TRAUMATIC INDUCTION OF EARLY MATURITY IN TETRAHYMENA*

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ABSTRACT

Exposure of conjugating pairs of *Tetrahymena thermophila* to high temperature (37°) during macronuclear development causes an abortion of many macronuclei, but it also often induces an early appearance of sexual maturity in clones completing macronuclear development. Lines become mature after about 15 cell divisions rather than after 50 or more cell divisions in untreated pairs. The phenotype resembles that associated with *Em* (early maturity) mutants but, because it is not transmitted to the progeny in the next generation, it must be considered a phenocopy. The hypothesis is developed that an early genotype-environment incompatibility, whether associated with an abnormal genotype or an unusual environment, activates a shunt mechanism permitting the organisms to undertake quickly an ordinarily forbidden sexual lottery.

BLEYMAN and SIMON (1967) reported the occurrence of a dominant mutation (*Em-1*) in *T. thermophila*, formerly syngen 1 of the *T. pyriformis* complex (NANNEY and MCCOY 1976), which greatly reduced the stage of sexual immaturity in heterozygotes, and which was lethal in homozygotes. Whereas normal strains require 50 or more fissions to reach full maturity under ordinary growth conditions, the *Em-1* heterozygotes were mature by 10–15 fissions. Such mutants apparently occur frequently. BLEYMAN and SIMON (1967) reported that 4–10% of the progeny produced at conjugation during inbreeding in different strains may be early mature, even though their parents have the normal immaturity interval. Not all early mature lines can be shown to carry *Em* mutations however, for some of them fail to survive a subsequent conjugation and some fail to manifest the condition in their progeny. Nevertheless, independent *Em* mutants are apparently common. BLEYMAN (1971) analyzed two other mutants, *Em-2* isolated after nitrosoguanidine treatment, and *Em-3*, another spontaneous variant. The three mutants are not closely linked to each other, but another mutant was mentioned as being allelic to *Em-1*. Linkage tests were apparently not carried out on yet another mutant called *Em-4* (BLEYMAN 1972). *Em-2* differs from the other mutants in being viable in the homozygous state, but it is like them in what appears to be an important characteristic: all *Em* mutants show a marked

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reduction in growth rate, and erratic divisions during the period immediately following conjugation. After about 15 divisions all the cells are capable of mating, and after 20–30 cell divisions the cell cycle becomes approximately normal.

At first the *Em* mutants were expected to provide information on the mechanisms regulating long term temporal patterns in *Tetrahymena* (BLEYMAN 1971; NANNEY 1974). It was hoped that genic effects on maturity would also be reflected in the timing of other life cycle events, such as the pattern of initiation of allelic assortment. When such effects were sought, however, they were not found (BLEYMAN 1971). The *Em* mutants appeared to affect *only* the timing of maturity and the rate of early growth. Moreover, their erratic growth and other deficiencies prevented their use in experimental studies.

Recent experiments in which various treatments were applied to developing macronuclei, have now suggested that phenocopies of *Em* mutants are readily obtained. When conjugating cells are placed under adverse conditions, so as to interfere with early growth processes, a substantial fraction of such cells manifest the mating types which they would not ordinarily express for 40 or more fissions. This observation permits a unifying view concerning the early maturity phenotype, and suggests an hypothesis concerning an evolutionary tactic in these ciliates. It is also of practical importance in that it permits a more economical study of the composition of macronuclei mosaic for mating type elements than has been possible thus far (NANNEY and ALLEN 1959). The present report is concerned primarily with the induction of early maturity by treatment of late conjugants with high temperature.

MATERIALS AND METHODS

The strains used in this study were representatives of strains A and B of *Tetrahymena thermophila* (formerly syngen 1 of *T. pyriformis*). Cultures to be crossed were grown in 1% proteose peptone (PP) at 24°; they were washed from late exponential phase growth and mixed for mating in DRYL's (1959) salt solution. In some cases PP was added to the mating mixture at 6 hours after mixture, and in other cases only an hour before the beginning of isolations. Nutrients block the formation of new pairs (thus synchronizing the mating to some extent) and reverse early pairing events (thereby preventing the isolation of nonspecific "nonconjugants" when pairs are transferred directly to nutrient medium). In the standard protocol, individual pairs were isolated by micropipette into depression slides from the mating mixtures in the interval between 11 and 13 hours after mixing. In a few experiments they were isolated into PP medium, but in all the later experiments they were isolated directly into the bacterized media necessary for mating tests. The bacterized peptone (BP) medium was 1% proteose peptone, inoculated with *Enterobacter aerogenes*, incubated for 24 hours and stored at 4° for up to one week before use, when it was diluted 1/80 with deionized water. Immediately after isolation the pairs were placed in pre-warmed moist chambers at the temperatures indicated and left for 24 hours. In the case of $CaCl_2$ treatment, pairs were isolated into the solutions prepared in BP.

