks. The induced reactivity is presumably temporary, although Vivier does not say if this is so; nor has he investigated whether the breis would specifically inhibit the ability to agglutinate of sexually reactive cultures of the opposite mating type. It is difficult to understand why a mating type substance should have the property of conferring mating reactivity on non-reactive paramecia of the same type.

The existence of mating type substances has been inferred from the results of experiments which involved treating paramecia about to enter a state of sexual reactivity, or which were already sexually reactive, with antibiotics known to inhibit RNA or protein synthesis (Nobili and Kotopolus, 1963; Bleyman, 1964; Cohen, 1965). These processes were essential to bring the paramecia into a state

of sexual reactivity, and sometimes necessary to maintain the state of reactivity, a conclusion that is compatible with the view that mating type substances are synthesised while the individual becomes reactive, and may continue to be synthesised thereafter.

There is one example of a soluble 'sex-substance' in Ciliates. Kimball (1942) demonstrated that the fluid from culture of Euplotes patella of one 'mating type' would induce selfing in cultures of a different mating type. The idea that these substances correspond to the ciliary mating type substances of other Ciliates is supported by the observations of Miyake (1964) who induced conjugation in Paramecium multimicronucleatum by mixing substances of one mating type with washed cilia detached from paramecia of the opposite mating type.

There are two features of the mating reaction of P. bursaria which agree with the hypothesis of the presence of more than one mating type substance on each individual.

Intervarietal mating reactions.

The significance of intervarietal reactions in P. bursaria for the interpretation of the mating reaction was first appreciated by Metz (1954) who, on the basis of the previously mentioned results of Jennings and Opitz (1944) on the reactions between syngens 2 and 4, suggested that these reactions will occur when two syngens have a pair of mating type substances in common. As an extension of this

hypothesis it can be predicted that, if the mating type genes control the synthesis of the mating type substances, the mating types of a syngen involved in an intervarietal mating reaction should have the same genotype at one of the mating type loci. This is the case for the mating types of syngen 4 which react with syngen 2. (Table 9).

Considering strong reactions alone, four of the mating types of syngen 2 (I, II, III and IV) react with four of the mating types of syngen 4 (II, III, VI and VII). The genotypes of the latter are:-

II, aa bb cc III, aa B- cc VI, A- bb cc VII, A- B- cc

i.e., they are all homozygous recessives at the C locus.

The interpretation of this intervarietal reaction is that the recessive allele at the C locus in syngen 4 controls the synthesis of a mating type substance Z_2, which is complementary to a substance Z_1 whose synthesis is controlled by the dominant allele at a C locus in syngen 2.

It is curious that the remaining four types of syngen 4 (I, IV, V, and VIII) do not react with the other four types of syngen 2 (V, VI, VII and VIII); presumably the substance z_1 of syngen 4 is not complementary to the substance z_2 of syngen 2.

Some tentative remarks can be made about the weak inter-:varietal mating reactions between syngens 2 and 4. Stock B29, mating type VII, of syngen 2 reacts with types II, IV, V and VI of syngen 2 (Table 9), which are all homozygous recessives at

the B locus. The other stocks of syngen 2 which show signs of the same pattern of reactivity are B20 and B32 (type III), and B23 and B30 (type IV). All these stocks were collected from the same locality (Karakashian, personal communication). The stocks of mating types III, IV and VII of syngen 2 which originate from a different locality (16, 67 and 93) do not react with types IV and V of syngen 4. This could be due to intravarietal differences in the specificities of the mating type substances.

The strongintervarietal mating reactions between syngens 2 and 7 (Table 10) conform to the same pattern as those between syngens 2 and 4; four of the types of one syngen react with four of the types of the other.

Adolescence

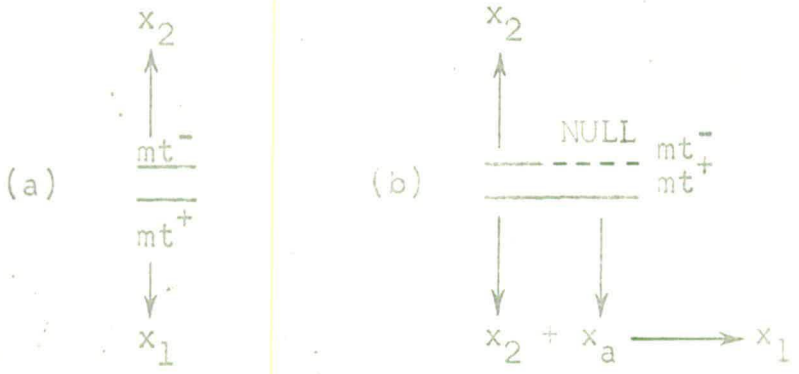
Jennings (1942) discovered some adolescent clones in syngen 1 which mated with only two of the four mating types of this syngen. Metz (1954) postulated that only one of the two mating type substances that will finally appear are present on the cilia of adolescent clones. Siegel & Cohen (1963) were able to relate the pattern of adolescence displayed by a clone to its genotype at the mating type loci; and, in conformity with the idea that only one substance is present on adolescent individuals, they found only four types of adolescent clones. They conclude that the mating type genes are expressed sequentially during the maturation of a clone.

These ideas can be made clearer by reference to the observations that were made on adolescence in syngen 4. (Table 16). Each adolescent clone failed to react with one other mating type apart from the one it finally became; e.g. the type I clone, when adolescent, did not react with type III. Furthermore in each case the genotypes of the two mating types with which the adolescent clone will not react differ from one another at one mating type locus only, at least in respect of the presence or absence of a dominant gene. Thus the genotypes of mating types I and III (aaB- C- and aa B- cc) are distinguished at the C locus only; and if the C locus were not expressed in a type I individual it would not be able to react with type III.

In the right hand column of table 16 are listed the gene loci which it must be assumed are not expressed in each of the adolescent clones. The limited nature of the data does not permit any generalisations to be made about the order in which the loci come into expression, except that different loci may be expressed last in different clones. Siegel (1965) has evidence that genetic factors can regulate the order of expression of the mating type genes in syngen I.

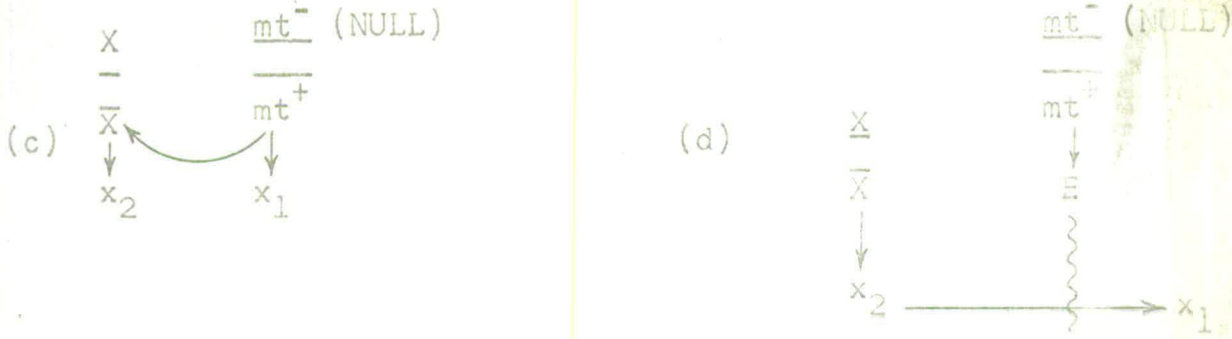
Adolescent clones of syngen 4 in which only one of the mating type loci is expressed would be expected to react with only four of the eight mating types. No clear instances of this have yet been observed which suggests that this stage of adolescence, if it exists, must be of relatively short duration.

In summary of this section it can be said that the phenomena of intervarietal mating reactions and adolescence, in both of which paramecia show mating reactions with only a selection of the total



(a) Alleles at mt locus code for complementing any mating type substances x_1 and x_2.

(b) The mt^- allele of the mt locus consists of an active region coding for mating type substances x_2, and a null region. The mt^+ allele codes for substance x_2, and also for another substance x_a : x_2 and x_a combine together to form x_1 which is complementary to x_2.



