Relationship between the Temperature Given at the Time of Fertilized Egg Formation and Bisexual Reproduction Pattern in the Deriving Strain of the Rotifer *Brachionus plicatilis*^{*1}

Akinori HINO^{*2} and Reijiro HIRANO^{*2}

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Temperature given at the time of fertilized (resting) egg formation in the rotifer *Brachionus plicatilis* was discussed, from a point of view that it may cause great difference in bisexual reproductivity among the deriving strains.

Fertilized eggs were produced at five temperatures (17, 20, 25, 27 and 30°C) in sea water. Neonates from the eggs were employed as stem mothers of respective experimental strains. Every strain consisted of isogenic individuals through keeping parthenogenesis, which was ensured by the individual culture in 0.1 ml sea-water medium. Media were renewed at regular intervals of 12, 24 or 36 hours at 30, 25 or 20°C respectively in order to prevent the accumulation of metabolites which is an exciting cause of bisexual reproduction.

The temperature given in the process of fertilized egg formation determines the bisexual reproduction pattern of deriving strains. Among the strains derived from the fertilized eggs which were produced at high temperature over 25°C, the appearance rate of mictic females has no relation to temperature. Among those, however, derived from the eggs originating in low temperature below 25°C, it varies inversely with temperature below 25°C, but not affected above 25°C.

Bisexual reproduction in the rotifer *Brachionus plicatilis* is clearly induced under high population density¹⁻³⁾ or a low temperature.⁴⁾ The authors observed in the further inquiries that the change of mictic female production rate with temperature differs among strains, although they commonly produce mictic females at a low temperature. In this study, the origin of the phenomenon was investigated, taking notice of the temperature conditioned at the time of fertilized egg (resting egg) formation. Fertilized eggs were produced at five temperatures and stored, and then mictic female production rates of the new strains originated from those fertilized eggs were compared at three temperatures.

The similar phenomena that the history of resting eggs affects the mode of reproduction among deriving descendants are well known in insects, and also studied by STROSS and HILL⁵⁾ in the Cradoccean *Daphnia* which has similar life cycle to rotifers. *D. pulex* bore more male offsprings in the swarm originating from those resting eggs which had been produced under short-day periodicity.

Materials and Methods

B. plicatilis was the same stock as examined in previous reports^{3,4)} and cultured in sea water of which chlorinity was adjusted at 16‰. All rotifer cultures were kept in the dark.

Massculture for Fertilized Egg Formation

Massculture for collecting fertilized eggs was carried out in 500-2000 ml of culturing media as shown in the left half of Fig. 1. *Chlamydomonas* sp. of marine species was supplied once a day for food so as to be 3×10^5 cells/ml after centrifuged. Yeast could not be utilized, because few fertilized eggs were produced by the strains examined when it was provided with. Rotifer density was adjusted at 100 individuals/ml also once a day through eliminating excess rotifers with a plankton net cloth.

Temperature condition was graded into five: A; 17°C, B; 20°C, C; 25°C, D; 27°C and E; 30°C.

Successive Culture for Testing Bisexual Reproductivity

From each egg lot, 7 to 20 neonates, that is the

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^{*2} Department of Fisheries, Faculty of Agriculture, The University of Tokyo, Yayoi 1-1, Bunkyo, Tokyo 113, Japan (日野明德・平野礼次郎: 東京大学農学部水産学科).

Fig. 1. Procedures in testing the effect of temperatures which were given in the process of fertilized egg formation.

left half: process of fertilized egg formation under five temperature conditions.

right half: successive culture in testing the bisexual reproductivity under three temperature conditions.

first generation were hatched out through incubating at 25°C for two days under continuous illumination (2000 lx). Straining was made by employing the first generation as the stem mother of a strain. After that, neonates were produced successively by parthenogenesis. Accordingly, any individual in the same strain has the identical gene. In every generation of a strain, the first daughter was employed as the mother of next generation (right half of Fig. 1).

Culturing medium was food suspended sea water of which chlorinity was 16‰. Dry yeast for baking was provided as food without any activation for testing bisexual reproductivity at different temperatures, intending to avoid the possible interference caused by physiological conversion of live green algae. The food density was adjusted at 10^7 cells/ml.

