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Size Variation and Cyclomorphosis of the Rotifer, *Brachionus rotundiformis*, Isolated from Cheju Island, Korea

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A rotifer strain has been isolated from tide pools located near Sogwipo, Cheju Island and the effects of environmental factors (water temperature and salinity) and clonal differences have been examined with regard to size variation and cyclomorphosis. Adult female rotifers varied from 141.61 to 173.97 μm in their mean lorica length under different rearing conditions in the laboratory and also had pointed anterior spines. Thus, this strain was classified as *Brachionus rotundiformis*. Mean lorica length tended to be smaller as rearing temperature increased although there were some exceptions. Statistical analysis indicated that lorica length was largely influenced by both clonal differences and rearing temperatures although the former as a genetic factor affected lorica length a little more than the latter did; overall effect of salinity on lorica length was not statistically significant; furthermore, cyclomorphosis was also largely influenced by both clonal differences and rearing temperatures, but clonal differences as a genetic factor affected rotifer cyclomorphosis much more than temperature did.

Key words : Rotifer, *Brachionus rotundiformis*, Size variation, Cyclomorphosis, Cheju island

Introduction

Rotifers are critically important for intensive rearing of most marine fish larvae and no adequate substitute has been found yet (Hoff and Snell, 1987). Since Ito (1960) showed the possibility of the use of rotifers as a larval feed for several fishes and invertebrates, many authors have investigated rotifers with regard to various biological aspects including the roles of rotifers in larval rearing (Eda et al., 1990; Gatesoupe and Robin, 1982), food requirements (Yu et al., 1989), nutritional improvement of rotifers (Hirayama and Funamoto, 1983; Fukusho et al., 1984), resting egg production (Hagiwara and Lee, 1991) and its application on aquaculture (Hagiwara and Hirayama, 1993; Lubzens, 1989).

In terms of body size variability of rotifers, a few researchers illustrated size ranges of different strains (Fukusho and Okauchi, 1982, 1983) and effects of culture conditions on the size of small- and large-type rotifers (Fukusho and Iwamoto, 1980, 1981; Snell and Carrillo, 1984), which are now classified as *Brachionus rotundiformis* and *B. plicatilis*, respectively (Segers, 1995). Recently, small, tropical marine *Brachionus* (so-called SS strain) was investigated with regard to its morphometric characteristics and classified as *B. rotundiformis* (Rumengan et al., 1998). However, no investigation has been attempted to determine the effects on size variability of Korean rotifer strain.

On the other hand, more than 50 private hatcheries for seed production of marine fishes

are located along the coast of Cheju Island, and they rely on rotifers as a feed for larvae to produce seeds of olive flounder (*Paralichthys olivaceus*) and rockfish (*Sebastes schlegeli*). However, the rotifer strains using at hatcheries are usually mixed together and their sources are unknown. In addition, although rotifer size is not a limiting factor for seed production of the two fish species mentioned above, it is sometimes an important factor for certain marine fishes which have especially small mouths by influencing the survival and growth rate of fish larvae (Kohno et al., 1997).

Therefore, this study aimed to investigate the effects of environmental factors (water temperature and salinities) and different clones on the size variation and cyclomorphosis of a rotifer strain isolated from Cheju Island.

Materials and Methods

A strain of the rotifer, *B. rotundiformis*, were collected from three different tide pools of Cho-do located near Sogwipo City, Cheju Island on June 20, 1997. Salinities measured at each tide pool were 3.2, 4.5 and 5.6 ppt, respectively. Body sizes of 50 females randomly selected from the field samples were measured because there were not many adult amictic females carrying eggs for some reason. Each sample was transferred to 250 ㎖ flasks with a culture medium consisting of sterilized seawater and distilled water to adjust its salinities to those observed in the wild, and then rotifer was allowed to grow in a 32℃ incubator for 7 days by feeding freshwater *Chlorella* (Chlorella Industry Co., Ltd.) everyday at the concentration of 1×10^6 cells/㎖. Again, body sizes of adult female were determined by measuring lorica length and width, and then they were compared with those of the wild rotifer. The differences in their body size were tested by the two-sample t test. In order to determine the biological differences among the clones, four different clones were isolated from the culture of 4.5 ppt. The isolated clones were placed in the 250 ㎖ flasks and grown at 4, 16 and 30 ppt salinities as well as at 22, 27 and 32℃. A total of the 36 cultures were maintained under constant light of 3,000 lux for three months before the initiation of this experiment.

