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Influence of the age of algae fed to rotifers (*Brachionus plicatilis* O.F. Müller) on the expression of mixis in their progenies

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Summary. The sequence of the appearance of mixis in the rotifer *Brachionus plicatilis* was followed among the descendents of amictic rotifers transferred from a high salinity media (40 S) to a low one (9 S). All the neonates that hatched from the amictic eggs, after being transferred to a low salinity, were amictic. Each one of these neonates was cultured individually and its offspring removed periodically every 8–10 h. It was observed that throughout their reproductive phase, these parental females retained their potential to produce either mictic or amictic offspring. All the first produced neonates developed into amictic females, but among those produced later, three patterns were prevalent. The prevalent pattern (type A) was one in which the probability of a neonate being mictic increased towards the middle of the parents' reproductive phase and was followed by a slow decline. In the second pattern (type B), the probability of a daughter being mictic was constant throughout the parents' reproductive phase, except for the initial neonates produced. In type C, mixis was maximal at the end of the parents' reproductive phase. It is suspected that the quality of food supplied to the rotifers determines the appearance of patterns, A, B or C. It is postulated that the innate capacity of rotifers to undergo mixis is genetically controlled, while its expression is modulated by environmental conditions.

Key words: Rotifer – Mictic pattern – Food quality

Several environmental factors have been implicated in promoting sexual reproduction of Brachionidae rotifers: changing photoperiod (Laderman and Gutman 1974), algal species fed to rotifers (Pourriot and Rougier 1979), exposure to low temperatures (Gilbert 1977a), population density (Gilbert 1963, 1977b), and the salinity in which algae fed to rotifers were cultured (Ben-Amotz and Fishler 1982). The salinity of the culture medium was also found to promote or prevent the appearance of mictic females (Lubzens et al. 1980, 1985; Lubzens 1981).

Internal factors, such as maternal effects and genetic variation among clones, have also been found to determine the occurrence of sexual reproduction in Brachionidae (Buchner 1977; Hino and Hirano 1977). Rougier and Pourriot (1977) demonstrated a negative correlation between maternal age and the rate of mictic females which produce the F_1 generation in *Brachionus calyciflorus*. In recent publications, it was reported (Lubzens et al. 1985; Snell 1986) that sexual reproduction occurred at environmental conditions which support relatively high asexual reproductive rates of the rotifer population. More specifically, sexual reproduction in *B. plicatilis* occurred at salinities lower than 30 S, when food was abundant.

The following experiments were designed to follow the sequence of the appearance of mixis in rotifers (*B. plicatilis*) after their transfer from a high salinity culture medium to a low salinity one and evaluate how this is modulated by food quality.

Materials and methods

Sea water, algae and rotifer culture medium

Sea water (salinity 40 S) and algae (*Chlorella stigmatophora*) were used and cultured as described previously (Minkoff et al. 1983). Throughout the experiments, rotifers were cultured in sea water to which log phase algae were added at a concentration of 3×10^6 cells ml. This culture medium was freshly prepared every 24 h unless otherwise stated.

Rotifers

Two clones (Cl. 6 and B12) isolated in the laboratory from single resting eggs of a wild type *Brachionus plicatilis* (O.F. Müller) were routinely cultured in the laboratory in 40 S sea water. Culturing was always done in a 25° C incubator with 24 h illumination of 40 $\mu Em^{-2}\ sec^{-1}$.

Experimental design

A uniform procedure was employed which permitted typing of whole progenies from single parthenogenic rotifers as mictic or amictic, in relation to their hatching order. For this, parthenogenically reproducing females, each carrying one egg, were transferred from a normal sea water medium (salinity 40 S) to a diluted sea water (salinity 9 S) culture medium. After 16 h incubation in a flask at a density of 1 individual/ml, the neonates, designated *P* in Fig. 1, were removed and placed individually with 1 ml fresh culture medium (salinity 9 S) in single wells of culture plates (cul-

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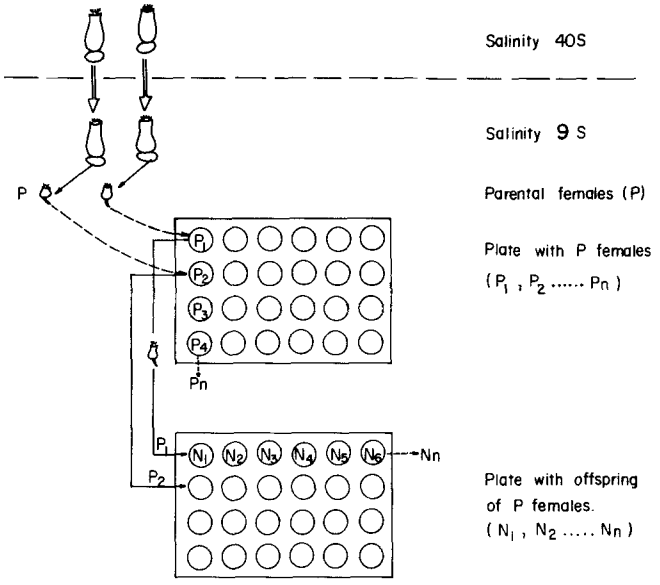