Any rotifer was cultured individually in a glass tube containing 0.1 ml culture medium, and transferred into new medium at regular intervals (T). If a newly hatched neonate was observed then, it was transferred into another culture tube. The time interval "T" was determined in proportion to the metabolic level varied with each temperature in order to nullify the density effect on mictic female production as shown in the previous report,⁴⁾ which was 12, 24 and 36 h at 30, 25 and 20°C respectively.

In the course of successive culture, substraining was made twice in order to be assigned for the

Fig. 2. Effect of the temperature given in the process of fertilized egg formation on the variation of bisexual reproduction pattern in deriving new strains.

egg origin: the temperature at which fertilized eggs as ancestry of experimental strains were formed.

Table 1. History of fertilized egg formation and the frequency of bisexual reproduction in the deriving strain

Egg origin*	Temp. experimented			Temp. experimented		
	20°C	25°C	30°C	20°C	25°C	30°C
series						
A: 17°C	52/136 (38.2%)	8/172 (4.7%) (7 strains)	3/315 (1.0%)			
B: 20°C	56/366 (15.3)	71/752 (9.4) (13 strains)	28/325 (8.6)	58/392 (14.8)	56/638 (8.8) (14 strains)	46/446 (10.3)
C: 25°C	123/512 (24.0)	90/1410 (6.4) (20 strains)	57/868 (6.6)	31/272 (1.4)	56/576 (9.7) (14 strains)	18/285 (6.3)
D: 27°C	53/447 (11.9)	117/1092 (10.7) (16 strains)	59/463 (12.7)			
E: 30°C	33/335 (9.9)	79/1059 (7.5) (17 strains)	60/457 (13.1)	46/352 (13.1)	52/582 (8.9) (12 strains)	16/197 (8.1)

* temperature given at the time of fertilized egg formation.

experimentations at 20 and 30°C respectively. The next available daughter other than the first was employed as the stem mother of a substrain then. Consequently, the identical strains could be allotted to all three temperature conditions.

In each temperature condition, bisexual reproduction rate, namely the rate of mictic females was calculated after summing up both numbers of amictic and mictic females which had appeared among the eldest five daughters of each generation.

Results and Discussion

The data before the seventh generation after hatching of fertilized eggs were omitted, in the light of past informations^{4,6)} that the ability of bisexual reproduction responding to some external factor could not be acquired till near then. Also, the data from the two generations since transferred into different temperature were omitted, in order to avoid the effect of temperature alteration.⁴⁾

Table 1 shows the ratio of mictic females appearing among 13086 females which could be distinguished whether amictic or mictic under three temperature conditions. The number of those strains which could be examined under all three temperatures in common was 113.

In each identical strain, the percentages of mictic females were compared among those three temperatures, and the remainders of percentages were found between adjoining two conditions. These remainders were arranged according to

tested strains and analyzed by Wilcoxon's signed rank test or paired sample t-test, which enabled to disregard the hereditary differences in bisexual reproductivity existing among strains. In Fig. 2, solid line indicates the significant difference above 95% confidence level.

Commonly to any experiment, there is no significant difference in the percentage of mictic female production between 30 and 25°C. In contrast, the results of mictic female production in 25 and 20°C depend on egg origin. When the rotifer strains are derived from the fertilized eggs which had been produced at low temperatures (20 and 17°C in this study), more mictic females are produced in the rearing at 20°C. In the case of those eggs which were produced at high temperatures (30 and 27°C in this study), there is no significant difference between 20 and 25°C in the rearing of deriving strains. The strains deriving from the eggs which have been produced at 25°C show an eclectic tendency; 25°C is thought to be critical temperature.

In a different expression, it is summarized as follows. The temperature given in the process of fertilized egg formation determines the bisexual reproduction pattern of hatched strains. Among the strains derived from the fertilized eggs which had been produced under a high temperature (over 25°C in this study), the appearance rate of mictic females would not be affected by culturing temperature. Among those, however, derived from the eggs originating in a low temperature (under 25°C in here), it varies inversely as temperatures

below 25°C, but not affected above 25°C.

It is suggested that the environmental conditions which had been given in the course of fertilized egg formation can be the origin of the internal factor that there lies great difference in bisexual reproductivity among the deriving strains. This may assure the breeding of *B. plicatilis* for massculture and resting egg formation, because bisexual reproduction is interruptive for massculture but essential for resting egg formation as well.

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