(c) Complementary mating type substances x_1 and x_2 coded for at gene loci mt and X respectively. Substance x_1, product of active mt^+ allele at mt locus suppresses X locus.

(d) Mating type substance x_2 coded for at gene locus X, and converted to complement any substance x_1 by product E of active mt^+ allele of mt locus.

FIGURE 7: Relation between mating type genes and substances.

of available mating types, demonstrate that the mating type genes act independently to confer on the organism separate surface specificities. These specificities are most easily imagined to reside in mating type substances, for the existence of which there is some rather inconclusive evidence.

3. The relation between the mating type substances and the mating type genes.

The purpose of this section is to outline the various possible mechanisms whereby the mating type genes might control the synthesis of the mating type substances.

Genes are believed to act by directing the assembly of amino acids into polypeptides via the synthesis of messenger RNA (Jacob & Monod 1961a). Therefore the simplest imaginable relationship between the mating type substances and mating type genes would be to suppose that the complementary substances of a pair are the polypeptide products of the two alleles at a mating type locus, as illustrated in fig. 7(a).

With this scheme it is difficult but not impossible to account for the dominance of one allele over the other. Two alternatives suggest themselves; that the dominant allele inhibits the activity of the recessive in some way, e.g. the messenger RNA or polypeptide product of the dominant allele acts as a repressor of the recessive; or that both mating type substances are synthesised in heterozygotes, the product of the dominant gene masking the function of the product of the recessive.

The schemes of figs. 7(b), (c) and (d) have been designed to solve the problem of the dominance of one mating type allele over the other by so adjusting the relationships between the mating type genes and mating type substances that the recessive gene can be assumed to be null, or to contain a null region.

In the scheme of fig. 7(b) the allele mt^- codes for the polypeptide x_2: the mt^+ allele is divided into two regions (cistrons), one coding for the polypeptide x_2, and the other for the polypeptide x_a. These two products of the mt^+ allele can combine together to form the x_1 protein, which is complementary to the x_2 polypeptide. The region of the mt^- allele which is homologous to the x_a cistron of the mt^+ allele is null.

The schemes of figs. 7(c) and (d) are similar in that in both of them there are two gene loci, mt and X, concerned with the synthesis of one pair of mating type substances, x_1 and x_2. According to scheme 7(c) the X locus, at which only functional wild type alleles are usually found, codes for the substance x_2; and the mt locus, at which functional mt^+ alleles or null mt^- alleles can be present, codes for the substance x_1. This latter, as well as being a mating type substance also acts as a repressor of the X locus, so that when there is a mt^+ allele at the mt locus the X locus will not be expressed.

In the scheme of fig. 7(d) the x_2 substance coded for by the X locus is converted to the complementary member of the pair, x_1, through the activity of the mt^+ allele, by some such mechanism as the enzymatic addition of a carbohydrate moiety.

The other property of the mating type genes which is perhaps relevant to a discussion of the mechanisms by which they control the synthesis of the mating type substances is their proneness to be affected by heritable modifications in a manner dissimilar to what is usually understood by mutation (Section I(ii) of this discussion). To accommodate a phenomenon of this sort in the scheme of fig. 7(a) would require assuming that relatively frequent and directed changes in the base sequences of the DNA of the genes were taking place. On the other hand, in the schemes of figs. 7(b), (c) and (d) it would only be necessary to suppose that the null alleles could become active or vice versa. This need not be accompanied by any changes in the base sequences of the DNA of the genes. (A discussion of the exact mechanisms which could be involved is deferred until section 6).

A knowledge of the chemistry of the mating type substances could be crucial in deciding between the schemes of fig.7. As previously noted (section 2 of this discussion) it is probable on comparative grounds that one member of each pair of substances will turn out to be a glycoprotein. The only scheme which allows for this contingency is that of fig. 7(d).

The schemes of fig.7 have been kept as simple as possible, and obviously the chain of reactions between the mating type genes and substances may be much longer than has been represented in any of them. None the less it will be seen below that these schemes can be used to explain some of the features of mating type transformation (section 4 (ii) of this discussion) and of mating type determination in other species of Ciliates (section 5 of this discussion).

4. Mating type transformation

The discussion of the phenomenon is divided into two parts; the first is concerned with the mechanism of transformation, or the means whereby a transformed individual is able to induce transform-:ation in a normal individual during temporary pair formation; and the second is devoted to the changes in phenotype observed in individuals of different genotypes after transformation.

(i) Mating type transformation as an example of infective heredity.

An obvious mechanism for mating type transformation is the transmission of an infective self-replicating agent from one paramecium to another during temporary formation. The infective agent would interact with the paramecium macronuclear genes to bring about a change of phenotype. This situation would resemble the phenotypic transformations that can be induced in vertebrate cell lines by infection with viruses (Rous, 1965).

If mating type transformation were the result of viral infection it is at first sight surprising that transformation could not be caused by exposing normal paramecia to homogenates of trans-:formed paramecia (Results, section 2 (vi)); but this could easily be because the right conditions for infection have not yet been discovered. For example, at one time great difficulties were encountered in infecting symbiont-free individuals of P.aurelia with bacterial-like symbionts (kappa) from breis, but this was later accomplished successfully (Sonneborn, 1959).

There are two ways in which temporary pair formation might facilitate the passage of an infective agent from one paramecium to

another, and which might explain why transformation cannot be brought about without pairing. The first is simply by the establishment of close proximity between the members of a pair. Since temporary pairs can be forced apart at any time without injury (Results, section 2(i)) it is certain that the channel through which the gamete nuclei pass during normal conjugation is not formed; but Vivier and Andre (1963), in an electron microscope study of conjugation in P.caudatum, discovered that, early in conjugation, the cuticles of the conjugants adhere so that homologous parts are opposed, and the pellicular ridges of each conjugant fuse with the formation of channels about 0.25u wide between the conjugants. Connections of this sort might be formed during temporary pair formation in P. bursaria .

Secondly, temporary pair formation could serve as an activator for the transmission of the infective particle; i.e. one or the other of the cellular changes associated with temporary pair formation, itself not directly concerned with transformation, could induce the transformed paramecium to release the particles, or the normal paramecium to receive them. The obvious candidates for this role are the nuclear modifications that take place during temporary pairing (Results, section 2 (ii)).

None the less, if mating type transformation is to be considered as a pathological symptom of viral infection it should be possible to prepare an infective extract of transformed cells; perhaps the separation of the crude brei into different cell fractions would be effective. Also it might be possible to detect the infective

particle, or morphological changes in infected paramecia, with the electron microscope.

A well-known example of a self-replicating factor which can be transferred by cell to cell contact in the F factor controlling sexuality in the bacterium E.coli (for review see Hayes, 1964).

It was the demonstration by Hirota (1960) that the F factor can be eliminated from F^+ bacteria by treatment with acridine dyes that inspired the unsuccessful attempt to cause transformed paramecia to revert to their original type by exposing them to acridine orange (Results, section 2(v)).

There are some reasons for believing that the agent of transformation might be an episome; i.e. might become associated with the paramecium genes at the chromosomal level as well as existing freely in the cytoplasm. These will be discussed in section 6 of this discussion. But it can be noted at this point that there could obviously be no real analogy between the agent of transformation and bacterial episomes. The latter have the quite unique property of integrating themselves into the bacterial chromosome and facilitating chromosome transfer.

Mating type transformation can also be usefully compared with the various cytoplasmically-controlled phenotypic modifications that have been discovered in Fungi (for review see Fincham and Day, 1963). Two examples will be considered in some detail here.

Roper (1958) induced a modified phenotype called 'mycelial' in several strains of Aspergillus nidulans by treatment with acriflavine. If an heterokaryon was constructed from a normal strain with marked nuclei, which may be designated as A(+) and a mycelial strain with differently marked nuclei, B(myc), conidia could be isolated that gave rise to colonies of type A(myc), but no conidia of type B(+) were ever found. When the mycelial character was transferred through heterokaryons to a number of different strains, i.e. C(myc), D(myc) etc., the expression of the character was found to differ in each strain; but if the mycelial character was then returned to the original strain, this still developed its characteristic phenotype. The factor controlling mycelial had not undergone modification by passage through different strains.