Fig. 1. The experimental design permitting typing of progeny from single parthenogenic females as mictic or amictic. For details refer to text

ture plates with 24 wells of 3 ml volume, Costar, USA). Once these females attained maturity, their progeny (designated *N* in Fig. 1) were removed every 8–10 h and placed individually and sequentially in fresh culture wells. These neonates were typed as mictic or amicitic according to the eggs they bore on reaching maturity.

In the first experiment, the order of appearance of mictic females was followed in the progeny (designated *N*) of 14 *P* females from Cl. 6. In the second experiment, an attempt was made to evaluate whether the mictic pattern observed in the first experiment resulted from gradual conditioning of the culture medium by the parental females or from the aging of the algae in their culture medium. For this purpose, *P* females of C1. B12 were divided into four treatment groups:

Group 1 (TN). This treatment was a repeat of the first experiment, in which *P* females ($n=21$) were maintained throughout the experiment in the same culture medium and their progeny removed sequentially to new wells.

Group 2 (TP). In order to evaluate the possible effects of medium conditioning by parental females, *P* females ($n=18$) were transferred every 8–10 h to fresh medium and their progeny was handled as in TN.

Group 3 (TPA). For evaluating the possibility that aging algae have an effect on the manifestation of the mictic pattern, *P* females ($n=23$) were transferred every 8–10 h to new wells containing aged culture medium which was prepared as explained below. The progeny were handled as in TN.

Group 4 (TNT). *P* females ($n=23$) were sham operated by withdrawing each one of them every 8–10 h into a pasteur pipette before being returned to their original well, thus exposing them to the same mechanical treatment experienced by TP and TN females. Their progeny were handled as described in TN.

Aged rotifer culture medium was prepared by suspending centrifuged log phase *Chlorella stigmatophora* in 9 S sea water at a concentration of 3×10^6 cells ml, and then allowing it to age for 84 h at 25° C under constant illumination, before being used as food for group TPA rotifers. During the course of the experiment, two batches of aged algae were used. The first one was prepared 84 hours before the initiation of the experiment and used up to 129 h from the time that *P* females were established in their individual wells, and the second one, which was prepared at 45 hours of the experiment, was used thereafter.

Results

Life table parameters of clone 6 females used in the first experiment are presented in Table 1. All *P* females examined produced mictic female descendents. These *P* females originated from the amictic eggs carried by their parents, when they were transferred from a high (40 S) to a low (9 S) salinity media. The proportions of mictic offspring from the total offspring born at each 8–10 h were fitted into a normal (Gausian) distribution curve, shown in Fig. 2. The probability of an offspring being mictic increased from 0 to a maximum of 0.63 towards the 86th hour of the experiment. As each female produces a different number of offspring, a better comparison between females could be achieved by dividing the reproductive period of each female into 5 equal phases. Thus, if one female produces 20 offspring and another only 16, each phase consisted of 4 offspring in the first female and 3.2 for the second one. Summarizing the mictic and amictic daughters in each phase for all *P* females (Fig. 3) shows that the highest proportion of mictic births occurs at the middle (phase III) of the parent's reproductive period. The percent of mictic females produced by *P* parents was 38.1 ± 12.2 (mean $\pm$S.D.).

Life table parameters of females from clone B12 employed in the second experiment are summarized in Table 2. The *P* females from groups TP, TN and TNT showed similar longevities which amounted to 60% survival, up to 220 h from the beginning of the experiment. Females of group TPA lived longer and 88% of them survived beyond 220 h. Also, in the present experiment, TP females showed a significantly shorter (ANOVA, $P\leq0.05$) average reproductive period lasting 95 h compared with averages ranging from 106–108 h in the other groups. However, there were no significant differences in progeny size or in the rate of their production between all treatments, during the course of the experiment. No significant differences were found in the total percent of mictic females produced in the course of the experiment in each of the treatments. These ranged from an average of 18.2% (group TPA) to 26.8% (group TP).