The clones derived from conjugating pairs (synclones) were treated somewhat differently depending upon whether the pairs were isolated into PP medium or BP medium. If a strong culture was not developed after three days, the synclone was classified as dead. Synclones growing in BP may be scored directly for the presence or absence of conjugating pairs. Such pairs may either result from "nonconjugation" or from early maturity. Nonconjugation is a general term referring to any interruption of the conjugation process before nuclear reorganization is completed. As noted, even normal pairs in early conjugation may be interrupted by the addition of nutrient, but such interruptions were avoided by the addition of nutrient an hour or more

prior to the isolation of pairs; at the time of isolation all persisting pairs are then fully committed to meiosis and subsequent nuclear events. Interruptions at later stages can be associated with cytogenetic irregularities (ALLEN 1963; NANNEY 1963), or with adverse environmental conditions (NANNEY 1976). The evidence for an aborted conjugation is the persistence of the phenotype of the parental strains into the progeny, assured by the persistence of the somatic nucleus from the parental strain. Maturity and mating types are the most commonly used indicators of somatic persistence, but in some of the crosses to be presented, the parents were also of different antigenic type, and questionable progeny could also be examined for heterozygous serotypes (NANNEY and DUBERT 1960). Because mating type determination is "caryonidal" (NANNEY 1956), synclones usually contain two or more mating types and parental mating types can be generated occasionally by chance, even when new macronuclei develop; this second test for completion of conjugation can be useful when available. The two exconjugant clones composing a true synclone are genetically equivalent and nearly all the cells produced during the early divisions respond to antisera against both parents.

The appearance of conjugation in the first synclonal culture is ordinarily considered evidence for genetically conditioned nonconjugation, and under ordinary circumstances most such cultures can be shown to be nonconjugant by the appropriate tests. The high frequencies of selfing synclones in these experiments showed, however, that the treatment caused the selfing synclones, either by aborting conjugation or by inducing early maturity. To determine the nature of the effect, three single cell isolations were made from some of the cultures and these secondary cultures were tested for mating type. Most of the synclones with large numbers of selfing pairs were found to manifest only parental phenotypes, while most of those with fewer pairs expressed nonparental mating types and/or serotypes. The lower frequency of mating pairs in mixtures with Em phenotypes reflects in part their early poor growth, and in part the fact that the clone is just becoming mature and is not strongly reactive. In some experiments all the synclonal selfers were not analyzed, however, but were interpreted as aborted conjugants unless compelling evidence to the contrary was available. Hence, the frequencies of early mature lines are underestimated, and perhaps underestimated to different extents in different experiments; the frequencies of aborted conjugants tend correspondingly to be slightly overestimated.

Synclonal selfing cannot be observed in cultures developing in PP medium, because this medium prevents conjugation. For analysis of such cultures, a drop of the synclone was inoculated into a test tube containing about 5 ml of BP medium, and this derived culture was tested for mating type. Selfing at this time was again considered *prima facie* evidence for nonconjugation, but sublines isolated from the original synclone and later tested for mating type sometimes contradicted this interpretation. Again, the scored frequency of early mature lines from pairs grown in PP is an underestimate of the actual frequency.