The main experiment was conducted with the pre-adjusted rotifers mentioned above using 75 ㎖ test tubes containing 30 ㎖ culture medium. 10 amictic females having eggs were inoculated into test tubes and grown by feeding freshwater *Chlorella* at the concentration of 2×10^6 cells/㎖ everyday. After exponential growth of the rotifer populations for 6 days, a 2 ㎖ culture was sampled and preserved in 10% formalin for measuring. Body length and width of 100 egg-carrying females were measured with an ocular micrometer with a inverted microscope (×250, Olympus IX70).

A mixed model or Model II, three-factor analysis of variance, ANOVA (see Zar, 1984) was carried out to discover the effects of temperatures, salinities and clones on lorica size. As four clones were chosen at random, the clone was considered as a random effect, while the other two factors were considered as fixed effects because certain temperatures and salinities were selected specifically. Thus, the null hypothesis was stated as that there is no clonal difference in lorica size of rotifers affected by three different temperatures and by three different salinities. If ANOVA rejected the null hypothesis mentioned above, multiple comparison testing was performed by the Duncan test

(Duncan, 1955). A three-factor ANOVA was also conducted to examine cyclomorphosis phenomenon using the ratios of lorica width to length. Statistical analyses were performed with the aid of software called SYSTAT 6.0 for Windows (SPSS Inc.).

Results

The mean lorica size of rotifer in the nature was small, ranging from 117.18 to 125.02 ㎛ in lorica length and from 92.49 to 95.89 ㎛ in lorica width (Table 1). However, the size of rotifer became significantly larger ($p<0.05$), ranging from 130.99 to 135.78 ㎛ in lorica length and from 103.25 to 104.41 ㎛ in lorica width, after a 7-day culture at 32℃ by daily feeding freshwater *Chlorella* at the concentration of 1×10^6 cells/㎖ in the laboratory.

The mean lorica length and width of the four different clones of adult female rotifers reared at different environments (3 temperatures and 3 salinities) are shown in Table 2. With this data, a three-factor ANOVA was performed to determine the major effects and interactions of temperature, salinity and rotifer clone (Table 3). As a result, two factors, temperature and rotifer clone, had the significant effect on lorica length, but salinity did not. The effect of temperature was revealed with an F of 41.28 and $p<0.001$ and that of the rotifer clone with an F of 65.92 and $p<0.001$. The two way interaction of temperature×salinity (F = 27.78, $p<0.001$) and temperature×clone (F = 47.00, $p<0.001$) were both significant, as was the three-way interaction (F = 40.53, $p<0.001$). These results indicated that lorica length was largely influenced by both clonal differences and temperature.

When comparing mean lorica length of the rotifers reared at different salinities, lorica size changes estimated with the size at 4 ppt salinity as a denominator ranged from -2.1% (clone II) to +5.8% (clone IV) at 22℃, +2.4% (clone III) to +18.2% (clone I) at 27℃, and -0.6% (clone II) to +2.6% (clone III) at 32℃. Thus, the effect of salinity on lorica size variability was highest at 27℃, whereas it was lowest at 32℃. However, overall effect of salinity on lorica length was not statistically significant ($p>0.05$) as shown in Table 3.

Mean lorica length tended to be smaller as rearing temperature was increased except for both clone I and clone III cultured at 16 ppt salinity (Fig. 1). The mean lorica lengths of Clone I were 173.97, 162.34, and 145.64 ㎛ at 27, 22, and 32℃, respectively, whereas those of clone III were 156.54, 144.71, and 141.61 ㎛ at 22, 32, and 27℃, respectively. At 30 ppt, which is the usual salinity of the normal seawater, the smallest mean lorica length turned out to be 143.06 ㎛ from clone IV at 32℃ and the largest one was 163.51 ㎛ from clone IV at 22℃. In addition, multiple comparisons among the cultures grown at 30 ppt showed that there were distinct dif-

Table 1. Comparison of rotifer body sizes (mean±SD; n=50) measured with field samples and 7-day cultures in the laboratory

Salinity	Field sample		After 7-day culture	
	Lorica length	Lorica width	Lorica length	Lorica width
3.2ppt	117.18±13.31	94.68±10.11	-	-
4.5ppt	125.02±12.34	92.49± 9.56	135.78±15.80	103.25±14.36
5.6ppt	122.05±11.78	95.89±11.05	130.99±10.74	104.41± 9.31