The proportion of mictic offspring produced was analyzed statistically (ANOVA) by grouping them into five reproductive phases as described previously for C1. 6 (Fig. 4, Table 3). The handling of rotifers by pasteur pipettes had no effect on the number of mictic offspring produced (compare group TNT to group TN). The *P* females in a constant (TN) or exchanged (TP) medium produced a similar number of mictic offspring in all phases except phase III (22% vs. 48%, $P\leq0.001$, Table 3). Also, females of group TP produced a similar number of mictic offspring to females of group TNT. The most striking result is that of females belonging to group TPA, which were

Table 1. Life table characteristics of 14 parental females (*P*). Rotifers (clone 6) were individually incubated in 1 ml sea water (salinity 9 S), fed *Chlorella stigmatophora* (3×10^6 cells ml^{-1}) and incubated at $25 \pm 1°$ C under constant illumination. New offspring were separated from their mothers every 8–10 hours and placed individually in new wells. Females were also transferred every 8–10 h to new wells containing freshly prepared medium. Values are given as Av. $\pm$S.D.

Life span (hrs)	Number of offspring (female^{-1})	Time from hatching to first produced egg (hrs)	Reproductive period (hrs)	Duration from last produced egg to death (hrs)	Innate capacity to increase[a] (r_m, females^{-1} day^{-1})
169.6±31.4	21.5±2.6	52.1±6.1	84.9±12.9	32.9±25.6	0.81

[a] r_m was calculated according to Birch (1948)

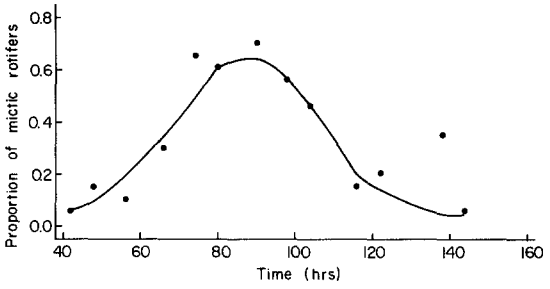

Fig. 2. The proportion of mictic offspring produced at 8–10 h intervals during the course of the experiment by females of clone 6, after they were transferred from sea water media (salinity 40 S) to diluted sea water media (salinity 9 S). The probability of a neonate being mictic within the progeny of a single female fits a Gausian distribution where:

$Y = K((1/(\sigma\sqrt{2\pi}) \times e^{-(x-\mu)^2/2\sigma^2})$

and values are $K = 30.08$, $\mu = 86.35$ and $\sigma = 18.93$

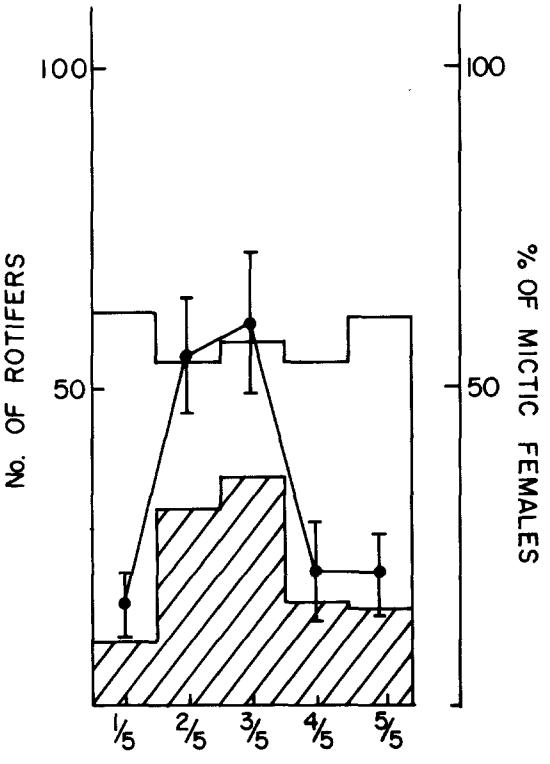

Fig. 3. The distribution of the total number of females (unshaded histogram), the number of mictic females (shaded histogram) and the percent of mictic females (line) produced by rotifers of clone 6 ($N = 14$). The reproductive period of each female was divided into five equal phases

transferred periodically to an aged medium. Except for phases I and IV, where no significant differences were observed, females of group TPA produced significantly less ($P \leq 0.001$) mictic offspring in phase II and in phase III. After being transferred into the second batch of aged medium (during phase IV), mixis reappeared in their progeny, so that by phase V they produced significantly more (66%) mictic offspring when compared to all other treatments (Table 3).

