Maternal Effect on Salinity Tolerance in Newborn Guppy *Poecilia reticulata*

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Salinity tolerance was examined in 14 strains of the guppy. Salinity tolerance was measured as LD₅₀ 24 h after transfer to serial concentrations of salinity. The LD₅₀ showed high values at birth and significantly decreased within 5 days after birth. The LD₅₀ was higher at birth than at 10 days after birth and at an adult stage in all of the strains. Strain differences were observed in the LD₅₀ at birth, 10 days after birth, and at an adult stage. Positive correlation was not observed between the strain differences at birth and at the adult stage but was observed between 10 days after birth and at the adult stage, suggesting that genetic control for salinity tolerance at birth differs from that of specimens over 10 days after birth. Cross experiments revealed that survival rates of F₁ hybrids in 35 ppt seawater at birth showed a positive correlation with rates of female parents but not with male parents. This indicated a maternal effect on salinity tolerance of newborn guppies at birth. The results of backcross suggested that the salinity tolerance of newborn guppies is not controlled by cytoplasmic genetic factors but by the mother's genotypes.

Key words: salinity tolerance, osmoregulation, maternal effect, strain difference, cross experiment, ovoviparous fish, newborn, guppy

It has been reported that salinity tolerance and osmoregulatory function at the adult stage differed among strains of the guppy, suggesting that salinity tolerance is genetically controlled.^{1,2)} The realized heritability of salinity tolerance of the guppy was calculated as 0.655 from a selection experiment.³⁾ Shikano *et al.*⁴⁾ reported that F₁ hybrids between strains showed significantly longer survival times in 35 ppt seawater than their parental strains, indicating heterotic effect on salinity tolerance of adult guppies.

A previous paper⁵⁾ showed that newborn guppies have higher salinity tolerance than adult guppies and that the high salinity tolerance plays an important role in successful propagation of the guppy in seawater. High salinity tolerance of euryhaline teleosts at early life stages has also been reported in the Atlantic salmon *Salmo salar*,⁶⁾ the ayu *Plecoglossus altivelis*,⁷⁾ and the tilapia *Oreochromis mossambicus*.⁸⁾ Hwang and Hirano⁷⁾ reported that the survival of the larvae of the ayu at the time of hatching in seawater correlated to the elaboration of chloride cells in the skin and branchial epithelium. Ayson *et al.*⁹⁾ also reported that mitochondria-rich cells on the yolk-sac membrane of the embryos and newly hatched larvae of the tilapia were larger in seawater than in fresh water. Several studies have been done concerning salinity tolerance and osmoregulatory action at the early life stages of teleosts.^{5–12)} However, it was not clear what factor induces the high salinity tolerance and the high osmoregulatory function at the early life stages in euryhaline teleosts.

Cross experiments will be useful to clarify the genetic controls for the high salinity tolerance at the early life stages. The present study examines relationships between strain differences in salinity tolerance of newborn and adult guppies and demonstrates the maternal effect on sa-

linity tolerance of newborn guppies at birth using cross experiments.

Materials and Methods

Animals

Fourteen guppy strains, S, S3, SC, M1, O, F22, T, T1, G, D, D1, A, B, and C, were used in this study. A description of the guppy strains, how they were produced and maintained, was given in earlier papers.^{1,13,14)} Each strain was maintained as a closed colony in a 60 l aquarium with a density of 200–300 individuals at a temperature of 23±2°C. The fish were fed twice daily with ground dry carp pellets and dried *Daphnia* as a supplementary diet. Newborn guppies of each strain were obtained from the gravid females transferred into 500 ml conical beakers in a random fashion.

Cross Experiments

Diallel and reciprocal crosses were performed among the A, F22, SC, and S strains. Backcross was also performed between the A and S strains. Immature guppies were taken from the parent stocks at random and reared in 2.5 l aquaria in order to obtain virgin females for crossing. Each virgin female mated with a male in a 2 l aquarium. The litters produced were used for the seawater challenge tests.

Seawater Challenge Tests

Day 0 (newborn within 6 h of birth), Day 1, Day 2, Day 3, Day 5, Day 7, Day 10, Day 15, and adult (over 60 days of birth) guppies of the F22, S3, and S strains and Day 0, Day 10, and adult guppies of other strains were trans-

ferred directly from fresh water to saline water with 15.0 ppt, 17.5 ppt, 20.0 ppt, 22.5 ppt, 25.0 ppt, 27.5 ppt, 30.0 ppt, 35.0 ppt, or 40.0 ppt salinity (Aquasalz, Nissei). Day 0 guppies of F₁ hybrids and backcross were transferred to 35 ppt seawater. As control, guppies were transferred to fresh water. Up to 20 individuals were held in a 1 l aquarium filled with saline water at a temperature of $23.0 \pm 0.5^\circ\text{C}$. Survival rates were measured 24 h after the transfer. The detection of death was determined by the complete cessation of opercular movement and the non-reaction against stimulations. Fifty percent lethal dose (LD₅₀) was calculated from the regression line drawn from the data of the survival rates.