These facts are strikingly analogous to the basic features of mating type transformation, in which the new phenotype is absolutely stable, depends on the genotype of the transformed paramecium, but is independent of the phenotype of the paramecium used to bring about transformation.

Roper favours the view that the mycelial character is controlled by a cytoplasmic particle that becomes irreversibly associated with the nuclear membrane.

In order to demonstrate that mating type transformation is controlled by a self-replicating particle which is a modified version of a normal cell constituent it would, at least, be necessary to find a means of inducing transformation de novo. A number of chemical and physical treatments were applied to transformed paramecia in an attempt to make them return to normal (Results, section 2(v)). Perhaps it would now be profitable to treat normal paramecia in the same way.

The non-conjugating stocks of P.bursaria described by Ehret (1948), and those of P.aurelia (Metz and Foley, 1949), both appeared amongst the progeny of crosses. The only example of this in syngen 4 of P.bursaria is the type II clone of the exceptional synclone of cross 6, table 11, that underwent mating reactions with clones of other mating types, but did not form pairs (Results, section 1(ii).

Mahoney & Wilkie (1962) studied some strains of A.nidulans whose conidia gave rise to self-sterile colonies called 'alba' at variable rates. If alba and normal hyphae were allowed to anastomose, the normal phenotype spread into the alba mycelium; but alba colonies could not be transformed to normal by growing them on media containing extracts of normal mycelia.

Mahoney & Wilkie point out that the spread of the normal phenotype along the hypha is explicable by the migration of a self-replicating genetic unit; but they also consider the possibility that

it could be accounted for in terms of self-perpetuating metabolic cycles. Later Wilkie (1964) has formulated this hypothesis in terms of the regulator-operator concepts of gene regulation of Jacob & Monod (1961b). In his scheme the activities of two genes are mutually exclusive because the product of either gene functions as a repressor for the other.

Applying this idea to mating type transformation, it can be imagined that, in transformed paramecia, a super-regulator gene is active whose product, directly or indirectly, controls the activity of other genes including the mating type genes, so that the transformed phenotype is expressed. Transformed paramecia produce an effector molecule capable of derepressing the inactive super-regulator gene of normal paramecia. The super-regulator gene then remains active because its product can repress its own regulator.

It has also been proposed that embryonic induction, another example of stable phenotypic change following cell to cell contacts, could be the result of the transfer of effector molecules (Grobstein, 1963).

At present there is no evidence for the operation of mechanisms of this sort in mating type transformations; the hypothesis would become testable if stocks of P.bursaria insusceptible to transformation were found. Insusceptibility would be expected to be associated with mutations of the super-regulator gene, or the regulator of the super-regulator.

In summary, by comparing mating type transformation with other examples of infective heredity it is possible to distinguish three alternative causes for the phenomenon; infection by a virus; infection by a modified cytoplasmic particle (this could, in practice, by indistinguishable from a virus); or the transfer of effector molecules. The information needed to choose between these hypotheses is not yet available.

(ii) The phenotypic changes after mating type transformation.

Changes of mating type after transformation.

The arguments presented in the previous sections of this discussion have led to the conclusion that the mating type specificities are conditioned by mating type genes which control the synthesis of mating type substances. On this basis it is possible to envisage three different ways whereby the mating type of a paramecium might become changed after transformation.

(a) By the acquisition of new mating type genes.

It is certain that mating type transformation is not accompanied by segregation amongst the pre-existing genes in the paramecium (Results, section 2(i)). It is, however, conceivable that new mating type genes could be introduced into a paramecium during transformation by some transduction-like process; but this is scarcely credible in view of the fact, for instance, that a syngen 2 paramecium transformed by a syngen 4 paramecium expresses a syngen 2 mating type. (Results, section 2(iii)).

(b) By the direct modification of the mating type substances.

The same mating type genes remain functioning after trans-:formation, and the same mating type substances are synthesised, but the steric properties of these are altered by interactions with the agent of transformation of its products.

While admitting that this is possible, it seems most improbable that the modified mating type substances should correspond in their specificities to those produced by normal paramecia with a different genotype; e.g. in syngen 4 transformed paramecia carrying dominant mating type genes usually have a mating type the same as that of normal paramecia in which recessive mating type genes are being expressed (Results, section 2(iii)).

The reactions of transformed syngen 2 paramecia with syngen 4 (table 22) do however indicate that, in this case, the specificity of at least one of the new mating type substances differs from that of any substances that are found on normal syngen 2 paramecia. This could be the result of a modification to a completely synthesised mating type substance; but, as will be explained below, this is not the sole possible cause.

(c) By the non-expression of mating type genes.

In transformed paramecia mating type genes that were former-:ly expressed have their functioning interrupted, either by interference with their transcription, or with the translation of their messenger RNA, or with some later stage of the synthesis of the mating type substances.

This mechanism will explain most of the transformation results if the appropriate assumptions are made about the relation between the mating type genes and substances in each case.

The easiest way to handle the results from syngen 4 is to consider each gene locus in turn:-

C locus: Paramecia which are homozygous dominants at this locus can transform to type IIS (table 19). They then are expressing a mating type which is characteristic of normal paramecia carrying recessive alleles at the C locus.

This could not happen if the relation between the mating type genes at the C locus and their mating type substances were as in the scheme of fig. 7(a). A homozygous dominant would not possess any genes determining the specificity of the mating type substance controlled by the recessive allele.

The situation at the C locus is, however, readily explicable according to any of the other schemes of fig.7. It is only necessary to suppose that in scheme 7(b) the x_a cistron of the mt^+ allele, or in schemes 7(c) or (d) the mt^+ allele, are not expressed in transformed paramecia.

B locus: Homozygous dominants at the B locus transform to type II-IIIS (tables 19, 20, 21). This type behaves as if it lacks either of the mating type substances synthesised under the control of the B locus. (The genotypes of mating types II and III, aa bb cc and aa B- cc, differ at the B locus only, and

so a paramecium without either of the two substances controlled by this locus, whose effective genotype would be aa - cc, could react with neither types II or III).

A loss of a mating type substance without replacement would occur in a homozygous dominant if, in the scheme of fig 7(a), both the mt^+ alleles were unexpressed. The same result would follow from the non-expression of the mt^+ alleles in scheme 7(b). In this case it would, however, have to be assumed that in hetero-:zygotes, which transform to type IIS, only the x_2 cistron which is in a cis position to the x_a cistron of the mt^+ allele is unexpressed.

The fact that heterozygotes at the B locus transform to type IIS also makes it difficult to apply schemes 7(c) and (d) to this locus. It would be necessary to suppose that the X loci are linked to the mt loci, and that the alleles at the X locus are only unexpressed after transformation when in a cis position to a mt^+ allele.

The explanation according to the scheme of fig. 7(a) is to be preferred on grounds of simplicity.

A locus: No paramecia known to be homozygous dominants at this locus were transformed (table 19); but it is likely that such paramecia transform to type IIS. If homozygous dominants at the A locus were affected by transformation in an analogous manner to homozygous dominants at the B locus they would express the phenotype II-VIS. (The genotypes of type II and VI, aa bb cc and A- bb cc, differ at the A locus only). No such phenotype has yet been observed.

Transformation in syngen 2 appears to follow the same lines as a transformation in syngen 4. The types VIIIS(2) and VII-VIIIS(2) of syngen 2 could be the equivalents of types IIS and II-IIIS of syngen 4; i.e. normal type VIII paramecia of syngen 2 should be triple recessives at the mating type loci, and normal type VII paramecia should carry dominant alleles at one of the mating type loci. This prediction would be easy to verify by making a few crosses.

One explanation why transformed paramecia of syngen 2 differ in their pattern of reactivity with syngen 4 from any normal paramecia has already been given - the post-synthetic modification of the mating type substance concerned. The alternative is to suppose that the relevant mating type substance is of different specificity because only part of the molecule gets synthetised, perhaps because only a portion of the gene locus concerned is unexpressed.

The loss of the ability to conjugate

Whatever their mating type, all transformed paramecia share the property of being unable to complete conjugation.

Metz (1954) has pointed out that the successive stages of conjugation are organised into a causal relationship. This implies that, in non-conjugating stocks, one of the earlier stages is blocked in some way. In the absence of any facts about the underlying mechanisms of the cellular changes that accompany conjugation it is not possible to be any more precise about this.