RESULTS

1. *Effects of high temperature during late conjugation on viability and macronuclear development*

The major results are displayed in Table 1 and represent crosses carried out over a period of approximately one year. For each cross the fraction of pairs dying is calculated as the number that failed to establish a flourishing culture within three days of isolation, divided by the total pairs isolated. The fraction of pairs assumed to be aborted is calculated as the number that produce mature progeny manifesting only parental phenotypes, divided by the total pairs isolated less those that failed to survive. Finally, the fraction of early mature pairs consists of those that are mature within 15–20 cell divisions but which manifest nonparental phenotypes with respect to mating type and/or serotypes, divided by the total of late mating and nonparental early mating synclones. Calculated

TABLE 1

Summary of effects of temperature on macronuclear abortion and early maturity in T. thermophila

Parental strains	Exp. no.	Temp. °C	Time exposed	Died fraction	%	Mature-parental fraction	%	Mature-nonparental fraction	%
A × A	75–29	24		4/72	6	9/68	13	2/59	3
	76–3	24		1/90	1	8/89	9	0/81	0
	76–6	24		1/72	1	3/71	4	1/68	1
	76–3	35	M	5/120	4	6/115	0	0/115	0
	76–6	36	M	3/144	2	1/141	1	0/140	0
		Total		14/498	3	21/484	4	3/463	1
	75–29	37	E	3/72	4	16/69	23	1/53	2
	75–29	37	M	11/69	16	29/58	50	7/29	24
	75–29	37	L	2/72	3	18/70	26	6/52	12
	75–34	37	M	15/36	42	5/21	24	3/16	19
	76–1	37	M	126/422	30	109/296	37	2/187	1
	76–10	37	M	6/108	6	3/102	3	10/99	10
		Total		163/779	21	180/616	29	29/436	7
	76–6	39	L	70/72	97	2/2	100	——	—
B × B	76–4	24		0/90	0	7/90	8	0/83	0
	76–7	24		0/92	0	4/72	6	1/68	1
	76–4	35	M	2/120	2	12/118	10	0/106	0
	76–7	36	M	6/144	0	6/144	4	0/138	0
		Total		2/426	<1	29/424	7	1/395	<1
	75–30	37	M	29/108	27	7/79	9	10/72	14
	76–1	37	M	85/180	47	69/95	73	16/26	62
	76–11	37	M	2/108	2	9/106	8	3/97	3
		Total		116/396	29	85/280	30	29/195	15
	76–7	39	L	48/72	67	9/24	38	0/15	0
A × B	75–26	24		2/72	3	2/70	3	2/68	3
	75–27	24		27/288	9	40/261	15	3/221	1
	76–2	24		15/180	8	9/165	5	0/156	0
	76–5	24		1/90	1	4/89	4	0/85	0
	76–8	24		4/72	6	0/68	0	1/68	1
	76–5	35	M	3/120	3	6/117	5	0/111	0
	76–8	36	M	2/144	1	4/142	3	0/138	0
		Total		54/966	6	65/912	7	6/847	1
	75–26	37	M	29/108	27	7/79	9	10/72	14
	75–27	37	M	22/307	7	82/285	29	5/203	2
	76–1	37	M	47/180	26	46/133	35	36/87	41
	76–12	37	M	2/108	2	2/106	2	23/104	22
	76–13	37	M	1/108	1	9/107	8	9/98	9
	76–14	37	M	5/144	3	3/139	2	32/136	24
	76–15	37	M	3/144	2	2/141	1	6/139	4
		Total		109/1099	10	151/990	15	121/839	14
	76–8	39	L	56/72	78	0/16	0	0/16	0
	75–27	40		90/108	83	17/18	94	1/1	100

Pairs were retained at 24°C unless otherwise noted. M pairs were exposed for 24 hours at a higher temperature beginning 11–13 hours after mixture. E pairs began exposure 2–3 hours earlier. L pairs began exposure 2–3 hours later.

in this way, each measure is allowed to vary from 0 to 100% and is independent of the others.

The A × A crosses carried out entirely below 37° gave an inviability percent of approximately 3%, an abortion rate of about 4% and an early maturity rate of about 1%. The corresponding figures for pairs exposed to 37° late in conjugation are 21%, 29% and 7%. Exposure to 35° and 36° had no demonstrable effect with respect to any of these measures, while an exposure to 39° at about the same time is almost completely lethal to the pairs. Considerable variability is observed within the 37° series. Some of the variability may be associated with the time the temperature treatment was begun. In one experiment three times of exposure were used, one (E) 2–3 hours earlier than the standard treatment (M) and one 2–3 hours later (L). All measures of trauma were highest when the temperature treatment was initiated at 9–11 hours after mixture of the cells. This factor does not explain all the variability, however. Exp. 76–1, for example, had a high frequency of death and abortion, but very little early maturity. In contrast, Exp. 76–10 had relatively low frequencies of death and abortion, and yet had substantial early maturity. Such variability might be expected of threshold phenomena, particularly if the various measures represent somewhat dissociable effects. Moreover, the temperature control equipment was perhaps not sufficiently sensitive for maintaining the temperature within narrow ranges.

