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Transmission of a genetic trait through total conjugation in a hypotrich ciliate *Paraurostyla weissei*. Genetic basis of the multi-left-marginal mutant

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SUMMARY

The genetic basis of slow growth rate and aberrations in the ciliary pattern was studied in the multi-left-marginal variant of *Paraurostyla weissei*. The 3:1 segregation in F2 sibling crosses and 1:1 segregation in test crosses indicate that the aberrant phenotype is controlled by a recessive allele at a single gene locus termed *mlm*. The phenotypic change from wild type to *mlm* takes place about 5–8 cell cycles after conjugation. The study established that total conjugation in *P. weissei* is a true sexual process in which meiosis, fertilization and Mendelian segregation occur.

1. INTRODUCTION

In the ciliated protozoans used in genetic studies (*Paramecium*, *Tetrahymena*, *Euplotes*), the migratory nuclei exchanged in the sexual process move between cells that retain their structural individuality. In this paper we report genetic studies of a cortical pattern abnormality in a ciliate *Paraurostyla weissei* that undergoes 'total conjugation'. During total conjugation the two mates fuse completely and give rise to only one progeny cell (Heckmann, 1965; Heumann, 1975).

When cells of complementary mating types are mixed, tight pairs are formed. The left partner is called 'donor', the right 'recipient'. The association appears to be random, each mating type could be either donor or recipient. Concomitant with meiosis in both conjugating cells the resorption of ciliature takes place in a specific order characteristic for the donor and the recipient cell respectively, the donor is gradually absorbed by the recipient and a zygocyst is formed (Jerka-Dziadosz & Janus, 1975). Fertilization, development of the new nuclear apparatus and the final degradation of the old nuclei occur in the zygocyst. The remnants of the old ciliature are completely resorbed and only the subcortical microtubular cytoskeleton of the recipient cell is retained throughout the zygocyst stage (Jerka-Dziadosz, 1984). In the zygocyst the development of the new macronuclear anlagen takes place, 8–10 days after cell union the primordia of ciliature are formed and a *single* exconjugant develops from the zygocysts.

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P. weissei, therefore, offers the opportunity to study the acquisition of cortical pattern abnormalities after conjugation when the ciliature is formed anew, without constraints imposed by the pre-existing ciliature (Heckmann & Frankel, 1968; Frankel, 1973*a*), and when the materials of two previous morphogenetic domains have been combined.

In a previous study an abnormal strain of *P. weissei* was described (Jerka-Dziadosz & Banaczyk, 1983), that possessed supernumerary rows of the left marginal cirri and was thus termed multi-left-marginal (*mlm*). The phenotypic aberrations were postulated to be caused by a recessive mutation of a single gene. The genetic analysis presented in this paper shows that the *mlm* phenotype is indeed controlled by a recessive gene which we termed *mlm*. The *mlm* mutant of *P. weissei* is the first case in the literature in which a genetically based abnormality in the cortical pattern is described in a fresh-water hypotrich ciliate undergoing total conjugation. A preliminary report of this study was published elsewhere (Dubielecka & Jerka-Dziadosz, 1984).

2. MATERIAL AND METHODS

(*a*) *Material.* Cells used in this investigation were clones of *P. weissei* isolated from natural sources in Poland. Lines Z6, Z8, G and line N-1 were all phenotypically wild type. Other lines studied were progeny lines obtained after conjugation. The *mlm* mutant of *P. weissei* was first discovered in the progeny derived from a cross between lines Z6 and Z8. An F1 progeny line, Z68, was back-crossed to Z6. Among the progeny lines one mutated line was isolated and grown further (C6). This line was then crossed to line G. The progeny of this cross (E) was the starting material for this investigation (Jerka-Dziadosz & Banaczyk, 1983).

Lines labelled with letter B are progenies of E4 × E5′, lines K from a cross between B16 and N1. Lines D are progenies of K1 × K6, line M6 is a progeny line from cross B16 × K6 (see also Table 1).