In syngen 4 the non-conjugating phenotype has only been observed in paramecia of mating types II or II-III. Jennings (1944) who discovered the non-conjugating stocks of syngen I whose cytology was studied by Chen (1946a), notes that the majority of his non-conjugating stocks were of mating type III (aa bb), but a few of them were mating type II (aa B-). This observation demonstrates that there is no necessary relationship between the inability to conjugate and the expression of the mating type controlled by recessive mating type genes. It can be suggested that Jennings' non-conjugating type III stocks carried dominant genes at the B locus which were resistant to the influence of the agent of transformation.

Concluding remarks on mating-type transformation.

The most satisfactory explanation for the changes of mating type that occur after mating type transformation is the non-expression of dominant mating type genes; although the application of this hypothesis does require supposing that the relation between the mating type substances and genes is different for the B and C loci of syngen 4.

There is no direct evidence as to at what level the expression of the dominant genes is prevented. The fact that all transformed stocks are non-conjugating means that even the micro-nuclear mating type genes are beyond the reach of genetic analysis.

The previously-discussed observations of Siegel (1965) on heritable modifications affecting the dominant allele at the B locus in syngen 1 demonstrate, at least, that a mechanism for changing the expression of the mating type genes, which operates at the chromosomal level at a frequency higher than that expected from mutation, is available.

The assumption that the agent of transformation acts by repressing the dominant mating type genes does not immediately allow any conclusions to be drawn about the nature of the agent of transformation itself. The question becomes one of imagining the possible causes of stable gene repression. This will be attempted in section 6 of this discussion.

One of the surprising features of mating type transformation is that at least three gene loci are affected. In general it might be expected that any mechanism for the control of gene expression would be rather specific in its action. But it has been tacitly assumed throughout this discussion that the agent of transformation is a single unit; if there were two or more separate agents selective infection might be expected to occur. The single agent of transformation must either be sufficiently complex to affect the expression of three gene loci each in a specific way, or else the three gene loci must share a feature which renders them susceptible to the action of the agent.

5. Mating type determination in Ciliates - A general review.

The purpose of this section is to try and use the ideas about the relation between the mating type substances and

mating type genes, that have been here developed for P.bursaria, to elucidate some of the obscure features of mating type determination in other species of Ciliates.

(i) Caryonidal systems of mating type determination.

The caryonidal system of mating type determination is exemplified in its most simple form in the so-called 'A group' syngens of P.aurelia. The earlier work has been summarised by Beale (1954). In each syngen there are two mating types and, after autogamy, in paramecia that are completely homozygous, the macronuclear analgen are independently differentiated to control either one of them. There is no reason to believe that there can exist any qualitative or quantitative differences between the genes in macronuclei controlling one or the other mating type; and so the only remaining possible basis for the macronuclear differentiation is differential gene expression.

Butzel (1955) studied two allelic recessive genes in syngen I of P.aurelia which allowed the expression of mating type I only. The dominant allele at the locus gives the paramecium the potentiality to express mating types I or II. Butzel postulated that the dominant allele controls a "terminal reaction" leading to the expression of mating type II; and that the differentiation of the macronucleus to control mating type I or II depends on whether the dominant allele is activated in the macronuclear analgen.

Butzel based his idea of a terminal reaction on the following considerations. In each of the many syngens of P.aurelia there are two mating types. Some intervarietal mating reactions have been discovered which indicate that there are similarities between mating types of different syngens (for the most recent account, see Sonneborn 1957). One of the mating types of a syngen is called the 'odd' type, and the other the 'even' type, after the numbers given to the mating types: syngen I with types I and II, syngen 2 with types III and IV etc. Intervarietal mating reactions are observed only between the odd type of one syngen and the even type of the other. Several recessive 'one-type' mutants have been discovered in syngens I and 7 which allow the expression of mating types I and XIII only, i.e. the odd mating types of each syngen. No one-type mutations restricting paramecia to expressing an even mating type have ever been found. This suggested that the known one-type mutations interfere with a terminal reaction necessary for the expression of the even mating type.

This idea can be interpreted in terms of mating type substances and mating type genes according to the schemes of fig. 7(d). The terminal reaction is the conversion of the mating type substance x_2 into the substance x_1. The mt^- allele corresponds to a one-type mutation; and the mt^+ allele is the dominant gene whose activation decides whether the even mating type will be expressed.

In the B group syngens of P.aurelia the inheritance of mating type is cytoplasmic. The mating type of an exconjugant or exautogamous individual is nearly always the same as its parent. Nanney (1954) suggested that in the B group syngens the parental cytoplasm exercises its influence by directing the differentiation

of the macronucleus of the next generation. This hypothesis was confirmed experimentally by Sonneborn (1954).

Taub (1963) studied two one-type mutations in syngen 7, a group B syngen. These two recessive mutants, mtXIII and n, which were located at two unlinked gene loci, both allowed the expression of mating type XIII only. In addition mt XIII had the dominant effect of causing the cytoplasm to be type XIV determining. Thus an individual of genotype mtXIII/mtXIII can only express mating type XIII, but if the wild type allele mtXIII-XIV is introduced at conjugation the exconjugant becomes type XIV, although normally in the group B syngens the mating type of an exconjugant is the same as its cytoplasmic parent.

Taub postulates that the mt and N loci "control different steps in the formation of the type XIV mating type substance", and also that mating type XIII determining cytoplasm might contain a repressor substance for the mtXIII-XIV allele. However, he does not combine these ideas into a scheme that will explain all his observations.

It is possible to formulate an exact interpretation of Taub's results according to the scheme of fig. 7(d).

The mt locus of syngen 7 corresponds to the mt locus of fig. 7(d), and the N locus to the X locus. The product E of the mt locus not only converts x_2 to x_1, but also activates or derepresses the mt locus in the developing macronucleus of the next generation, so that the type XIV phenotype, the outcome of an active mt locus, is inherited cytoplasmically. Under normal circumstances an inactive mt locus does not become active unless stimulated to do so by its own product.

The mtXIII mutation controls the synthesis of an altered product E′ which, although it can still bring the mt locus of the next generation into expression, has lost the power to convert x_2 to x_1. Therefore individuals of genotype mtXIII/mtXIII remain mating type XIII, but their cytoplasm is type XIV determining. In addition it must be assumed that the MtXIII allele is self-activating; i.e., it must come into expression spontaneously in a macronucleus developing in an individual that was formally mating type XIII, with mating type XIII determining cytoplasm.

The n mutation codes for a modified mating type substance x_2' which retains its mating type specificity, but is not susceptible to conversion by E; so n/n individuals must be mating type XIII. Their cytoplasm may be type XIII or XIV determining depending on the state of affairs at the mt locus. Individuals of genotype N/n with an active mtXIII-XIV allele must be assumed to synthesise both mating type substances x_2' and x_1, but must behave as type XIV.

The only addition to the scheme of fig. 7(d) that has been made to include mating type determination in the B group syngens is the assumption that the agent which converts x_2 into x_1 also brings the mt locus of the next generation into expression. There is one important prediction which follows from this. If cytoplasmic exchange were to be induced in a conjugating pair of syngen 7 paramecia the substance E will be distributed in both exconjugants, the mt loci of both of them should be expressed, and they should therefore both become mating type XIV. Taub has not performed this experiment, but Sonneborn (1954) has done it in syngen 4

with mating types VII and VIII. After cytoplasmic exchange both exconjugants became type VIII. Mating types VIII of syngen 4 and XIV of syngen 7 are both even mating types and hence, as previously explained, are probably equivalent.

In a further experiment Sonneborn (1954) induced macronuclear regeneration (i.e. the growth of one of the fragments of the old macronucleus to form the macronucleus of the exconjugant) in an exconjugant that had previously been type VII, but which had received cytoplasm from its type VIII mate. In contrast to the behaviour of similar exconjugants whose macronuclear anlagen were allowed to develop normally, the exconjugants with regenerated macronuclei remained type VII. This shows that the differentiation of the macro-:nucleus to control mating type VII is irreversible. According to the ideas about this differentiation that have just been discussed, the product of an active mt locus must prevent the irreversible repression of the mt locus of the next generation.