The results in the B × B crosses are similar to those with the A × A crosses. The frequency of death in the controls (below 37°) is slightly lower and the frequency of abortion slightly higher. Again treatment with 37° generally increases all the measures of trauma. At 39° survival is greatly reduced, but among the few survivors no early mature lines were detected.

The largest amount of data is available for the A × B crosses, and it agrees with that for the other crosses. All measures of trauma are increased at temperatures above 36°, but considerable variability is apparent from experiment to experiment. Very few pairs survive with newly developed macronuclei at 39° or 40°.

2. *Effects of laboratory misadventures on viability and early maturity*

The crosses in Table 1 are characteristic of most of the crosses in this laboratory with these strains. Occasionally, however, a control cross performs significantly less well. Sometimes the precise cause of the aberrant behavior can be identified—as inadequately cleaned glassware or improperly prepared media, etc.—but often the cause is obscure. On one occasion during the interval represented by the crosses already discussed, such misbehavior was simultaneously observed with two crosses conducted on the same day (Table 2). The problem did not reflect the intrinsic capabilities of the strains, which performed normally both before and after, and must have arisen from some common environmental factor. The controls of both crosses (24°) showed a high frequency of dead pairs, and one of them (75–36) had high frequencies of mature parental and mature nonparental pairs also. In comparison, pairs from the same cross exposed to 37° showed even greater effects of trauma. The death and abortion rates were increased in 75–36, while the abortion and early maturity rates were increased in 75–35. The chief reason for presenting these data, however, is to indicate that early maturity may

TABLE 2

Summary of temperature effects on crosses simultaneously exposed to an unidentified environmental hazard

Parental strains	Exp. no.	Temp. °C	Time exposed	Died fraction	%	Mature-parental fraction	%	Mature-nonparental fraction	%
B × B	75–36	24		13/72	18	11/59	19	14/48	29
		37	L	31/72	43	13/41	32	9/28	32
		37	M	74/108	69	11/34	32	5/23	22
A × B	75–35	24		26/72	36	0/46	0	1/46	2
		37	E	32/72	44	3/40	8	8/37	22
		37	M	17/108	16	5/91	5	25/86	29

Symbols as in Table 1.

be augmented without heat exposure under conditions indicative of other adverse environmental factors. Early maturity does not always accompany high death and abortion rates, but it often does; never has a high rate of early maturity been found without some other indication of trauma.

3. *Calcium chloride effects on early maturity*

The general linkage between early maturity and trauma has been observed on other occasions, particularly with the use of concentrated cell extracts, but only one other set of data will be offered in documentation of the association. We recently reported (NANNY 1976) that calcium chloride increases the rate of macronuclear abortion when conjugating pairs are isolated into 0.1 N solutions (not 0.1 M solutions as erroneously reported) under circumstances very similar to those reported here. Table 3 summarizes experiments in which systematic efforts were made to distinguish between macronuclear abortion and induced early maturity. They demonstrate that $CaCl_2$ does indeed provoke macronuclear abortion, but that it also induces early maturity.

Cross 75-26 employed three concentrations of $CaCl_2$. At a concentration of 0.05 N, the results were scarcely distinguishable from the control series (see same experiment in Table 1), but at 0.1 N, all three measures of abnormality were

TABLE 3

Summary of effects of $CaCl_2$ on macronuclear abortion and early maturity

Parental strains	Exp. no.	$CaCl_2$ conc.	Died fraction	%	Mature-parental fraction	%	Mature-nonparental fraction	%
A × B	75–26	0.05 N	7/72	10	4/65	3	2/61	3
		0.10 N	70/144	49	21/74	28	6/53	11
		0.15 N	118/144	83	17/25	68	1/8	12
A × B	76–16	0.10 N	40/72	56	7/32	22	24/25	96
A × B	76–17	0.10 N	48/72	67	2/24	8	12/22	55

Exposure for 24 hours beginning 11–13 hours after mixture.

increased. The mortality rate was greatly increased at 0.15 N, thus reducing the sample sizes and the statistical significance of the other measures.

