Relationship between Water Temperature and Bisexual Reproduction Rate in the Rotifer *Brachionus plicatilis*^{*1}

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Brachionus plicatilis was cultured individually and successively under four (15, 20, 25 and 30°C) temperature conditions, and the frequency of mictic female production was examined. In order to avoid the density effect derived from accumulation of metabolites, culture medium was renewed at regular intervals proportionally to the metabolic rate which was specific to each temperature.

The hereditary differences in bisexual reproductivity existing among strains could be negligible through experimenting on identical strain. A hundred substrains were made from the same stock, and cultured for 7-8 generations, picking up five offsprings in one generation-one strain. Data from the initial two generations were rejected, because a temporary lowering of mictic female production was observed just after the transference into new environmental condition.

Bisexual reproduction rate increases in lower temperature below 25°C. There is no significant difference between 25 and 30°C groups.

Rotifers have been very attractive since the last century among biologists because of their world-wide distribution and peculiarity in life cycle. In recent years, they have become indispensable as food organisms in seedling production of aquaculture. Particularly in Japan, several million fish seedlings are annually released in natural waters even by a single prefectural institute. Consequently, many informations have been accumulated particularly on *Brachionus plicatilis*, supposing a large scale production as in a 100-200 m³ tank. There are also many fundamental studies on the biology of *B. plicatilis*, such as growth rate in experimental environments, filtering rates, morphological variety etc.^{*3} In addition, the inducing factors of bisexual reproduction have been also studied relating to continuation of parthenogenesis which is prerequisite to massculture. Bisexual reproduction produces male offsprings or only two or three fertilized dormant eggs, which does not contribute to population growth.

On the bisexual reproduction of *B. plicatilis*, it was reported that high density culture induces relatively high mictic female production.¹⁻³⁾ In addition to this external factor, HINO and HIRANO⁴⁾ made clear the preceding action of internal factors that the ability of bisexual reproduction differs

among strains and it is acquired as generation passes in a single pure line. In this study the relation between bisexual reproduction rate and temperature was experimented after considering the action of internal factors. Concerning the effect of temperature on the bisexual reproduction of *B. plicatilis*, IMAMURA *et al.*⁵⁾ succeeded in a large scale production of dormant eggs after stimulating through relatively high temperature. The mechanism of dormant egg formation is not, however, completely clear, because many steps must be existing in a population from the beginning of bisexual reproduction till the completion of dormant eggs. This study treats the appearance of a mictic female that is the first step of bisexual reproduction.

Materials and Methods

B. plicatilis experimented was the same stock as examined in previous studies,^{3,4)} and all individuals belonged to the identical strain which had only one stem mother as the ancestry that had hatched from a fertilized egg. Culture medium was food suspending seawater of which chlorinity was adjusted to 16‰. In this study, dry yeast for baking was applied to food without any activation, in order to

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^{*3} These are generally reviewed in "Shiomizu tsubo wamushi" (ed. by Japan. Soc. Sci. Fish.), Suisangaku series No. 44, Koseisha Koseikaku, Tokyo, 1983.

Fig. 1. Experimental method—Substraining and successive culture.

avoid the interference of physiological conversion of live food, such as green algae, in different temperature conditions.

A hundred females were picked up from the massculture of the aforesaid strain, and allotted individually to a hundred substrains. Each twenty or forty substrains were incubated in four temperature conditions (15, 20, 25 and 30, each $\pm 0.5^\circ\text{C}$) down to seventh or eighth generation in

Fig. 3. Rate of mictic female production under four temperature conditions.

total darkness after substraining. These successive cultures were inevitable particularly in the initial stage of the experiment in order to distinguish the mictic female production whether it was induced by the experimental condition itself or the results of transference into different temperature.

Any rotifer was cultured individually in a glass tube containing 0.1 ml/ medium, and transferred into new medium at regular intervals (T). Then a newly hatched offspring was received into another culture tube. The first daughter of each generation was employed as the mother of next generation and cultured till she produced five offsprings (Fig. 1). In case that five offsprings could not be obtained from the mother because of sterility, death or being mictic, the next available daughter was employed.