(*b*) *Cultures.* Cells of *P. weissei* were kept in Petri dishes in a modified Pringsheim solution (Jerka-Dziadosz & Frankel, 1969) and fed daily with the green alga *Chlorogonium* grown on earth medium (Heckmann, 1963).

(*c*) *Cytological preparation.* Cells were stained with silver proteinate (Protargol) using two protocols described in detail elsewhere (Jerka-Dziadosz & Frankel, 1969; Jerka-Dziadosz, 1985).

(*d*) *Crosses and isolation of progeny lines.* In order to make a cross, two lines of complementary mating types were poured into one Petri dish and a little fresh food was added. Pairs appeared in the mixture usually the next day, when the cells were slightly starved. Zygocysts (i.e. late conjugants) were isolated from crosses of cells with wild-type phenotype. From crosses of mutant clones the tight pairs (i.e. early conjugants) were isolated, in order to avoid confusing zygocysts with rounded up monstrous mutants (Jerka-Dziadosz & Banaczyk, 1983).

Single pairs or zygocysts were placed in depressions of the three spot depression slides in Pringsheim solution and were kept in moist chambers. During the next 7–10 days a single exconjugant emerged from each zygocyst. The exconjugants were then fed and after a culture developed it was transferred to a test-tube or

to a Petri dish. In experiments involving the analysis of expression of phenotypes, the division rate of exconjugants was noted in each depression over a period of several days. When a given line manifested simultaneously the decline in growth rate, change in cell shape and presence of cortical reorganization (i.e. a morphogenetic process in which the whole set of ciliature is replaced by new one), instead of cell division, the line was assessed as a mutant line. Samples of these lines were then stained with Protargol. The presence of additional marginal rows on the left side confirmed the *mlm* phenotype. The remaining exconjugant lines showing neither morphological changes nor decline in growth rate were considered as phenotypically wild type. Lines selected at random were stained in order to confirm this diagnosis.

3. RESULTS

(i) *The morphology of the wild type of* P. weissei

A detailed description can be found in earlier reports (Jerka-Dziadosz, 1976; Jerka-Dziadosz & Banaczyk, 1983). Here only the important features relevant to the mutant morphology will be given.

The non-dividing cell of wild-type *P. weissei* is an elongated oval, dorso-ventrally flattened, and measures approximately 200 μm. The oral apparatus and locomotory cirri are located on the ventral surface (Pl. 1). The oral ciliature consists of an adoral zone of membranelles (AZM) which bounds the left side of the peristome and the anterior margin of the cell. The right side of the peristome is flanked by two (inner and outer) undulating membranelles (UM's). Seven or eight frontal cirri are situated posterior to the distal portion of the AZM in two oblique rows. The middle of the ventral surface is occupied by four or five (sometimes six) longitudinal rows of cirri. Posterior to the ventral cirri is located an oblique row consisting of seven to nine transverse cirri. A single row of marginal cirri is located at both the right and left margins of the ventral side.

The dorsal surface is covered by five or six rows of dorsal bristle units (Jerka-Dziadosz, 1982). On the posterior tip of the cell a small group of caudal cirri are present which originate at the posterior ends of the dorsal bristle primordia. Although the general pattern of arrangement of the ciliature is fairly stable, the number of cirri and membranelles and their constituent basal bodies is directly related to the cell size (Jerka-Dziadosz, 1976).