Table 2. Mean lorica length and width (mean±SD; n=100) of Cheju rotifer strain reared at different culture conditions

Clone	Salnity	22℃		27℃		32℃	
		Lorica Length	Lorica Width	Lorica Length	Lorica Width	Lorica Length	Lorica Width
I	4ppt	164.32±8.59^{B}	125.65±13.31^{b}	147.16±13.56^{A}	111.22±10.84^{a}	143.76±7.21^{A}	113.59±9.15^{a}
	16ppt	162.34±9.53^{B}	124.70± 6.47^{b}	173.97±11.15^{C}	123.03± 9.73^{b}	145.64±5.57^{A}	116.52±6.21^{a}
	30ppt	161.22±7.99^{C}	125.09± 5.53^{b}	156.67±10.02^{B}	122.70± 7.94^{b}	146.18±6.05^{A}	113.86±7.20^{a}
II	4ppt	165.79±9.15^{B}	127.00± 5.80^{b}	151.99± 9.66^{A}	117.46± 8.00^{a}	148.39±5.70^{A}	115.59±6.88^{a}
	16ppt	164.32±9.20^{C}	125.30± 6.90^{b}	155.60± 6.88^{B}	115.56± 7.27^{a}	147.47±5.98^{A}	116.56±6.28^{a}
	30ppt	162.28±7.36^{C}	124.99± 5.90^{b}	156.56± 7.09^{B}	117.06± 7.24^{a}	148.12±7.56^{A}	115.27±6.95^{a}
III	4ppt	158.23±6.30^{B}	122.99± 7.15^{b}	152.76±10.68^{B}	117.00± 7.91^{a}	143.67±6.32^{A}	114.46±7.66^{a}
	16ppt	156.54±7.82^{B}	121.60±12.51^{b}	141.61± 7.67^{A}	114.59± 7.08^{a}	144.71±6.87^{A}	113.81±7.16^{a}
	30ppt	159.16±7.95^{B}	122.89± 7.29^{b}	156.50± 6.59^{B}	122.57± 8.37^{b}	147.35±6.79^{A}	116.27±6.82^{a}
IV	4ppt	154.52±7.40^{B}	118.98± 6.34^{b}	153.44± 6.15^{B}	112.53± 6.14^{a}	143.40±9.47^{A}	110.95±8.02^{a}
	16ppt	159.68±6.88^{B}	123.93± 5.27^{b}	158.41± 5.36^{B}	122.95± 5.47^{b}	145.18±8.28^{A}	114.07±7.20^{a}
	30ppt	163.51±9.05^{B}	126.24± 6.79^{b}	158.62± 5.63^{B}	122.45± 6.78^{b}	143.06±6.16^{A}	112.61±7.13^{a}

Different superscript letters at the same raw data indicate that they are statistically different from each other ($p<0.05$); lorica length and width are distinguished with each other by big and small superscript letters.

Table 3. A three-factor ANOVA of the effects of temperature×salinity×clone on lorica length.

Source	*df*	SS	MS	*F*	*P*
Temperature	2	145,773.82	72,886.91	41.28	<0.001
Salinity	2	5,040.42	2,520.21	0.84	n.s.
Clone	3	12,566.31	4,188.77	65.92	<0.001
Temperature×Salinity	4	4,862.45	1,215.61	0.47	n.s.
Temperature×Clone	6	10,593.31	1,765.55	27.78	<0.001
Salinity×Clone	6	17,920.29	2,986.71	47.00	<0.001
Temperature×Salinity×Clone	12	30,903.71	2,575.31	40.53	<0.001
Error	3,564	226,469.55	63.54		

The model III or a mixed model, ANOVA (Zar, 1984, p. 212) with temperature (A) and salinity (B) fixed and clone (C) random variables. N=100, A=3, B=3, C=4. n.s. indicates that there is no statistically significant effect.

ferences in lorica lengths of clone I and II between all three different temperatures ($p<0.05$), whereas no difference in lorica length of clone III and clone IV between 22 and 27℃ were detected ($p>0.05$) (see Table 2 for more detail).

