

シオミズツボワムシの両性生殖と飼育水塩素量の関係

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Relationship Between Water Chlorinity and Bisexual Reproduction Rate in the Rotifer *Brachionus plicatilis*

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Bisexual reproduction of the rotifer *Brachionus plicatilis* was investigated at three different chlorinity levels. Special attention was given to the origin of the strains examined.

Strains were isolated by hatching fertilized eggs produced at chlorinities of 4 and 16‰. All rotifers were cultured individually throughout the experiment in 4, 8 and 16‰. The appearance rate of mictic females was used as an indicator of bisexual reproduction activities. Culture media of 4 and 8‰ were changed every 12 or 18 h, while 16‰ cultures were changed every 24 and 30 h.

The bisexual reproduction rate in the strains originating from 4‰ varied markedly depending on the chlorinity of the culture. At chlorinities of 4, 8 and 16‰, the bisexual reproduction rates were 33, 18 and 6%, respectively. Conversely, in strains originated at 16‰, no correlation was found between the bisexual reproduction rate and the culture chlorinity.

Differences in the pattern of response between L- and S-type *B. plicatilis* were inferred as the result of adaptation to environmental factors such as temperature or chlorinity.

Nearly 2000 freshwater rotifer species have been identified worldwide. Only about ten species have been found, however, that can inhabit brackish or seawater regions. Therefore, most research on the rotifer life cycle have been restricted to freshwater species. Few studies have been done on the effect of chlorinity on bisexual reproduction. Luntz¹⁾ reported that the rate of mictic female appearance among *B. bakeri*, a common freshwater species, increased to 100% among neonates when the NaCl concentration was elevated from 0 to 0.09%.

B. plicatilis is one of the few species which inhabits regions of varying chlorinity. Because of its adaptability, it is used as a primary food not only for marine, but also for freshwater juvenile fish and crustaceans. The chlorinity of culture media used for this species in Japan varies from less than 3 to more than 16‰. Previous investigations²⁾ have reported an inverse relationship between salinity and the rate of parthenogenetic reproduction (*i.e.*, low salinity produces a high rate of parthenogenetic reproduction).

Several factors are known to induce bisexual reproduction in *B. plicatilis*. Internal factors include genetic differences among strains and the capacity for bisexual reproduction acquired with subsequent generations.³⁾ One external factor which influences bisexual reproduction is a density

dependent phenomenon that has been previously described.⁴⁻⁶⁾ This phenomenon is frequently observed during mass culture of this species. It complicates investigation of bisexual reproduction because the density effect is often inseparable from other external factors being investigated. Hino and Hirano⁷⁾ differentiated the effect of temperature from that of density on mictic female production by increasing the frequency of culture media renewal at higher temperatures. It is felt by the authors that the accumulation of metabolites in the culture is the principal cause of the density dependent phenomenon. They⁸⁾ also investigated the relation between temperature during formation of fertilized eggs and the bisexual reproductive pattern in the derivative strain. It is reported that the culture conditions experienced by a strain of rotifers influences the bisexual reproductive pattern of the derivative strain. To investigate the influence of one external factor on the reproductive rate and/or pattern of rotifers requires a complex design.

The effect of chlorinity on bisexual reproduction was examined in this study using two groups of rotifer strains. These strains originated from fertilized eggs formed in two different seawater concentrations. Mictic female production was used as an indicator of bisexual reproduction. In order to eliminate density-dependent factors,

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rotifers were cultured individually and the interval of media renewal was shortened in proportion to growth rates at specific chlorinities.

Materials and Methods

Seawater obtained near the coast of Hachijo Island in the Kuroshio Current was sterilized by boiling and the chlorinity was adjusted to 16‰ by adding deionized water.

The same *B. plicatilis* stock was used in this experiment as in previous reports.^{3,4,6-8)} In our laboratory, rotifers are generally cultured in seawater with 16‰ chlorinity and fertilized eggs are stored in a refrigerator. The eggs are hatched when needed for experimental purposes. All rotifer cultures used in this study were maintained in seawater with 16‰ Cl' at 25°C in the dark.