Experiments 76-16 and 76-17 were simultaneous crosses using different parental lines and only one $CaCl_2$ concentration. Both crosses gave high proportions of death, abortion and early maturity.

4. *The breeding performance of induced early mature lines.*

These studies established clearly the induction of early maturity by means of high temperature or $CaCl_2$ solutions. They do not reveal the nature of the alteration in the cells, particularly with respect to its heritability. Does the treatment induce a heritable change capable of being transmitted to sexual progeny, perhaps even localizable to nuclear genes? Or does the treatment only induce phenocopies, so that the progeny manifest the usual interval of sexual immaturity following conjugation?

The breeding performance of 15 early mature lines from as many pairs from Exp. 75-35 was studied by means of crosses to strain B. Early maturity mutants thus far studied are all dominant; if the induced early maturity lines bear newly induced mutations, they should segregate in a cross to a normal strain to give 50% early mature and 50% normal progeny. If, however, the effect is transitory (or if, paradoxically, new mutations are dominant in the first generation and recessive in a second generation) the progeny should include no higher proportion of early maturity than crosses between wild-type strains.

The frequencies of both death and abortion (Table 4) in the backcrosses were higher than had been observed in the original crosses among the inbred strains (Table 1), but not exceptionally high for randomly selected strains produced in crosses. The "standard" breeding strains are selected from sibships because of their superior breeding performance. The progeny of such strains usually, however, manifest a wide distribution of performance. The "standard" strains are in a sense "phenodeviants," manifesting unusual properties not reliably transmitted to their progeny. That, however, is a secondary consideration. For our purposes, we need only note that none of the 15 lines tested showed the 50% early maturity progeny expected from a heterozygous dominant parent. The early maturity phenotype provoked by high temperature treatment is not transmitted to the progeny, and is most plausibly considered a phenocopy of the gene-associated *Em* phenotype.

DISCUSSION

Tetrahymena thermophila owes its specific name to its ability, unique among tetrahymenas, to grow at 40°, in contrast to strains of other species, which fail to sustain growth above 37°. Yet *T. thermophila* is not able to pursue all its affairs at the highest temperatures. Mating types are distributed at random among the four caryonides produced by each pair (NANNEY 1956) but the frequencies of the mating types are sensitive to the temperature at which the macronuclei developed. Studies on the effect of temperature on mating-type frequencies (NANNEY 1960) were limited to an upper range of 34°, because pairs

exposed to higher temperatures during the whole conjugal sequence were unable to survive. The present observations grew in part from an attempt to ascertain temperature effects on mating type determination at higher levels, by applying the temperature differentials only in later stages of conjugation, after the possibly more sensitive meiotic stages are passed. This procedure is at least partially successful, for no difficulties are observed when late conjugants are exposed to 35° or 36°, and many survivors are obtained at 37°. Late conjugation, probably macronuclear development, is however more sensitive to high temperatures than is the ordinary cell cycle, which can be sustained at 40° or slightly higher.

Of greater present interest, however, is the observation that certain adverse environmental conditions encountered during macronuclear development in Tetrahymena induce a precocious maturity. Since both $CaCl_2$ treatment and high temperature treatment have this effect, it may be a general response to adversity. Whether an environment is "adverse" depends of course, on the genotype of the cell; a perfectly appropriate environment to one cell may be inappropriate to another. This consideration permits the genotypic early maturers to be associated with the environmentally induced early maturers. The early maturing lines all show slow and erratic growth immediately following conjugation, as do those conjugants of normal genotype exposed to adverse environments. The hypothesis arising from this association is that early maturity is a general response of exconjugant tetrahymenas to an incompatibility between the genotype and the environment.