Discussion

Life table parameters for all treatments of clone 6 are consistent with those reported by Ruttner-Kolisko (1972) and show an average of 20 offspring per female born at a rate of one every 4 h at 25° C.

Throughout their reproductive phase, parental females retain their potential to produce either mictic or amictic offspring. The appearance of mictic offspring within the progeny of individual females was not necessarily a continuous event. This led to three distinct patterns of mixis expression. The prevalent pattern (type A) was one in which the probability of a neonate being mictic increased towards the middle of the parents' reproductive phase and was followed by a slow decrease (Figs. 2 and 3, and treatments TN and TNT in Fig. 4). This mictic wave as it appears in single rotifers could be the basis of the similar pattern observed in rotifer populations (Ito 1960; Gilbert 1963; 1977c; Ruttner-Kolisko 1972; Buchner 1977; Lubzens et al. 1980). We have found this pattern to repeat itself also in the third generation (i.e. the grand-daughters of *P* females; unpublished data). The second pattern (type B) was one in which, excluding the initial neonates, the probability of a daughter being mictic was constant throughout the parents' reproductive phase (TP in Fig. 4). Such a pattern is characteristic of steady state populations (Scott 1977) in which the culture medium is being renewed at a constant rate. The exception to these was a pattern (type C) in which mixis was maximal at the end of the parents' reproductive phase (TPA, Fig. 4) and minimal in the middle.

These patterns differ from those described for *B. rubens* and *B. calyciflorus* (Pourriot and Rougier 1976; Rougier and Pourriot 1977; Rougier et al. 1977), where mixis rates were highest during the initial period of reproduction. Such differences could be due to species specificity or the methodology of the different experiments.

The factor causing the two different mixis patterns in our experiments is most likely the changes in the algal nutritional (caloric or specific metabolites) value on transition from the logarithmic to the senescent growth phase. We presume the influence of other abiotic (salinity, pH) or

Table 2. Comparison in life table characteristics between parental females (*P*) of four treatment groups of clone B12. For details see Materials and methods

	Group 1 (TN)	Group 2 (TP)	Group 3 (TPA)	Group 4 (TNT)	Statistical analysis (ANOVA)
1. Number of replicates (n)	21	18	23	23	
2. Time from hatching to first produced egg (hrs, Av ±S.D.)	61.5±4.15	60.5±5.1	63.9±4.15	62.4±4.9	N.S.
3. Duration of reproductive period (hrs, AV ±S.D.)	108±7.25	95±11.5	106±9.7	108±7.9	TN=TNT=TPA TP+TN, TNT, TPA ($P<0.05$)
4. Reproductive rate (hr^{-1}, Av ±S.D.)	0.2±0.02	0.2±0.02	0.19±0.02	0.2±0.02	N.S.
5. Number of offspring (Av ±S.D.)	20.4±2.3	20.2±2.8	20.7±2.2	20.7±2.2	N.S.
6. Percent of survival up to 220 hrs	60	60	88	60	
7. Percent of mictic females produced (Av ±S.D.)	25.2±15.0	26.8±16.7	18.2±18.6	19.7±17.3	N.S.

Table 3. Comparison between the percent of mictic offspring produced by *P* females in the four treatment groups presented in Table 2. The reproductive period of each rotifer was divided into five equal phases and the clustered results of each treatment pooled for comparison. An analysis of variance and Duncan's Multiple Range Test were used at 5% or 1% significance level for comparison between the averages of the four treatment groups

Reproductive phase	Probability[a] (*P*)	Significance level 5%	Significance level 1%
1/5	0.5778	N.S.	N.S.
2/5	0.0067	TN=TP=TNT TPA<TN, TP, TNT	TPA<TN, TNT
3/5	0.001	TN=TNT; TP=TNT TN>TP TPA<TP, TN, TNT	TPA<TN, TNT
4/5	0.1348	N.S.	N.S.
5/5	0.0000	TP=TN=TNT TPA>TP, TN, TNT	TPA>TP, TN, TNT

[a] *P*=probability for means being equal in a one way analysis of variance

biotic (food level) factors on mixis expression within this system to be negligible, as such factors should also have influenced population reproduction rates and progeny size (Edmondson 1964; King 1967; Hu 1980; Lubzens et al. 1985). In our experiments, mixis is promoted in type A by a continual transfer of *P* females to fresh, log phase medium, or as long as the algae in the culture medium had not aged beyond a certain point, which, under the particular experimental conditions, was 100–120 hrs. Algae senescing beyond this point suppressed mixis as in phases IV and V of type A and phases II, III and IV of type C.