Mass Culture for Fertilized Egg Formation

The strain designated 7904 A1 began with an offspring hatched from the fertilized egg mentioned before. This strain was then divided into three substrains. One substrain was set aside for checking the capability of bisexual reproduction in the same manner that will be described later for the new strains derived from fertilized eggs of this strain. The remaining two substrains were used in mass culture to produce fertilized eggs in 4 and 16‰ Cl'. The three cultures were fed *Chlamydomonas* sp. which was also cultured at 4 and 16‰ Cl'. All individuals in these cultures were assumed to have the same genotype because they were produced parthenogenetically.

Fertilized eggs were collected in the manner described in a previous report³⁾ and transferred to seawater of 16‰ Cl'. They were then stored in a refrigerator for several weeks.

Successive Culture for Testing Bisexual Reproduction in the Derivative Strains

The preserved eggs were retrieved from the refrigerator and hatched under continuous illumination in an incubator. Neonates from the eggs produced in 4 and 16‰ Cl' were employed as stem mothers, producing 10 and 9 new strains, respectively. These strains were cultured successively at 16‰ Cl' as described in previous reports.^{7,8)} This method uses glass test tubes containing 0.1 ml of seawater in which *Chlamydomonas* sp. is suspended. Rotifers were individually cultured. At constant time intervals, individuals, including hatching offspring, were transferred into fresh culture medium. The first daughter of

each generation was employed as the mother of the next generation and cultured until she produced five offspring. Mictic and amictic females were determined by the size of the eggs they produced. The interval between transfers was based on the amount of time required for a parthenogenetic egg to hatch. This ranged from 24 to 30 h at 16‰ Cl' and from 12 to 18 h at 4 or 8‰. These transfers served to circumvent the density effect caused by hatching neonates.

In successive culture, substrains were separated out for tests at 4 and 8‰ Cl'. The second and third amictic daughters of each generation were employed as stem mothers of these new substrains. Identical strains could then be tested in all three chlorinities.

The rate of bisexual reproduction in each chlorinity was expressed as a ratio of mictic females to total females. The total number of females was determined by summing the number of each female type in each generation. Previous research³⁾ indicated that data from the first seven generations after hatching should be omitted. This was done so that only the generations capable of bisexual reproduction would be counted. Also omitted were data from the first two generations after transfer into different chlorinities so that the effect of a change in chlorinity was avoided.

Results

Bisexual Reproduction in the 7904 A1 Strain

Data were obtained from six generations at 4‰, and from ten generations at both 8 and 16‰ Cl'. Fig. 1 demonstrates that the mictic female appearance rate decreased as chlorinity increased.

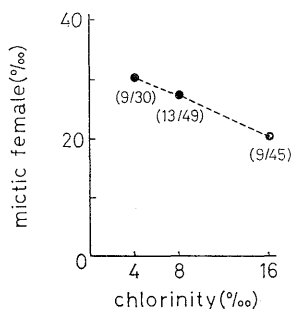


Fig. 1. Percentage of mictic females determined in 7904 A1 strain. Data were obtained from the individual successive culture at 25°C. No significant difference was found among chlorinities. Numbers in parenthesis indicate the ratio of mictic offspring to the total of mictic and amictic ones.

Table 1. Ratio of mictic females to total females determined in the course of successive culture

strain	Egg origin: 4‰ Cl'			strain	Egg origin: 16‰ Cl'		
	Culture condition (‰ Cl')				Culture condition (‰ Cl')		
	4	8	16		4	8	16
1	13/34	12/58	1/21	1	9/37	4/30	2/34
2	5/31	8/62	2/39	2	9/32	10/32	2/28
3	13/21	8/59	4/17	4	6/34	7/41	2/23
4	13/29	10/55	3/28	7	3/18	6/34	0/24
6	9/16	7/36	0/42	8	10/39	10/38	1/33
8	9/30	12/39	1/31	9	4/23	6/40	3/36
10	9/22	10/24	2/24	11	5/25	7/31	7/16
11	6/34	3/41	0/38	14	3/25	2/29	0/8
12	8/30	7/43	1/31	16	7/34	10/57	4/35
13	6/28	5/39	3/40				
total	90/275	82/456	17/311	total	56/267	62/332	21/237
(%)	(33)	(18)	(5)	(%)	(21)	(19)	(9)
paired				paired			
t-test	t=2.92		3.34	t-test	t=0.78		2.00

Strains originated from fertilized eggs formed at 4 or 16‰ Cl' by 7904 A1 strain.

Analysis by χ^2 test, however, shows that the difference was not significant.