The other species of Ciliate in which the caryonidal system of mating type determination has been shown to operate is syngen I of I.pyriformis (Nanney & Caughey, 1953, 1955; Nanney, Caughey & Tefankjian, 1955; Nanney, 1959, 1960, 1963). The special features of the situation in this species are:-

(a) Altogether seven mating types exist.

(b) The range of types which an individual may potentially express depends on its constitution at a mating type locus mt. Individuals homozygous for the mt_A allele can develop all types except IV and VII; and individuals homozygous for the mt_B allele all types except I.

Heterozygotes for the two alleles can express any of the seven mating types.

Nanney, Caughey & Tefankjian (1955) and Nanney (1959) discuss the possible structure and function of the mt locus with respect to Metz's ideas about mating type substances. They are puzzled by the fact that there is no eighth mating type, and also that the restrictive mutants do not remove the potentiality to express four of the mating types, as they might be expected to do if they prevented the synthesis of one of the mating type substances. They do not suggest any explanations for these facts.

A formally satisfactory scheme for mating type determination in syngen I I.pyriformis can be developed from the following assumptions. There are three pairs of mating type substances and so potentially eight mating types. The mt locus (of I.pyriformis) fulfils the same function as the mt locus of fig. 7(d), except that it is responsible for the conversion of three different mating type substances instead of one. For this purpose it is divided into three cistrons, each of which controls the conversion of one mating type substance. The mating type of an individual will therefore depend on what combination of the cistrons of the mt locus is activated in the developing macronuclear anlagen.

In order to account for the fact that the alleles at the mt locus restrict the range of mating types that can be expressed (mt_A forbids the expression of types IV, VII and VIII and mt_B of types I and VIII) it is necessary to devise a mechanism that would

No. restrictions on combinations	mt_A	mt_B	Mating types of combinations
ABC	ABC	ABC	
ABc	(ABc) ⟶ ABC	(ABc) ⟶ ABC	VIII
aBC	aBC	aBC	
AbC	(AbC) ⟶ ABC	AbC	IV or VII
Abc	(Abc) ⟶ ABC	Abc	IV or VII
aBc	aBc	(aBc) ⟶ aBC	I
abC	abC	abC	
abc	abc	abc	

TABLE 26: Combinations of active (capital letters) and inactive cistrons in mating type alleles of syngen 1. T.piriformis. Forbidden combinations are bracketed.

limit the possible combinations of active and inactive cistrons within an allele to five in one case and six in the other, with one of the forbidden combinations being shared. This can be done by assuming that the cistrons are activated in a coordinated manner in each allele in the developing macronucleus. In table 26 the three cistrons are designated as A, B and C (active cistrons) or a, b and c (inactive cistrons). In the mt_A allele (second column of table 25) the activation of the A cistron is always accompanied by the activation of the B and C cistrons as well, so that the combinations ABc, AbC and Abc are not permissable, and only five combinations are left. In the mt_B allele the activation of the B cistron is linked to the activation of the C cistron, which reduces the total combinations to six. The combination ABc is forbidden in both mt_A and mt_B, and so this combination would control the elusive eighth mating type.

This theoretical scheme could be tested by the discovery of new alleles at the mt locus, potentiating new ranges of mating types. Nanney (1959) secured two new stocks of syngen I T.pyriformis from different localities in the United States. They both turned out to possess mt alleles which allowed the expression of the same range of mating types as mt_A, but at different frequencies.

(ii) Genetic systems of mating type determination

A genetic system of mating type determination has been shown to operate in four other species of Ciliates apart from P.bursaria: in E.patella (Kimball, 1942), E.vannus and E.crassus (Heckmann, 1963, 1964) and syngen 8 of T.pyriformia (Orias, 1963). Only the last

three will be considered here.

Each of these three species has a number of mating types controlled by a series of multiple alleles at a single locus. Each allele controls a single mating type, and the alleles fall into a hierarchy of dominance. In E.vannus and E.crassus five mating types have so far been discovered, and in syngen 8 of T.pyriformis, three. The alleles of each species are designated, using the authors' notations, as below:-

Eupbtes: mt_5 mt_4 mt_3 mt_2 mt_1

T.pyriformis: mt_A mt_B mt_C

In interpreting these results Heckmann (1963) pointed out that the mt locus could be considered as a complex locus; and Orias (1963) remarks "the mt locus controls, at least, the mechanism of expression of the genes responsible, directly or indirectly, for the specificity of the mating reaction". He suggests that each of the alleles at the locus could inhibit the expression of all the alleles recessive to it.

Another way of accounting for the dominance relationships of the alleles at the mt locus in these species is to assume that the mt locus fulfils the same function as in all the other cases that have been cited in this section; i.e. it controls the conversion of mating type substance into complementary substances. As in syngen I of T.pyri-:formis the alleles of the locus are divided into a number of cistrons which are, however, stable in their state of activity or inactivity.

Since the number of mating types is not equal to a term of the series $(2)^n$ there must exist restrictions on the combinations of cistrons that can occur in any one mating type allele, and these

combinations must be such as to ensure that the alleles fall into a hierarchy of dominance. A scheme for the arrangement of the mating type alleles which satisfies this requirement is set out below (capital letters for active cistrons):-

T.Pyriformis		Euplotes	
Mating type Allele	Cistrons	Mating type Allele	Cistrons
mt_A	AB	mt_5	ABCD
mt_B	Ab	mt_4	ABCd
mt_C	ab	mt_3	ABcd
		mt_2	Abcd
		mt_1	abcd

It should be noted that to accommodate the five mating types of Euplotes it is necessary to postulate the existence of four pairs of mating type substances; so there would be sixteen mating types if all the possible combinations of cistrons were allowed.

For a system such as this to work it is essential that there should be little or no crossing over within the mt locus. In the heterozygote mt_5/mt_1 in Euplotes a cross-over anywhere within the gene would produce a new mating type; and this would not fit into the dominance hierarchy.

Equally the mating type cistrons must be immune from regular changes of state between the active and inactive conditions. There is in fact, surprisingly, some evidence for the instability of the mating type alleles both in E.crassus and syngen 8 T.pyriformis.

Heckmann (1964) observed selfing in some cultures of E.crassus when the clones became aged (had passed through many divisions since the last conjugation); and he was also able to induce selfing in these cultures by lowering the temperature to 12°C. All the cultures in which selfing occurred were heterozygotes at the mating type locus. This suggests that the selfing could be the result of a change in mating type of some individuals in the culture brought about by an active mating type cistron becoming inactive. Heckmann does not report whether he was able to identify new mating types in selfing cultures, nor has he yet investigated what mating types are expressed by the progeny from the pairs in selfing cultures.

Orias (1963) obtained some type II (mt_C/mt_C) progeny clones from crosses where these should not have appeared. As one of the parents was heterozygous for the mt_C allele in each case, these exceptional progeny could be the products of cytogamy; but Orias does not favour this explanation as he did not observe any of the other progeny classes that would be expected if cytogamy were general (cf. the arguments about the origin of the exceptional synclones of syngen 4, P.bursaria; section I(i) of this discussion). He prefers to believe that the exceptional clones are the result of a modification of the mt_A or mt_B alleles to the mt_C condition. If the mt_A allele changes to mt_C this must, according to the hypothesis under discussion, be brought about by the inactivation of two cistrons simultaneously. This recalls the coordinated regulation of the activity of the mating type cistrons that was postulated to take place in the mating type alleles of syngen I of I.pyriformis.

(iii) The relation between the caryonidal and genetic systems of mating type determination

Orias (1963) has suggested that the genetic and caryonidal systems of mating type determination must be variants of the same basic organisation because syngens 1 and 8 of I.pyriformis, which possess different systems of mating type determination, are closely related by morphological taxonomic criteria. Siegel & Larison (1960) pointed out that the difference could lie in the relative stability of expression of the mating type genes, these being sufficiently stable to function as Mendelian alleles in a genetic system, but not in a caryonidal system. However, they do not specify how the mating type genes are supposed to function, apart from noting that the could control the synthesis of the mating type substances by some means.