Fig. 2. Experimental method—Nullifying procedure of density effect.

Medium change and separation of neonate were made every "T" which corresponds to the time required for each hatching and maturing.

Table 1. Ratio of mictic females appearing among the first five offsprings of each generation under four temperature conditions

15°C						25°C						
Substrain	Generation after the Exp. was started					Substrain	Generation after the Exp. was started					Total
	4	5	6	7th	Total		4	5	6	7	8th	
01	3/5	0/3	1/3	0/2	4/13	41	0/4	0/4	0/5	1/5	0/5	1/23
02	0/3	2/5	1/5	0/4	3/17	42	0/5	0/3	0/5	0/3	0/2	0/18
03	0/3	0/3	1/4	0/4	3/14	43	0/2	0/2	0/4	0/5	1/4	1/17
04	0/4	1/3	1/3	1/3	3/13	44	0/2	0/5	0/4	0/1	0/3	0/15
05	1/5	3/5	0/3	1/4	5/17	45	0/4	1/3	0/3	0/3	2/4	3/17
06	0/1	2/5	1/2	2/3	5/11	46	0/3	0/5	0/5	0/3	0/5	0/21
07	0/2	1/1			1/ 3	47	1/5	0/3	0/5	1/5	0/3	2/21
08	1/5	0/4	3/4	0/1	4/14	48	1/2	1/5	0/4	0/3	0/4	2/18
09	1/1				1/ 1	49	0/4	0/2	0/3	0/2	0/2	0/13
10	2/5	2/4	0/5	0/3	4/17	50	1/4	0/3	0/5	0/2	0/5	1/19
11	1/3	1/3	1/4	1/2	4/12	51	0/2	0/2	0/5	0/3	0/2	0/14
12	2/5	3/4	0/1	1/1	6/11	52	0/4	0/2	0/5	0/4	0/3	0/18
13	0/5	0/3	1/3		1/11	53	1/1					1/ 1
14	0/5	2/5	2/5	2/2	6/17	54	0/4	0/5	0/3	0/5	0/5	0/22
15	0/5	2/3			2/ 8	55	0/5	0/3	1/5	1/4	1/4	3/21
16	0/4	1/2	1/2	1/2	3/10	56	0/5	0/5	1/4	0/5	1/3	2/22
17	0/4				0/ 4	57	0/4	1/5	0/3	0/3	0/2	1/17
18	0/3	1/5	0/2		1/10	58	0/3	0/4	0/4	0/5	0/1	0/17
19	1/3	1/5	0/3	1/1	3/12	59	0/5	0/3	0/3	0/5	0/5	0/21
20	0/2	0/1			0/ 3	60	0/2	0/5	0/5	0/4	0/4	0/20
					Total	61	0/1	1/5	0/4	1/4		2/14
					59/218	62	0/5	0/3	1/5	2/5		3/18
						63	0/4	0/5	0/4	0/5		0/18
						64	0/4	0/4	0/3	0/5		0/16
						65	0/5	0/4	0/5	0/4		0/18
						66	0/5	0/4	0/4	0/4		0/17
						67	0/4	0/3	0/5	0/4		0/16
						68	0/5	0/4	0/4	0/5		0/18
						69	0/5	0/4	1/5	0/5		1/19
						70	0/5	0/5	0/5	0/5		0/20
						71	0/5	0/5	0/5	0/3		0/18
						72	0/3	0/4	0/5	0/5		0/17
						73	0/4	0/2	0/5	0/4		0/15
						74	1/5	0/5	0/4	0/1		1/15
						75	0/3	0/5	0/4	0/5		0/17
						76	0/3	1/4	0/5	0/4		1/16
						77	0/1	0/3	0/4	1/3		1/11
						78	0/5	0/5	0/5	0/3		0/18
						79	0/3	1/5	0/4	0/4		1/16
						80	1/5	0/5	0/4	2/3		3/17
											Total	30/689

Table 1. (continues)