(ii) *Characteristics of the* mlm (*multi-left-marginal*) *mutant of* P. weissei

The *mlm* variant described previously (Jerka-Dziadosz & Banaczyk, 1983) shows several modifications in its cortical pattern in addition to slower growth rate and changes in body proportions. The most important feature of the cortical pattern that differentiates the *mlm* variant from normal cells is the multiplication of the number of rows of the left marginal cirri (LM) (Pl. 2). In a culture of *mlm*, cells are present which do not differ from normal wild-type cells as well as cells which have up to 8 rows of the left marginal cirri. The percentage of cells with an elevated number of marginal rows depends on the cell line and culture conditions, but cells

with 2 and 3 LM's constitute about 80% of a given population (Jerka-Dziadosz & Banaczyk, 1983). The extra marginal rows are located to the left of ventral cirri; sometimes the left margin is humped and marginal rows do not cover the whole margin but form short segments containing few cirri. Other defects in the cortical pattern are less frequent. These include: a variable number of frontal and/or transverse cirri, additional bristle unit rows or (rarely) a decreased number of rows

Table 1. *Genetic interpretation of the* mlm *phenotypic*

Cross	Genotypes of parents	Percentage of survival	Experimental results	Theoretical value	P
E4 × E5′	*mlm*/+ × *mlm*/+	54·0	Wild type 84	81	$P > 0{\cdot}6$
			mlm 24	27	
E4 × E5′	*mlm*/+ × *mlm*/+	56·4	Wild type 38	38·25	$P > 0{\cdot}9$
			mlm 13	12·75	
B16 × N1	*mlm*/*mlm* × +/+	77·3	Wild type 17	17	—
K1 × K6	*mlm*/+ × *mlm*/+	62·0	Wild type 24	23·25	$P > 0{\cdot}7$
			mlm 7	7·75	
K6 × K20	*mlm*/+ × mlm/+	60·0	Wild type 19	18	$P > 0{\cdot}5$
			mlm 5	6	
B16 × K6	*mlm*/*mlm* × *mlm*/*mlm*	47·9	Wild type 12	11·5	$P > 0{\cdot}5$
			mlm 11	11·5	

of the ventral cirri. Cultures of *mlm* have a tendency to form monsters, occasionally producing unstable doublets.

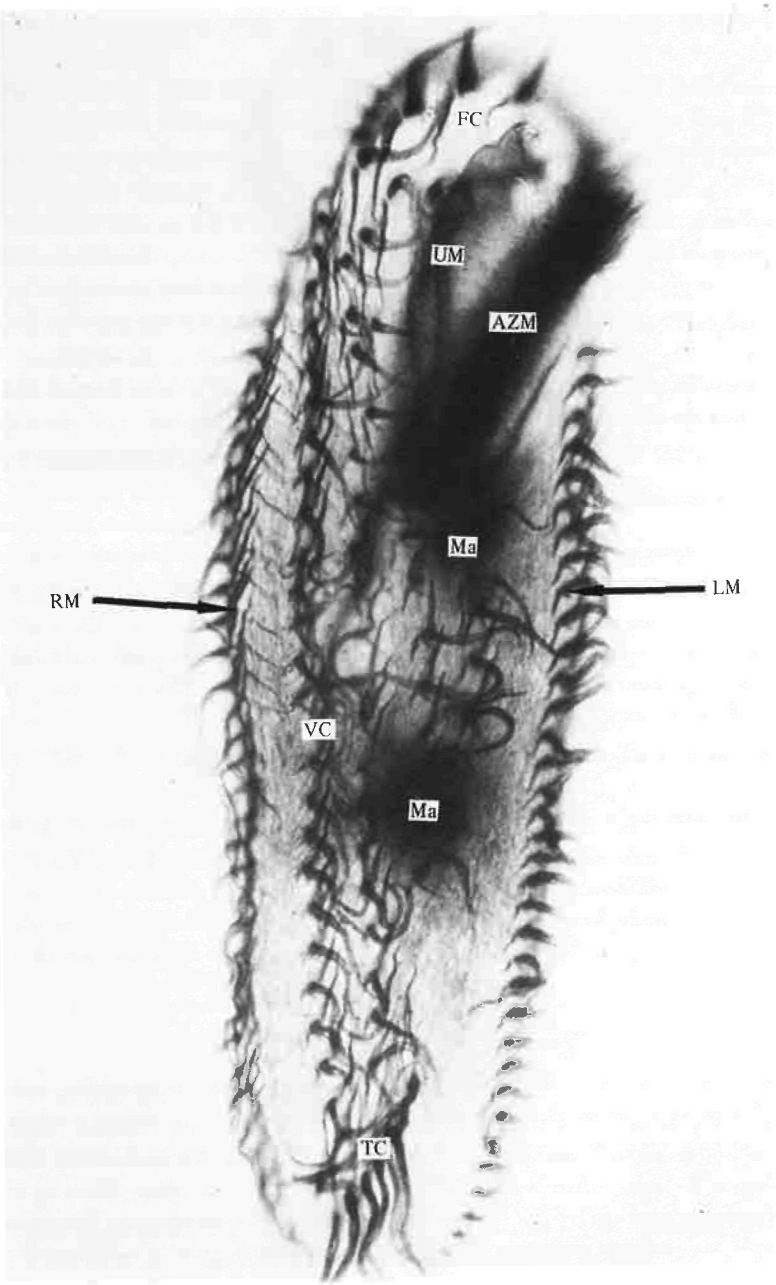
The previous study established that the growth rate of *mlm* lines is about half that of wild-type cells. The slow growth is caused by a prolonged cell cycle time and is influenced also by an increased frequency of cortical reorganization, monster formation and cell death. The relatively high mortality of the cells from *mlm* cultures is manifested first by rounding up of cells which are no longer able to feed.