The way in which Siegel and Larison's hypothesis can be completed should be obvious from the conclusions that have emerged in the earlier part of this section. The mating type genes whose stability of expression differs in the caryonidal and genetic systems are the alleles at the mt locus as represented in fig. 7(d); the potentially active alleles of this locus invariably come into expression in the macronucleus of the next generation if the system is genetic, but they are sometimes repressed in a caryonidal system.

6. The stable repression of gene loci

In this section an attempt will be made to define the mechanism of the inactivation of the mating type genes with reference to the known structure and organisation of the genetic material, and by comparison with other examples of stable gene inactivation.

The notion of the inactivation of the mating type genes has been here advanced in connection with three separate phenomena:-

(a) The stable 'non-mutational' alterations of the mating type genes in the micronucleus of syngen 1 of *P.bursaria*.

(b) The changes in expression of the macronuclear genes of syngen 4 of *P.bursaria* after mating type transformation.

(c) The differentiation of the macronuclear anlagen in species of Ciliates with a caryonidal system of mating type determination.

The changes of state of the micronuclear mating type genes of *P.bursaria* present some similarities to the heritable gene modifications associated with transposable elements (McClintock, 1956) and paramutation (Brink, 1960) in Maize, in that they are self-perpetuating modifications of a single gene locus that occur sporadically (Jennings' self-differentiation), or at a higher frequency amongst the progeny of certain crosses (Siegel's mutator factor).

The physical basis of these phenomena in Maize is still obscure. The transposable elements have been variously interpreted as genetic elements functioning in an analogous manner to the regulator and operator components of Jacob & Monod (McLintock,

1961), as episomes (Campbell, 1962) and as "heterochromatin dis-:arrangements" (Brink, 1964).

The problem is to imagine how the organisation of a gene locus can become modified without a change in the base sequence of its DNA in such a way that the new organisation is self-perpetuating. The easiest solution is that of an additional element, which is itself self-replicating and therefore presumably DNA, becoming associated with the locus and preventing its transcription. Any alternative solution would involve postulating heritable changes in the non-DNA portion of the gene. Although it is possible that the transmission of the structural organisation of sub-cellular constituents can sometimes depend on the molecular orientation of substances other than DNA, the present knowledge, or lack of knowledge, about chromosomal architecture does not permit the development of any precise hypotheses along these lines.

From the experimental point of view the situation will only become clearer if some means of changing the state of the mating type genes by a defined stimulus can be found. For this reason the repetition and extension of Lee's (1949) experiments on changes of mating type induced by X-irradiation of P.bursaria is imperative.

The macronuclear differentiation that is the basis of the caryonidal system of mating type determination has a number of features in common with a case of the control of gene expression through a variegated position effect studied by Baker (1963). He found that a gene controlling the synthesis of pigment in the ommatidia of the compound eye of Drosophila, when translocated to the heterochromatic region of another chromosome, was only expressed in a

proportion of the ommatidia. By studying the pattern of pigmentation of the affected eyes he was able to infer that the decision whether the gene was to be expressed was made at a particular early stage in the cell lineage of the eye. Temperature changes during this period of fixation of the future fate of the gene influenced the proportions of cells in which it was expressed.

The macronuclei of Ciliates with a caryonidal system of mating type determination become differentiated at a particular stage of the development of the anlagen. This can be identified as a temperature sensitive period, during which the proportion of caryonides which will in the future express each mating type is influenced by the prevailing temperature (for P.aurelia see Beale, 1954, and for syngen I I.pyriformis nanney, 1960).

The question now arises whether there is any connection between gene inactivation by episomal factors and by hetero-:chromatisation; or to rephrase the question, what could induce and maintain a state of heterochromatisation in a chromosome or chromosomal region? A possible answer to both questions has been provided by Grumbach (1964) who, speculating on the mechanism of induction of heterochromatisation in the mammalian X chromosome, suggests "that induction involves the incorporation of an episomal factor into an X chromosome at a specific receptive locus during early embryonic development".

It now remains to consider whether there is any reason to implicate episomal controlling factors in mating type transformation. First, it must be stressed that there is, in the case of mating type transformation, no direct evidence that the changes of gene

expression that are observed are the outcome of the inactivation of the mating type gene due to their modification at the chromosomal level. Because transformed paramecia cannot complete conjugation even the micronuclear genes are beyond the reach of genetic analysis.

The observed changes of mating type after transformation are, however, compatible with the inactivation of mating type genes (section 4 (ii) of this discussion); and it is a reasonable hypothesis that the agent of transformation itself is a self-replicating genetic unit (discussion, section 4(i)). The intriguing possibility then arises that the agent of transformation represents a modified version of an episomal factor that is normally engaged in the regulation of the activity of the mating type genes.

SUMMARY

I. The two characters of P.bursaria whose inheritance was studied were the ability to maintain symbiotic zoochlorellae in the cytoplasm and mating type.

2. An attempt was made to detect variation amongst stocks of P.bursaria with respect to their ability to maintain diverse strains of Chlorella. 21 stocks of P.bursaria were freed of their symbiotic algae, and then reinfected with foreign strains of algae. It is hoped that the stocks of P.bursaria would be distinguishable according to the strains of algae they were competent to accept as symbionts. This was found not to be the case, and the investigation was discontinue

3. The stocks of P.bursaria which had been collected in Scotland were classified into syngens and mating types. (With a few exceptions, stocks of paramecium conjugate together only if they are of different mating types within the same syngen). The scottish stocks were found to comprise three syngens, each containing eight mating types. These three syngens were identified as syngens 4, 6 and 7 on the basis of the mating reactions between their stocks and stocks belonging to known syngens. Intervarietal mating reactions between stocks of syngens 4 and 7, and between stocks of syngen 2 and syngens 4 and 7, were discovered.

4. The inheritance of mating type after conjugation in syngen 4 was studied. The results indicate that the eight mating types are controlled at three gene loci, the A,B and C loci. There are two alleles, one dominant to the other, at each gene locus. The genotypes

of the eight mating types are: type I, aa B- C-; type II, aa bb cc; type III, aa B- cc; type IV, aa bb C-; type V, A- bb C-; type VI, A- bb cc; type VII, A- B- cc; type VIII, A- B- C-. (The symbols A-, B- and C- represent the homozygous or heterozygous state of the locus).

5. A means was discovered of changing the mating type of a paramecium without segregation amongst the mating type genes. Some stocks of mating type II were collected, individuals of which were able to go through only the preliminary stages of conjugation with individuals of other mating types; the pairs which are formed fall apart prematurely, before any exchange of gamete nuclei can take place. The 'separating' type II stocks have been called type IIS.

Normal paramecia, after engaging in temporary pair formation with type IIS paramecia, usually grow up into clones of type IIS; i.e. their phenotype has been stably modified in two ways - with respect to their mating type and their ability to complete conjugation. This phenomenon has been called mating type transformation.

6. The following facts about mating type transformation were ascertained:-

(i) There is no nuclear reorganisation, and hence no segregation amongst the mating type genes, in paramecia separated from temporary pairs.

(ii) Paramecia which are homozygous dominants at the B mating type locus become type II-IIIS after mating type transformation. II-IIIS paramecia form temporary pairs with paramecia of all mating types except II and III.

(iii) Mating type transformation can occur in syngen 2. Trans-:formed stocks of syngen 2 express mating type VIII or VII-VIII (of syngen 2).

(iv) The outcome of mating type transformation depends on the genotype of the paramecium being transformed, not on the phenotype of the paramecium used to transform it.

(v) Newly-transformed paramecia can themselves effect further transformations by temporary pair formation with normal paramecia.

(vi) The transformed phenotypes are very stable. All attempts to cause transformed paramecia to revert to their original phenotype were unavailing.

(vii) Mating type transformation could not be brought about without temporary pair formation, e.g., by exposing normal paramecia to breis of transformed paramecia.

7. Mating type transformation is discussed as an example of infective heredity; and the changes of mating type that take place after transformation are explained by changes in the expression of the mating type genes.