30°C	Generation after the Exp. was started					Total
	4	5	6	7	8th	
81	0/5	1/5	2/5	0/3	0/4	3/22
82	0/4	1/4	0/5	0/2	0/3	1/18
83	0/5	0/5	0/4	0/3	0/4	0/21
84	1/5	1/4	1/4	0/3	0/5	3/21
85	0/5	0/3	0/5	0/5	2/2	2/20
86	0/5	0/5	0/5	0/2	0/4	0/21
87	0/5	0/5	0/5	0/2	0/4	0/21
88	0/4	0/4	0/5	0/4	0/5	0/22
89	0/3	0/3	1/5	0/3	0/4	1/18
90	0/5	0/4	0/4	1/5	0/5	1/23
91	0/5	0/3	0/4	0/5	0/3	0/20
92	0/4	0/4	0/4	0/4	1/5	1/21
93	0/5	0/4	1/5	1/3	0/2	2/19
94	0/5	0/3	0/5	0/3	0/3	0/19
95	0/5	0/4	0/5	0/5	0/3	0/22
96	0/1	0/5	0/5	0/3	0/4	0/18
97	0/5	0/5	0/3	0/5	0/3	0/21
98	1/5	0/5	0/5	0/4	0/3	0/22
99	0/4	0/5	0/4	0/3	0/2	0/18
100	0/5	0/5	1/4	0/4	0/5	1/23
Total						15/410

The denominator means total No. of offsprings which could be discriminated mictic or amictic.

Female type was discriminated by egg size. An amictic female lays a diploid egg which is larger than a haploid egg laid by a mictic female.

Nullifying the Density Effect

Mictic female production in *B. plicatilis* is a density dependent phenomenon as in the case of *B. calyciflorus*,⁶⁾ and the substance of the density effect was thought to be metabolites accumulating in culture medium. It may arise that the experimental condition of temperature may degenerate into density effect because of the difference in metabolic level. It is resolved, however, by means of renewing culture medium at regular intervals which are proportional to metabolic rate (Fig. 2). In this study, the interval was decided to the time required to get hatching of a parthenogenetic egg, which was 48, 36, 24 and 18 h. at 15, 20, 25 and 30°C respectively. These values were calculated with Q_{10} law⁷⁾ from the observation at 25°C.

Results and Discussion

Table 1 shows the ratio of mictic females to the total number of individuals which could be dis-

Table 2. Numbers of mictic and amictic females appearing under four temperature conditions

Female type	Temp. experimented				Total
	15	20	25	30°C	
Mictic	59	30	30	15	134
Amictic	159	296	659	395	1509
Total	218	326	689	410	1643
Mictic (%)	27.1	9.2	4.4	3.7	
$\alpha < 0.01$ $\alpha < 0.01$ N.S.					

tinguished whether mictic or amictic among the first five offsprings of each generation. The data from the 2nd and 3rd generations were rejected in view of the previous report⁴⁾ where rotifers made bisexual reproduction rate down over a few generation after they had been transferred into a new culture condition. On the 4th generations of 15 and 20°C groups, the appearance rates of mictic females are lower than those of later generations, which shows the lowering phase has not recovered yet. As to 25 and 30°C groups, the phenomenon is not obvious because of the low inductivity of mictic females.

The numbers of mictic and amictic females were summed up in Table 2. As the result of χ^2 -test between adjoining two conditions, significant differences are in evidence below 25°C, but it is not in existence between 25 and 30°C groups. Considering that *B. plicatilis* reproduces normally within the temperature from 15 to 30°C, it is given as a conclusion that bisexual reproduction rate increases in lower temperature below 25°C.

Generally in animals and plants, temperature has substantially two meanings. One is the induction of response specific to a certain temperature, and another is that caused by its fluctuation. In genus *Brachionus*, HALBACH and HALBACH-KEUP⁸⁾ reported that a fluctuation of temperature induced bisexual reproduction in *B. calyciflorus*, *B. rubens* and *B. angularis*, contrary to the experiment on *B. rubens* by LADERMAN and GUTTMAN.⁹⁾ As to *B. plicatilis*, as stated before, it exhibits a temporary lowering of mictic female production for a few generations just after the rearing condition is changed. Consequently, positive induction of bisexual reproduction caused by fluctuation of temperature could not be recognized through our methods.

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