(iii) *Analysis of crosses*

After mixing of two phenotypically wild-type lines of the complementary mating types a large number of pairs were formed. When a *mlm* line was crossed with wild type, only a few pairs were formed at the beginning. This poor mating is caused by a longer cell cycle time of the *mlm* cells. New pairs in the conjugating mixture appear over a period of several days.

In most experiments not all zygocysts develop successfully into new lines. In all crosses some isolated zygocysts die even before the exconjugant is formed. Exconjugants may develop and go through one or several divisions after which the line dies out. In a given experiment only those lines that produced mass cultures were analysed. Samples of these cultures were fixed and stained with Protargol.

The starting material for the genetic analysis performed in this study were the heterozygous lines E4 and E5′ obtained previously (Jerka-Dziadosz & Banaczyk, 1983). Two crosses between these lines were performed (Table 1), and both the phenotypic segregation ratio was 3:1. This suggested that the studied pheno-



PLATES 1 AND 2

Protargol stained cells of *Paraurostyla weissei* showing the ventral surface. The anterior of the cell is up, the left on viewers right hand side. Abbreviations: AZM, adoral zone of membranelles; UM, undulating membranelles; FC, frontal cirri; VC, ventral cirri; TC, transverse cirri; LM, left marginal cirri; RM, right marginal cirri. Plate 1 shows a cell from the wild-type line N1. Plate 2 shows a *mlm* cell from line B 21 with two cirral rows (1LM and 2LM) on the left margin.