Although the proximate mechanism for this "early maturity shunt" is unknown, a facile evolutionary rationalization is available. SONNEBORN (1957) has developed the thesis that the immaturity period in a ciliate is a device for the regulation of breeding patterns. For planktonic organisms, time and distance are

TABLE 4

Breeding performance of heat-induced early mature lines in back-crosses to strain B

F_1 line	Died fraction	%	Mature-parental fraction	%	Mature-nonparental fraction	%
19	9/28	32	2/19	11	1/17	6
24	6/30	20	4/24	17	0/20	0
32	12/57	21	9/45	20	0/36	0
69	6/28	21	12/22	55	0/10	0
71	7/30	33	12/23	52	0/11	0
155	13/30	43	2/17	12	0/15	0
164	9/30	30	1/21	5	0/20	0
177	3/30	10	8/27	30	0/19	0
181	0/30	0	3/30	10	1/27	4
184	4/30	13	9/26	35	0/17	0
197	9/30	30	3/21	14	2/18	11
204	4/30	13	10/26	38	0/16	0
225	3/28	11	3/25	12	0/22	0
234	7/30	33	2/23	9	0/21	0
238	5/30	17	11/25	44	0/14	0
Totals	97/471	21	91/374	24	4/283	1

closely related parameters. An organism which is able to mate soon after a previous mating will encounter "close" relatives, in both a genetic and geographic sense. An organism which mates only after a long interval will be more likely to be separated from its parents and siblings; it will be more likely to mate with a stranger. NYBERG (1974) has recently provided significant support for the relationship between the length of the immaturity period and the means whereby an organism responds to environmental variables.

In the present context, the early maturity shunt would fall into the same category of mechanisms as those responsible for "senile selfing." *Paramecium bursaria* species are ordinarily outbreeders which have long immaturity periods and strict prohibition of intraclonal mating, but JENNINGS (1941) noted that strains maintained in monastic isolation in the laboratory, as they near the ends of their life cycles, sometimes acquire the ability to mate within a clone (undergo selfing). A similar phenomenon has been described by HECKMANN (1967) in *Euplotes crassus*. In both cases, the loss of the "incest taboo" can be considered an adaptation to an atypical circumstance. Mating with a relative (or the self), while not ordinarily sanctioned, is evolutionary preferable to death from senility. The utility of this shunt, particularly for a colonizing population, should give it a selective advantage. In the present cases, a similar explanation might be applicable. Given that the genotype-environment relations of a new conjugant are inappropriate, a quick return to the genetic lottery might sometimes restore compatibility. And this early mating, even with close relatives, might be preferable to extinction.

The plausibility of this interpretation depends in part upon whether the induction of early maturity occurs too early for new gene action to have begun, and before incompatible genotype-environmental reactions could have been sensed. The experiments give only limited information on the timing of the effect. All the exposures to adverse conditions lasted for 24 hours. They differed only in the time at which exposure began, and all continued through the interval during which the first two or three postzygotic cell divisions usually occur. The most marked effects were found with exposure beginning at 11–13 hours after mixture, *i.e.*, about 1–9 hours after the initiation of pairing, and shortly after pronuclear exchange and zygote formation. Exposure beginning even 2–3 hours later, and near to the time of separation of the cells, still had a significant influence. These results, however, must be considered preliminary in view of the variability of the responses. Nothing in the observations, however, renders implausible the hypothesis of a period of genotype-environment testing following conjugation. Studies on the length of the "phenomic lag" associated with gene substitutions in Tetrahymena show that some gene substitutions at least are detectable before the first post-zygotic cell division (BRUNS 1974). More difficult to evaluate is a mechanism capable of sensing a wide variety of environmental incompatibilities.

Regardless of the mechanism of early maturity induction, or its evolutionary rationalization, the phenomenon itself is of practical utility in certain studies. Particularly to be noted are studies on the composition of macronuclei mosaic for mating types. Mosaic macronuclei usually comprise less than 10% of all macro-

nuclei examined, when their composition is determined by carrying 30 sublines through the period of immaturity. Hence, experiments to analyze mosaics with no useful information. Early maturity clones can be scored, however, very soon (by observing intraclonal selfing) and only the mosaic lines expanded (BLEYMAN 1973). Genetic early maturers have not been very helpful, however, because of their general debility. Early mature phenocopies provide greatly increased efficiency of analysis and should permit much more complete information on the associations of mating types in mosaic macronuclei (NANNEY and ALLEN 1959). Indeed, this procedure has already been used to greatly increase our sample of mating type mosaics. These studies will be reported subsequently.

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