Results

Strain Differences

Figure 1 shows changes in the LD₅₀ during the 15 days after birth in the F22, S3, and S strains. The LD₅₀ showed the highest values at birth in all of the three strains. With increase in the days after birth, the LD₅₀ decreased within 5 days after birth and then was stable there after. Table 1 shows LD₅₀ at birth, at 10 days after birth, and at the adult stage in 14 guppy strains. The LD₅₀ varied among the strains from 28.1 ppt to 34.2 ppt at birth, from 20.1 ppt to 23.1 ppt at 10 days after birth, and from 21.4 ppt to 26.9 ppt at the adult stage. The results indicate that salinity tolerance at birth was higher than at 10 days after birth and at the adult stage in all of the strains and that strain differences were observed at all three stages.

Figure 2 shows the relationships of the strain differences among the three stages. A positive correlation was not observed between the strain differences at birth and at the adult stage but was observed between the specimens at 10 days after birth and at the adult stage. The result indicates that the strain differences of salinity tolerance at birth differ from those at 10 days after birth and at the adult stage.

Cross Experiments

Cross experiments on salinity tolerance of newborn guppies were performed among the A, F22, SC, and S strains.

Fig. 1. Time-courses of the changes in the LD₅₀ from birth in the F22 (●), S3 (■), and S (▲) strains.

Table 1. LD₅₀ at birth, 10 days after birth, and at adult stage in 14 strains

Strain	LD ₅₀ (ppt)		
	Day 0	Day 10	Adult stage
A	34.2 (305)	21.3 (69)	23.2 (42)
F22	34.1 (1176)	21.5 (127)	24.9 (41)
O	33.6 (571)	23.1 (116)	26.9 (166)
C	33.2 (713)	21.7 (196)	24.1 (115)
T1	33.2 (388)	22.7 (77)	23.5 (63)
B	31.7 (754)	21.7 (169)	24.2 (57)
S3	30.6 (885)	21.1 (152)	23.0 (181)
D	30.2 (544)	20.7 (175)	22.1 (41)
G	30.1 (528)	20.1 (88)	22.8 (55)
D1	29.4 (515)	20.4 (59)	21.4 (97)
T	29.2 (429)	21.8 (93)	24.0 (152)
M1	28.8 (474)	20.5 (170)	23.5 (155)
S	28.2 (619)	20.2 (120)	22.5 (73)
SC	28.1 (526)	22.7 (88)	24.5 (91)

Number of tested guppies is shown in parentheses.

Fig. 2. Relationships between the strain differences in the LD₅₀ at birth and at the adult stage (A), and between those at 10 days after birth and at the adult stage (B).

Table 2. Survival rates of newborn guppies in 35 ppt seawater and fresh water in the A, F22, SC, and S strains and their F₁ hybrids

Cross (Female × Male)	35 ppt seawater					Fresh water (control)				
	N ₁	N ₂	Survived	Died	Survival (%)	N ₁	N ₂	Survived	Died	Survival (%)
P(A)	11	188	72	116	38.3	3	46	46	0	100.0
P(F22)	42	804	268	536	33.3	5	73	73	0	100.0
P(SC)	25	258	7	251	2.7	8	52	52	0	100.0
P(S)	23	254	3	251	1.2	4	80	80	0	100.0
F ₁ (A × F22)	6	113	25	88	22.1	4	80	80	0	100.0
F ₁ (F22 × A)	11	375	97	278	25.9	6	115	115	0	100.0
F ₁ (SC × S)	14	299	2	297	0.7	6	84	84	0	100.0
F ₁ (S × SC)	16	412	1	411	0.2	5	100	100	0	100.0
F ₁ (A × SC)	8	135	54	81	40.0	5	78	78	0	100.0
F ₁ (SC × A)	7	155	11	144	7.1	4	63	63	0	100.0
F ₁ (A × S)	9	267	93	174	34.8	5	110	110	0	100.0
F ₁ (S × A)	11	284	6	278	2.1	5	105	105	0	100.0
F ₁ (F22 × SC)	16	586	283	303	48.3	5	82	81	1	98.8
F ₁ (SC × F22)	15	255	2	253	0.8	7	66	66	0	100.0
F ₁ (F22 × S)	19	412	129	283	31.3	6	105	105	0	100.0
F ₁ (S × F22)	19	390	0	390	0	9	123	123	0	100.0

N₁: Number of crosses.N₂: Number of tested newborn guppies.

Salinity tolerances of newborn guppies were high in the A and F22 strains and low in the SC and S strains (Table 1). Table 2 shows survival rates of newborn guppies in 35 ppt seawater and in fresh water in the A, F22, SC, and S strains and their F₁ hybrids. All or almost all guppies survived in fresh water. The survival rates in 35 ppt seawater were 38.3% in the A strain, 33.3% in the F22 strain, 2.7% in the SC strain, and 1.2% in the S strain. The F₁ hybrids between the A and F22 strains showed more than 20% survival rates in reciprocal crosses. The F₁ hybrids between the SC and S strains showed low survival rates in reciprocal crosses. However, the survival rates significantly differed between reciprocal crosses in the F₁ hybrids between the A and SC strains, between the A and S strains, between the F22 and SC strains, and between the F22 and S strains. A positive correlation was observed between the survival rates of the F₁ hybrids and the female parents but not observed between the F₁ hybrids and the male parents (Fig. 3).