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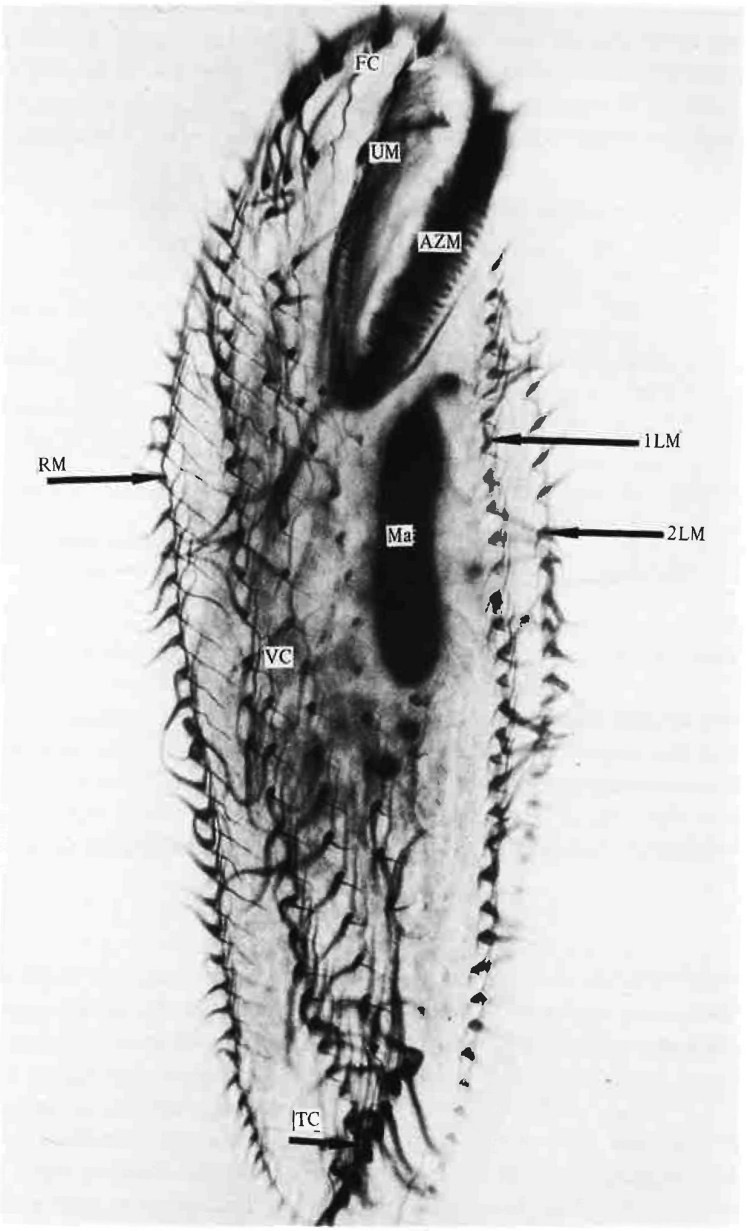

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type is controlled by a recessive allele which segregates in a simple Mendelian fashion. In order to confirm this conclusion several other crosses were performed (Table 1).

One of the progeny lines obtained in the previous experiment (B16) with an assigned genotype *mlm/mlm* was crossed to a presumably wild-type line N1 freshly collected from a natural pond. It was assumed that in the F1 progeny of the cross the *mlm* should not appear if the N1 line is a homozygous dominant line with respect to the *mlm* allele. All studied F1 lines were phenotypically wild type, and were probably heterozygous (Table 1). In order to prove this assumption the F1 daughters of the cross between N1 and B16 (labeled K) were sibcrossed to each other. In two crosses the phenotypic ratio of *mlm* and wild type was again 3:1 as in the first experiment.

In the next experiment a test cross was performed between the mutant line B16 (*mlm/mlm*) and line K6 (*mlm/+*). The pairs appeared in the mixture over a relatively long time and were isolated in a 4-day span. Half of the obtained progeny lines were wild type and the other half were multi-left-marginal, resulting in the phenotypic segregation ratio of 1:1.

Next, we performed a cross between two mutant lines (*mlm/mlm* × *mlm/mlm*). All attempts to rescue the progeny, however, failed.

Line B33 and B21 were crossed twice. In the first cross the zygocysts were kept at room temperature (20±2 °C), in the second cross they were kept in an incubator (27 °C). All pairs formed zygocysts, but only three zygocysts developed into exconjugants. Those exconjugants grew, moved around and fed, but they did not divide, and died. Therefore it was not possible to assess their morphology or division rate.

In a crosses of line B33 and M6, during 2 days 51 pairs were isolated. Among this sample 6 zygocysts developed in due time and exconjugants lived for 10 days. These however, did not divide and eventually died, after passing through several cortical reorganizations. In effect not a single exconjugant formed a line which could be analysed further.