The degree of cyclomorphosis can be determined using the ratio of lorica width to lorica length. As the ratio reaches close to 1, rotifers become round. The ratios estimated with 3600 measurements ranged from 0.595 to 1.015 and their mean ratio was 0.774. The ratio tended to be smaller as lorica length increased (Fig. 2). There were some clonal differences in mean ratio which were 0.771, 0.768, 0.785, and 0.772 in clones I, II, III, and IV, respectively. Thus, there was close similarity observed between clone I and clone IV. To determine the effect of genetic and environmental factors on cyclomorphosis in rotifer, a three-factor ANOVA was performed with the ratio of lorica width to lorica length

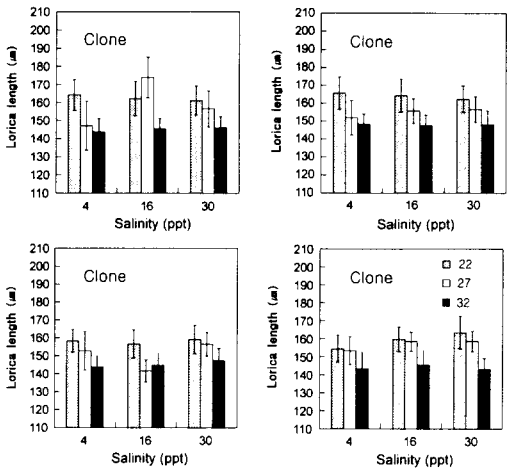

Fig. 1. Mean lorica length of Cheju rotifer strain, *Brachionus rotundiformis* at the different temperatures and salinities. Bars indicate standard deviations.

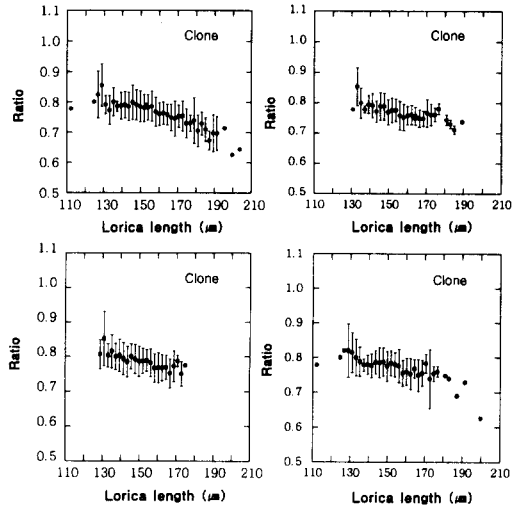

Fig. 2. The ratios of lorica width to lorica length estimated from the four different clones of Cheju rotifer strain, *Brachionus rotundiformis*. Bars indicate standard deviations.

(Table 4). The results showed that among the three factors, temperature and rotifer clone were statistically significant effects. That is, the effect of temperature was revealed with an F of 7.81 and $p<0.05$ and that of clone with an F of 24.39 and $p<0.001$. The two way interaction of temperature × clone (F = 10.72, $p<0.001$) and salinity × clone (F = 13.07, $p<0.001$) were both significant, as was the three-way interaction (F = 16.97, $p<0.001$). Therefore, cyclomorphosis is largely influenced by both clonal differences and rearing temperatures, but clonal differences as a genetic factor affected cyclomorphosis much more than temperature did.

Discussion

The rotifer, *Brachionus*, is a cosmopolitan spe-

Table 4. A three-factor ANOVA of the effects of temperature × salinity × clone on the ratios (lorica width/lorica length)

Source	*df*	SS	MS	*F*	*P*
Temperature	2	0.329	0.164	7.81	<0.05
Salinity	2	0.014	0.007	0.27	n.s.
Clone	3	0.144	0.048	24.39	<0.001
Temperature × Salinity	4	0.043	0.011	0.33	n.s.
Temperature × Clone	6	0.127	0.021	10.72	<0.001
Salinity × Clone	6	0.154	0.026	13.07	<0.001
Temperature × Salinity × Clone	12	0.401	0.033	16.97	<0.001
Error	3,564	7.022	0.002		

The model III or a mixed model, ANOVA (Zar, 1984, p. 212) with temperature (A) and salinity (B) fixed and clone (C) random variables. N = 100, A = 3, B = 3, C = 4. n.s. indicates that there is no statistically significant effect.

cies and an excellent first food for fish larvae. However, the lack of suitable size for a certain hatched fish larvae which have very small mouths is often an obstacle for larval rearing. Such a problem will be solved by either discovering a new strain or manipulating culture conditions that can affect the size of rotifer.