Induction of mixis via dietary factors is well documented for rotifers. In some *Brachionus* species, mixis rates can be altered according to the algal species on which these rotifers are fed (Pourriot 1957; Pourriot and Rougier 1979). Furthermore, a specific dietary factor, α-tocopherol, synthesized by photosynthetic organisms, has been shown to induce mixis in three *Asplanchna* species (Gilbert and Thompson 1968). It is conceivable that a similar substance present in log phase algae induces mixis in our strain of *B. plicatilis*. Alternatively, it is conveivable that the total nutritional value is in itself the mictic cue.

The above considerations are in accordance with Ruttner-Kolisko's (1974) statement that the stimulus which induces mixis must be unspecific and affect the animals through a general system, i.e. that of their metabolism. Gilbert (1977b, 1980) proposed a similar system for *Asplanchna*. In principle, the presence of an algal dietary factor (α-tocopherol) is indicative of abundant environmental energy resources, permitting the population to increase and contributing to successful mating encounters and the production of energy rich resting eggs. Gilbert's considerations are valid also for our rotifer species. From our results, the first neonates deposited by a female are more likely to be amictic, thus ensuring the immediate continuity of the population and the increase in population density. Resting egg production therefore, in this case, is a "long term investment", taking place while environmental conditions are most favourable. It is under these conditions, which also

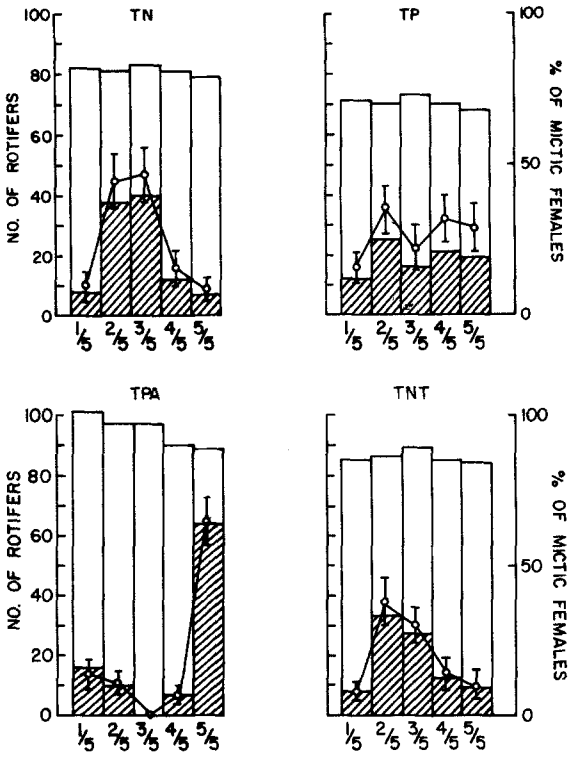

Fig. 4. The distribution of the total number of females (unshaded histogram), the number of mictic females (shaded histogram) and the percent of mictic females, produced by rotifers of clone B12 subjected to four different treatment groups (TN, TP, TPA and TNT). For details refer to text

ensure the increase of the population through asexual reproduction, that production of males and resting eggs takes place. The loss to the population through production of males and resting eggs, which have no immediate contribution, is thus greatly diminished. Also, these favourable environmental conditions ensure that once fertilization has taken place, the environment will support the production of energy rich resting eggs.

Another mictic inducing factor, that of specific medium conditioning ("density effect") proposed to operate in Brachionids (Gilbert 1963, 1977b) and in *Notomata copeus* (Clement and Pourriot 1973), does not exist in our system. A constant medium renewal (TP in Fig. 3) did not cause the expected reduction in mixis levels. On the contrary, it resulted in the prolongation of the mictic reaction. Sexual reproduction in *B. plicatilis* is also known to proceed during constant dilution rates in a chemostat (Scott 1977). It seems to us most likely that population density and mixis are connected through an environment supplying the energy required for a rapid population increase.

Our present results are in accordance with the hypothesis we put forward previously (Lubzens et al. 1985) and with the views of Snell (1986), namely that sexual reproduction is suppressed under extreme environmental conditions that may still support asexual reproduction. Our proposition and that of Snell (1986) is contrary to the concept that resting egg or dormant stage production appears at the onset of unfavourable environmental conditions (Williams 1975). Both concepts see the resting stages as a means of maintaining the species through periods of adverse environmental conditions. The differences lie in the mechanism operating in the timing ("cue") of the production of the resting stages. In a more general concept, the environmental factors modulate the ability of the rotifer to undergo mixis, while recent publications (Buchner 1977; Hino and Hirano 1977; Lubzens 1987) lead to the conclusion that this capacity is genetically determined.

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