(iv) *Expression of the* mlm *phenotype*

During the study on exconjugant lines we observed that the *mlm* lines began to express their mutant phenotypic traits some time after the exconjugants started dividing. The first phenotypic difference from wild type observed is the slowing down of the growth rate. In order to determine exactly the time of expression of the decline of the growth rate in the mutant clonal life cycle, the generation time of progeny lines from a cross between K1 and K6 was studied.

After mixing of two heterozygous lines (K1 × K6), 23 exconjugants were isolated singly into depression slides. Every second day a single cell was reisolated from each depression. The number of cell divisions occurring in each depression during 24 h was then calculated every day for 14 days. The numbers were added up and the mean numbers of divisions per day were calculated separately for wild type (20) and *mlm* lines (3). The data are presented in Fig. 1. When these data are recalculated into cell cycle times it appears that during the first 4 days of the

experiment both wild type and *mlm* lines have the cell cycle time about 17·1 h. During the fifth day there is a decline in the growth rate in the mutant lines and from that time on the mean cell cycle time for the *mlm* lines is about 30 h. This means that the phenotypic change from wild type to the mutant took place after about 5 cell divisions.

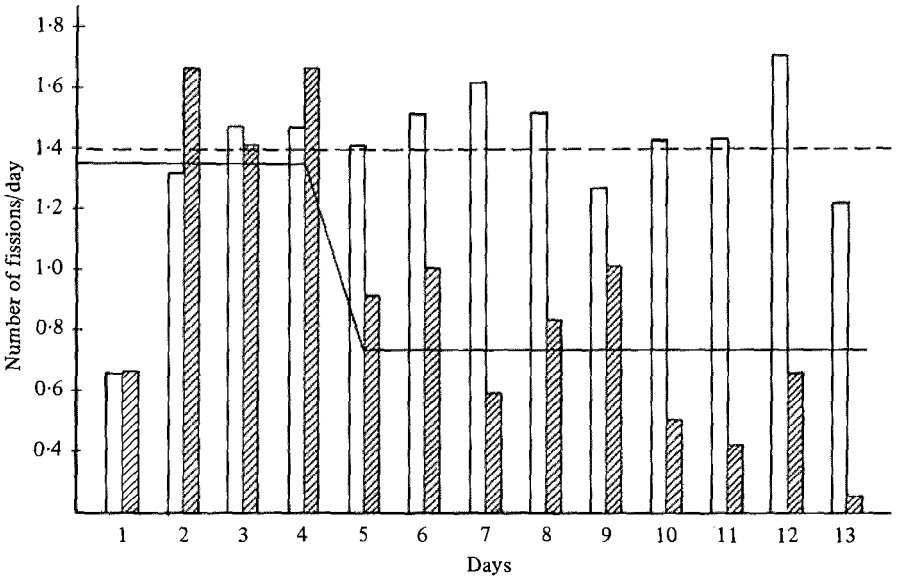

Fig. 1. A histogram showing the mean number of fission per day during first 13 days after conjugation in wild type (open bars) and in *mlm* (hatched bars) progeny lines of a cross K1 × K6. Solid black line shows the mean of the total number of fissions per day in *mlm* lines, broken lines shows the mean of the total number of fissions per day in the wild type lines during 13 days.

4. DISCUSSION

The study presented in this paper was aimed at establishing the genetic basis of the slow growth rate and the cortical pattern abnormalities found in the multi-left-marginal variant of the hypotrich ciliate *P. weissei*. The pattern of phenotypic segregation in progeny lines (3:1 in F2 sibling crosses and 1:1 in test crosses) clearly indicate that the multi-left-marginal phenotype is controlled by a recessive allele (*mlm*) at a single gene locus.

In spite of the clear-cut results (Table 1), some comments concerning the mating-type system, intraclonal conjugation and survival after conjugation are required.