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Infection of Alga-Free *Paramecium bursaria* with Strains of *Chlorella, Scenedesmus,* and a Yeast

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SYNOPSIS. Twenty-one different stocks of *Paramecium bursaria*, belonging to 4 separate varieties (syngens), whose endosymbiotic chorellae had been removed, were tested for reinfection by several strains of *Chorella,* some previously isolated from *P. bursaria*, and others free-living. In addition, infection of *P. bursaria* by a single strain of the green alga *Scenedesmus* sp., and an unidentified strain of yeast was attempted. Most combinations involving *Chlorella* yielded infected paramecia, and all those with *Scenedesmus* or the yeast did so. The failures with *Chlorella* were attributed to low infectibility of the stocks of *Paramecium* concerned, rather than to inability of the *Chlorella* to survive inside the paramecia. Little evidence was found that the strains of *P. bursaria* differed genetically in ability to maintain the symbiotic organisms.

INDIVIDUALS of *Paramecium bursaria* collected from the wild normally contain several hundred cells of the green alga *Chlorella* established in the cytoplasm as endosymbionts. The two partners in the symbiosis have been separated, grown independently, and reunited by several workers(5,7,9,10). Infection with *Chlorella* originally endosymbiotic in *P. bursaria* and in other species(5,9,10), with free-living strains of *Chorella*(7,10), and with other genera of green algae (7) has been successfully accomplished. Some workers record that they were unable to infect their stocks of *P. bursaria* with all the strains of algae they tested (5,7,10).

In particular Siegel & Karakashian(10), and Siegel (9) noted differences between 4 stocks of variety (syngen) 1 of *P. bursaria*. These differences concerned the proportion of animals infected by a strain of algae, and the rate of growth of the algae following infection.

All this previous work suggested that a given stock of *P. bursaria* might form a symbiotic relationship with some strains of algae but not with others; and that different stocks of *P. bursaria* may be distinguished according to which strains of algae they are competent to accept. In the present study 21 *Chlorella*-free stocks of *P. bursaria*, belonging to 4 varieties, were tested for infection by a number of strains of *Chlorella*, both symbiotic and free-living, by a single strain of the green alga *Scenedesmus* sp., and by a yeast. It was hoped that the *Paramecium* stocks would be distinguished as described above, so that the differences could be subjected to genetic analysis.

MATERIALS AND METHODS

Individuals of *P. bursaria* were collected from about 20 localities throughout Scotland and England, and from a few localities in Holland and Denmark. The clones thus obtained were grouped according to their mating reaction(4) into three varieties. Of the 7 previously described varieties of *P. bursaria* (2) reference stocks were available only for varieties 1 and 6 (by courtesy of Drs. R. W. Siegel and T. T. Chen, respectively). One of the 3 varieties obtained is variety 6. Samples of the other two, here designated as 'A' and 'B,' have been sent to Dr. Chen for identification.

Three free-living strains of *Chlorella,* LA25, Gr-B, and 1U20 (*C. ellipsoidea,* Indiana University culture collection) were kindly supplied by Dr. R. W. Siegel. In addition symbiotic

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algae were isolated (by the method of Siegel(9)) from 4 stocks of *P. bursaria*: CO4 and BL (Var. 'A'); CAI (Var 'B') and Sd (Var 1); and these 4 cultures were carefully purified by dilution techniques before use in infection experiments. The composition of the medium used for algal cultivation was,

$Ca(NO_3)_2 \cdot H_2O$	0.05	g/l
$MgSO_4 \cdot 7H_2O$	0.025	
Na glycerophosphate $\cdot 5\frac{1}{2}H_2O$	0.025	
KCl	0.0125	
TRIS	0.65	
Trace elements (3)	0.05 ml	
Distilled aq.	1 l	

This was supplemented with 0.5% glucose and 0.1% Proteosepeptone, and used in slants stiffened with 1.5% agar.

Repeated attempts to culture the Chlorellae from 5 other stocks of *P. bursaria* in this way were unsuccessful [Loefer(6) was successful with his strain, but Pringsheim(8) reported failures]. Algae from these 5 stocks for use in reinfection experiments were obtained directly from mass cultures of paramecia by homogenization with a syringe. There is no certainty that algae so obtained consist of a single strain.

The strain of *Scenedesmus* sp. was obtained by spinning down the organisms from a sample of water from a local pond, and spreading them on unsupplemented algal medium. The culture was subsequently purified by dilution.

The yeast used in these experiments was first noticed in the cytoplasm of one of the *Chorella*-free stocks of *P. bursaria*, where it had presumably arrived as a contaminant in the culture. A few of the paramecia were washed by transfer through sterile exhausted culture fluid and then placed on the surface of malt-extract agar where they ruptured, liberating the yeast. This formed pink colonies consisting of budding cells of approx. 5 μ diameter. No further identification of the yeast has been attempted.

The techniques for culturing *P. bursaria* with and without algae were those of Siegel(9). Colourless stocks were obtained by growing cell lines in the dark at 28°C. When a line was suspected to be free of its algae a single individual was isolated and allowed to divide once. One of the daughter cells was examined under the high power of the microscope for algae, the other allowed to divide again. One of the products of the second division was examined for algae. If both of the examined cells were free of algae the surviving cell was allowed to grow up into a clone in the light. All the *Chlorella*-free cultures were kept in the light, and no "revertant" cells were ever observed.

In addition some stocks of *P. bursaria* produced colourless individuals while growing in light. No differences in behaviour in the subsequent experiments could be detected in colourless stocks derived in this way.

The procedure of the infection experiments was as follows. Individuals of the alga-free stock to be tested were pipetted into fresh bacterized culture fluid and allowed to remain there overnight. Thus all the paramecia were in a fully fed condition at the outset of the experiment. Approximately 100 individuals were added to 2 drops of the algae solution in a depression slide. (The algae were suspended in Dryl solution: 0.1 M Na citrate $\cdot 2H_2O$, 20 ml; 0.1 M $Na\ H_2PO_4$, 10 ml; 0.1 M Na_2HPO_4, 10 ml; 0.1 M $CaCl_2$, 15 ml; dist. aq, 945 ml.) After 24 hr the mixture was transferred to a tube with an equal volume of bacterized culture fluid. The tubes were examined under a binocular 6 days later. In most cases a mixture of infected, green individuals and uninfected, white individuals could be seen. In cases where the number of algae per infected cell was low, e.g., the combinations involving the algae strains Gr-B and 1U20 (see Tables 1 and 2), and in all combinations involving *Scenedesmus* or the yeast, the individuals suspected to be infected were transferred into fresh bacterized culture fluid and allowed to remain there for 2 hr, removed, and replaced in fresh fluid for another 2 hr before being examined under the high power of the microscope. This ensured that algae or yeasts that had been ingested and were lying in food vacuoles would not be mistaken for symbiotic organisms.

In the later experiments a rough estimate was made of the proportion of paramecia infected. This was scored as "high" if few or no white animals could be seen when the tubes were examined, "low" if the number of green animals was smaller than about 20, and "medium" for intermediate conditions (L, M, and H in Tables 1, 2). Despite the approximate nature of this method, it has revealed the relatively low efficiency of infection of the algae strains Sd and LA25, a fact that became apparent only when Table 2 was compiled.

RESULTS

1. *Infection with* Chlorella. Nine stocks belonging to variety "A" were tested for infection by the strains of *Chlorella* shown in Tables 1 and 2. The only combinations which failed to yield infected individuals are those between *P. bursaria* stock M1 and algae of strains CA1, CO4, P3, and M1. Thus, it will be noted, paramecia of stock M1 could not be reinfected with algae derived from stock M1 itself. It was therefore concluded that the failures were due to the low infectibility of the stock M1, rather than to its incompatibility with some strains of *Chlorella*.