Bisexual Reproduction in Derivative Strains

Ten strains were derived from fertilized eggs produced at 4‰ and nine strains were derived from fertilized eggs produced at 16‰ Cl'. Each strain was divided into three substrains for tests at 4, 8 and 16‰ Cl'. The percentage of mictic females was compared among each set of substrains. The differences in percentages between substrains were compared by a paired t-test so that hereditary differences in bisexual reproduction among strains could be disregarded.

The chlorinity of egg origin influenced the bisexual reproduction rate after transfers to other levels of chlorinity. In the strains derived from fertilized eggs formed at 4‰ Cl', the percentage of mictic females decreased drastically when chlorinity increased, e.g., 33, 18 and 5% at 4, 8 and 16‰ Cl', respectively (Table 1 and Fig. 2). These differences were highly significant. In strains derived from fertilized eggs formed at 16‰ Cl', however, the mictic female appearance rate decreased gradually in response to increases in chlorinity (21, 19 and 9% at 4, 8 and 16‰ Cl', respectively). There was no significant difference among these substrains.

Discussion

In the nine new strains derived from fertilized eggs produced at 16‰ Cl', the bisexual reproduc-

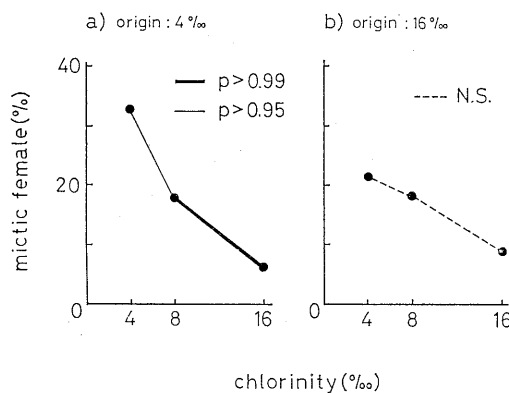


Fig. 2. Bisexual reproduction in derivative strains. Rotifers originated from fertilized eggs formed at 4 or 16‰ Cl' by 7904 A1 strain.

tion pattern was similar to that of the parent strain, 7904 A1. The most probable reason is that the 7904 A1 strain and the nine new strains were both derived from fertilized eggs formed under the same environmental conditions. The bisexual reproduction rate in the ten strains derived from eggs produced at 4‰ Cl' increased significantly with a decrease in water chlorinity. As previously reported,⁸⁾ cultures of eggs formed in low temperatures exhibit increased bisexual reproduction when water temperature is decreased. These results indicate that the rate of bisexual reproduction changes in relation to environmental factors and that this change is also dependent on the culture history of the parental strain.

Lubzens *et al.*⁹⁾ studied the mictic female appearance rate in salinities ranging from 2 to 40‰. They concluded that salinities optimal for bisexual reproduction also support mixis. Our results corroborate this finding in that our rotifer stock also grew faster in lower chlorinities ranging from 4 to 16‰ Cl'. Snell¹⁰⁾ examined salinities ranging from 5 to 40‰ Cl', but his results differed from the reports above. He found that in lower salinities, fewer male rotifers were produced. He continued to monitor the bisexual reproduction rate in batch culture. This meant, however, that response to chlorinity was indistinguishable from response to density.^{4, 6)} Also as Snell¹⁰⁾ pointed out, the rotifer stock he examined may differ from ours physiologically. Based on specific growth rate,¹¹⁾ the rotifer strains examined by Snell¹⁰⁾ seem to be S (small) type stocks, classified by Sudzuki¹²⁾ as *B. plicatilis rotundiformis* which is naturally distributed in coastal waters. Our stock is the L (large) type *B. plicatilis typicus* which originated in an inland eel culturing pond (2–3‰ Cl') in Mie Prefecture, Japan.¹³⁾ These subspecies differ markedly not only in size and lorica shape, but also in optimum temperature and salinity for growth.

Our results indicate that a change in the bisexual reproduction rate occurs in response to the culture history of a strain. In addition, the culture conditions can influence the bisexual reproduction rate in the successive strain. Differences between

rotundiformis and *typicus* may result from environmental adaptation over generations. Changes in bisexual reproduction as described by Snell¹⁰⁾ and in our previous report⁷⁾ may result from variation in temperature at the time of fertilized egg formation.

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