In *P. weissei* six complementary mating types were collected from natural sources during several years. Some of these stocks displayed intraclonal conjugation (Jerka-Dziadosz & Janus, 1975). Since repeated isolations of zygocysts from such lines failed to produce any exconjugants, it was concluded that intraclonal conjugation in *P. weissei* is lethal in a majority of cases. When cells of complementary mating types are mixed, homotypic pairs (i.e. where both partners belong to the

same mating type) are occasionally formed and in rare instances (conjugation of double cells) viable progeny may develop from them (Jerka-Dziadosz, unpublished observation). Though the lines used in this investigation (Table 1) did not show spontaneous intraclonal conjugation, formation of homotypic pairs after stimulation with complementary mating types is not excluded. The rather poor survival (about 60 %) after conjugation in most crosses (Table 1) might indicate that intraclonal conjugation did contribute to the total number of formed pairs, especially in cases where pairs were isolated during more than 1 day. Such pairs most probably did not produce viable progeny.

When two *mlm* lines were crossed with each other no viable progeny was formed. This is not surprising in the light of several reports that homozygous mutant lines frequently do not produce viable progeny (c.f. *janus* mutant of *Tetrahymena* (Frankel & Jenkins, 1979), early maturity mutant in *Tetrahymena* (Bleyman & Simon, 1967).

As in the basal-body-deficient mutant of *E. minuta* described by Frankel (1973*b*) the *mlm* cells were not impaired in any process related to conjugation, and yielded viable progeny when they were crossed with cells which were not *mlm*. It seems that the wild-type product of the *mlm* allele is indeed essential to carry on the cell to the first cell division after conjugation.

When the wild-type nucleus becomes replaced by the *mlm* nucleus it takes about 5–8 cell divisions (5 days) for the cells to show a decline in the growth rate and in some cases at least 3 weeks for the cells to acquire abnormalities in the cortical pattern. Delays in expression of the genically determined new cortical pattern have been described in other ciliates (Heckmann & Frankel, 1968; Frankel, 1973*a*; Ng, 1976). These delays were interpreted in terms of 'cortical inertia' (Nanney, 1980); that is, the phenomic lag was caused by the mode of perpetuation of the cortical structures (Aufderheide, Frankel & Williams, 1980), which tends to maintain the pre-existing pattern.

The transmission of normal cortical pattern into a homozygous *mlm* exconjugant in *P. weissei* cannot be attributed to direct local cytotaxis (Aufderheide *et al.* 1980), because during total conjugation all ciliary structures from *before* fertilization are resorbed during zygocyst formation.

The two basic features of the *mlm* mutant: the slower growth rate and the cortical pattern abnormalities, are expressed differently. The time of expression of the growth decline is rather uniform in all mutant lines (5–8 cell cycles after the formation of an exconjugant) whereas the expression of pattern abnormalities is more gradual and variable (about 8–30 cell cycles). It seems that the growth decline may be directly related to the RNA metabolism during and shortly after conjugation (Berger, 1976; Sapra & Ammermann, 1973, 1974), whereas ciliary pattern abnormalities are secondary pleiotropic effects executed via modifications in the control of positioning of the primordia similar to that found in mirror-image doublets of *P. weissei* (Jerka-Dziadosz, 1985) and *janus* mutants of *Tetrahymena* (Frankel & Nelsen, 1981; Frankel & Nelsen, 1985). In other cortical mutants thus far described, defects in ciliary patterns are not accompanied by decrease in the growth rate (Jerka-Dziadosz & Frankel, 1979; Kaczanowski, 1975) although Frankel (1973*b*) noted that some of the basal-body-deficient mutant lines of *E. minuta* did show slower growth rate.

From the study presented in this paper, the following conclusions can be drawn: first, it established that the multi-left-marginal phenotype of *P. weissei* is controlled by a single mutant gene and is inherited in a Mendelian mode. Second, the total conjugation in *P. weissei* is a true sexual process in which meiosis, fertilization and genetic segregation occur. The study also shows that the mutated *mlm* allele is expressed gradually during the clonal life.

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