The combinations carried out with stocks of *P. bursaria* of varieties "B," 1 and 6 are shown in Table 2. The only negative combinations are those between the 3 stocks belonging to variety 6—W, HW, and Pt—and the free-living strains of algae Gr-B and IU20. It will be noted that these strains of algae grew relatively badly, producing small populations per cell in

TABLE 1. *Infection of 5 stocks of variety 'A' of* P. bursaria *with symbiotic strains of* Chlorella *sp. from varieties 'A', 'B', 1 and 6 of* P. bursaria, *and with free-living strains.*

Paramecia	*Chlorellae*										
	Ex-paramecia							'B'	1		
	CO4	BA6	BL	LU	M1	BA13	P3	CA1	Sd	Gr-b	IU20
CO4	+	+	+	+	+	+	+	+	+	F	F
BA6	+	+	+	+	+	+	+	+	+	F	F
BL	+	+	+	+	+	+	+	+	+	F	F
LU	+	+	+	+	+	+	+	+	+	F	F
M1	—				—	+	—	—	+	F	F

TABLE 2. *Infection of stocks of* P. bursaria *belonging to varieties 'A', 'B', 1 and 6 with symbiotic strains of* Chlorella *sp. from* P. bursaria *of all four varieties, with free-living strains of* Chlorella *sp., with the green alga* Scenedesmus *sp., and with a yeast.*

Paramecia		*Chlorella*									Yeast	
		Ex-paramecia					Free-living					
		'A'		'B'	1	6						
Variety	Stock	W4	BL	CA1	Sd	HW	LA25	Gr-B	IU20	*Scenedesmus*	Control	and Yeast
	SM1	H	H	H	H	M	H	F	F	L	—	H
'A'	SM2	H	M	H	L	H	M	F	F	L	—	H
	SM9	H	H	H	H	H	H	F	F	L	—	H
	TV	H	H	H	L	L	H	F	F		—	H
	WL				H		M			L		
	P	H	H	M	M	H	M	F	F	L	—	H
'B'	BL16	H	H	H	H	H	M	F	F		+	
	H4a	H	H	H	M	H	M	F	F		—	H
	H6c	H	H	H	M	H	M	F	F		—	H
	H	H	H	H	H	H	H	F	F		—	H
	W	M			L	M	L	—	—			
6	HW	H	H	M	L	M	L	—	—	L	—	H
	Pt	H	H	H	L	H	H	—	—		+	
1	3	H	H	M		H	M	F			—	H
	8	M	M	M		M	L	F			—	H

Key to symbols in Tables 1 and 2. +: infected, but proportion of paramecia infected not noted. H: high proportion infected (for method of scoring see text). M: medium proportion infected. L: low proportion infected. F: infected, but few algae per paramecium. —: not infected. Blank: combination not tested.

all the other stocks of paramecium which they infected. This fact was noticed for algae strain IU20 by Siegel & Karakashian(10) with their stocks of *P. bursaria*.

Attempts were made to remove the chlorellae from several other stocks of variety 6, in the hope of obtaining a stock that could be infected with the algae Gr-B and IU20, but paramecia of this variety grew very badly in the dark, and the attempts failed.

2. *Infection with* Scenedesmus *sp.* As seen from Table 2, all the stocks of *P. bursaria* exposed to *Scenedesmus* became infected. Up to 400 algae per cell were recorded. Cultures of paramecia infected with *Scenedesmus* could survive without the addition of bacterized culture fluid for several months; such cultures contain large numbers of free *Scenedesmus* cells, which might serve as food.

The proportion of paramecia infected was always very low, and some of the combinations had to be repeated many times before a single infected individual was recovered.

It was much easier to remove symbiotic *Scenedesmus* than *Chlorella*, 6 days rapid growth in the light being sufficient, compared to 2 to 3 weeks in the dark for *Chlorella*. As an initial population of *Scenedesmus* of about 400 was eliminated after 9 divisions of the paramecium the algae could not be increasing in numbers, or only very slowly, under these conditions. However, *Scenedesmus* cells in the process of division could be seen in the cytoplasm of infected paramecia which had been growing slowly in tubes. This fact, and the low frequency of infection mentioned above, suggest that *Scenedesmus* can multiply in the cytoplasm of *P. bursaria* under appropriate conditions, and that the high populations of algae observed in some paramecia are not merely the result of algal accumulation.

When paramecia containing *Scenedesmus* were infected with *Chlorella* a mixed population of algae were at first produced, but later the *Scenedesmus* was eliminated. Table 3 shows an experiment with *P. bursaria* stock SM 9 and chlorellae derived from stock BL; it was known that this alga infects a high proportion of paramecia of stock SM 9. The technique of infection was the same as described above except that exactly 40 paramecia were placed in the control and experimental tubes, which were filled with exhausted culture fluid rather than bacterized culture fluid, and kept in a lighted chamber at 20°C. These modifications were introduced to keep the fission rate of the controls as low as possible, so that large number of *Scenedesmus* would be maintained. Ten individuals were removed from each tube at the intervals shown in Table 3 and, after being treated as described above to remove ingested algae, were examined under the microscope and the symbiotic algae counted.

At the end of the experiment (day 11), 0.1 ml of fluid was withdrawn from the tubes, and the para-

TABLE 3. *Super-infection of* P. bursaria *stock SM9 carrying* Scenedesmus *with* Chlorella. *Ten individuals were examined under high power to provide an estimate of the numbers of algae.*

Day after infection	Numbers of algae			
	Control		+ *Chlorella*	
	Scenedesmus	*Chlorella*	*Scenedesmus*	*Chlorella*
0	50–150	0	50–150	0
3	50–150	0	50–150	10–20
6	50–150	0	0	>100
11	50–150	0	0	>100

mecia counted. The totals were 32 for the *Chlorella*-infected culture and 2 for the *Scenedesmus*-infected culture. It was concluded that infection with *Chlorella* had raised the fission rate of the paramecia and that this increase was the cause of the elimination of the *Scenedesmus*, rather than any effect directly associated with *Chlorella*.

Paramecia with a full complement of chlorellae continue to ingest *Scenedesmus* when this is present in the culture, 20 to 50 cells being seen if the paramecia are examined before the "washing" process.

3. *Infection with yeast.* The stocks infected with the yeast are listed in Table 2. In two cases, BL16 and Pt, it will be noticed that the controls were found to be already infected, presumably as a consequence of contamination in the stock cultures. In all combinations 100% of the paramecia examined (the high power of the microscope was necessary to detect the presence of the yeast) were found infected, with between 100 and 300 yeast cells per paramecium. The yeast cells could be seen to be budding. Cultures of yeast-infected paramecia required to be fed regularly with bacterized culture fluid even when the yeast was growing freely in the culture. The yeast could not be eliminated from the paramecia by rapid growth in the dark, but was lost if the paramecia were subsequently infected with *Chlorella* and left unfed for several weeks. This would account for the absence of yeast endosymbionts in individuals collected from the wild.

DISCUSSION AND CONCLUSIONS

These results have not revealed any intra-varietal differences amongst the stocks of *P. bursaria* tested with respect to the strains of algae they are competent to maintain. What variation there is amongst the stocks of *P. bursaria* appears to concern the ability to become infected with algae, rather than the ability to maintain them as symbionts. The experiments were designed to scan a number of stocks for complete inability to support certain algae, and quantitative differences in the efficiency of maintenance such as those reported by Siegel(9) would not have been detected.

The results indicate that *P. bursaria* can tolerate a wide variety of endosymbionts, but novel combinations of paramecia with algae may not function equally well as judged by growth rate(5), as has been seen in the case of infection with *Scenedesmus*.

This tolerance is in contrast to the situation in *P. aurelia*, where most if not all of the different cytoplasmic symbionts (*kappa* etc.) require a specific nuclear gene to be supported(1). However, the endosymbionts themselves are specialized, and can only be cultured outside the paramecium under the most special conditions(11) if at all. Why have not similar specifications arisen amongst the stocks of *P. bursaria*? One difference between the two systems is in the selective advantage conferred by the presence of the endosymbiont, considerable in the case of *P. bursaria*(5) and *Chlorella*, uncertain for *P. aurelia* and *kappa*. If an individual of *P. bursaria* were to lose its chlorellae, perhaps during a period of rapid growth in the presence of abundant bacterial food (as noted, instances of this have occurred in the laboratory), it would require to re-establish the symbiosis as quickly as possible afterwards. Too many specifications as to the types of chlorellae that could be accepted would be detrimental in this respect. Stocks of *P. bursaria* which were too exacting in their requirements for specific strains of *Chlorella* would thus be at a disadvantage to other paramecia.

Even if complete loss of chlorellae does not occur in nature, the composition of the algae population in stocks of *P. bursaria* may change as more effective symbionts are selected. A crude and artificial example of this process is provided by the replacement of *Scenedesmus* by *Chlorella*.

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