Traditionally, two morphologically different varieties (S-type and L-type) of the rotifer, *B. plicatilis*, have been recognized (Rumengan et al., 1991) and considered to be genetically different strains (Fukuso and Iwamoto, 1980, 1981; Ito et al., 1981). These studies reported that the S-type strain is a small round shape, 140-220 ㎛ in lorica length, having pointed anterior spines of larica, and prefer high temperatures of more than 20℃, while the L-type strain is large with long orica (230-320 ㎛) and obtuse-angled spines of orica, and shows tolerance to low temperatures of less than 20℃. Later, with more detail data, - and L-type *B. plicatilis* have been classified s *B. rotundiformis* and *B. plicatilis*, respectively (Segers, 1995). Hagiwara et al. (1995) reported that three small, tropical marine rotifers, so called SS strains, were classified as *B. rotundiformis* because there was no evidence of a new species based on the study on its morphology, reproduction, genetics, and mating behavior. Lorica lengths of adult females were 179, 187, and 182 ㎛ in Thai, Fiji, and Okinawa strains, respectively.

On the other hand, Hur and Park (1996) isolated 16 Korean strains of the marine rotifers from salt pond, estuary, and lagoon, which were divided into two varieties, L-type (lorica length, 244.3-255.3 ㎛) and S-type (lorica length, 131.0-165.8 ㎛). Their study, however, mainly focused on the factors affecting resting egg formation. As the Cheju strain of adult female rotifers varied from 141.61 to 173.97 ㎛ in their lorica length under different rearing conditions in our laboratory (see Table 2), and had also pointed anterior spines, it is likely to be *B. rotundiformis*. Meanwhile, body size of field samples was much smaller than that of cultured rotifers in the laboratory, and showed 117.18-125.02 ㎛ in mean lorica length. However, due to the lack of egg-carrying adult females in field samples when these measurements were performed with randomly selected females whether carrying eggs or not, a more detailed field investigation regarding rotifer body size will be necessary in the future. In any event, Korean rotifer strains including the Cheju strain revealed rather smaller body size than most other strains that have been reported so far.

The effect of environmental conditions on rotifer body size has been studied by several investigators. Fukusho and Iwamoto (1981) investigated the influence of various feeds on the size and shape of the L-type rotifer, and reported that body size was increased when rotifers were fed with ω-yeast or a combination of baker's yeast and formula feed for prawn. In contrast, Yufera (1982) concluded that rotifer body size was primarily determined by its genetics, and not greatly influenced by environmental conditions (e.g. dietary manipulation). More intensive investigation of body size variability was conducted by Snell and Carrillo (1984) to determine the effect of salinity, temperature and rotifer strain on lorica length. They concluded that lorica size was largely determined by the genetic factor, but small environmental modification of lorica size is possible although an independent effect of either temperature or salinity turned out to be not statistically significant.

By the way, most studies on rotifer have been carried out with a clone which is generated by parthenogenetic propagation of one individual isolated from a strain. Investigators have thus focused on differences that occur between rotifer strains, and usually have ignored clonal differences within a strain. Monogonont rotifer has amictic and mictic cycles of reproduction depending on external and internal factors (reviewed by Gilbert, 1974; Pourrit and Snell, 1983). As the rotifer has a sexual reproductive cycle, genetic variation between clones would exist even within a population although it is unknown how many different clones constitute a rotifer population inhabiting a tide pool or how much difference exist between clones.

A recent study illustrated that there were some variations in isozyme pattern and number of chromosomes between S-type rotifer strains, as well as between two strains (clones) that originated from a single strain and were then parthenogenetically maintained for about the first six months (for more detail, see Rumengan et al., 1993). Therefore, this study has been designed to know the effect of clonal differences as a genetic factor. Our results indicated that lorica length was largely influenced by both clonal differences and temperature ($p<0.001$) although clonal differences as a genetic factor affected lorica length a little more than temperature did.

About the cyclomorphosis phenonenon, Fukusho and Iwamoto (1980) concluded that seasonal change of water temperature was one of the main factors inducing the strain replacement that occurred between the S- and L-type rotifer, whereas Kerfoot (1980) reported that clonal replacement likely occurred along with cyclomorphosis. Our results indicated that cyclomorphosis is largely influenced by both clonal differences and rearing temperature, but clonal differences as a genetic factor affected cyclomorphosis much more than temperature did.

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