Table 3 shows the survival rates of the backcross progeny of the F₁ hybrids between the A and S strains in 35 ppt seawater and in fresh water. All guppies survived in fresh water. The survival rates in 35 ppt seawater were 1.0% in the B₁[(A × S) × S] progenies and 0% in the B₁[(S × A) × A] progenies.

Discussion

A previous paper⁵⁾ reported that newborn guppies of the F22 strain showed significantly higher salinity tolerance at birth than adult guppies. The present study focused on the genetic control for the high salinity tolerance of the newborn guppy. Newborn guppies showed significantly higher salinity tolerance than at 10 days after birth or at the adult stage in all of the 14 strains used in this study. This suggests that the high salinity tolerance at birth is commonly observed in the guppy. It was reported that salinity toler-

ance of adult guppies differed among strains with no variation in relation to sexes and sizes.¹⁾ No correlation was observed between salinity tolerance and size in the newborn guppy, either (unpublished observation). The present study demonstrated that salinity tolerance differed among the strains at the three stages. Correlation analysis showed that the strain differences of salinity tolerance at birth differ from those at 10 days after birth and at the adult stage, suggesting that the genetic control for salinity tolerance of newborn guppies differs from that of specimens at 10 days after birth and at the adult stage.

Cross experiments on salinity tolerance of newborn guppies were performed among the A, F22, SC, and S strains. Salinity tolerance of newborn guppies was high in the A and F22 strains and low in the SC and S strains, although these strains had the same level of salinity tolerance at the adult stage. The results revealed that survival rates of the F₁ hybrids in seawater did not correlate with those of the male parents but corresponded to those of the female parents, indicating maternal effect on salinity tolerance of the newborn guppy. If the salinity tolerance of the newborn guppy was inherited through cytoplasmic genetic factors, salinity tolerance in backcross progenies of F₁ hybrids would show the same levels as that of their female parents and also the female parents of the F₁ hybrids. However, the B₁[(A × S) × S] progenies showed significantly lower survival rates in seawater. Therefore, the salinity tolerance of the newborn guppy may be genetically controlled by not cytoplasmic genetic factors but by the mother's genotypes. The notion was supported by the facts that paternal genetic contribution was not invaluable and also that the B₁[(A × S) × S] and the B₁[(S × A) × A] progenies, whose mothers have the same genotypes, showed the same level in salinity tolerance.

Shikano *et al.*⁴⁾ reported that F₁ hybrids between strains showed significantly longer survival time in 35 ppt sea-

Fig. 3. Relationships between the survival rates of newborn guppies in 35 ppt seawater in F₁ hybrids and female parents (A), and between those in F₁ hybrids and male parents (B).

water than their parental strains in adult guppies, indicating heterotic effect on salinity tolerance of the adult guppy. Judging from the present results and the above-mentioned study, salinity tolerance of the guppy and the genetic control for it may be characterized as follows: Newborn guppies have high salinity tolerance at birth, and the tolerance significantly decreases within 5 days after birth and then is stable there after. The high salinity tolerance of the newborn guppy is genetically controlled by the mother's genotypes within the mother's ovary. The

genetic controls gradually change to its own genotypes within 5 days after birth, and heterotic effect is observed. The characterizations suggest that the high salinity tolerance of the newborn guppy is induced by the maternal effect.

Shikano and Fujio¹⁵⁾ previously reported that the newborn guppies yielded by seawater-reared guppies showed significantly higher survival rates in 35 ppt seawater than those yielded by freshwater-reared guppies, and demonstrated that the mother's environmental salinity enhances the salinity tolerance of the offspring at birth through the medium of the mother. This indicates the effects of the mother's environmental salinity on salinity tolerance of the newborn guppy. Results from the present study and the above-mentioned study suggest that salinity tolerance of the newborn guppy is controlled by the interaction between the mother's genotypes and environmental salinity.

In oviparous fish, high salinity tolerance at early life stages has been reported in the Atlantic salmon,⁶⁾ the ayu,⁷⁾ and the tilapia.⁸⁾ Heuts¹⁶⁾ showed that two populations of the stickleback *Gasterosteus aculeatus* need physiologically different adjustmental salinity to hatch and the difference is genetically controlled by the maternal effect. Influence of the parents' environmental salinity on the salinity tolerance of their offspring at the early life stage has also been reported in the medaka *Oryzias latipes*¹⁷⁾ and *Silago sihama*.¹⁸⁾ These facts indicate the possibility that the salinity tolerance at early life stages in oviparous fish is also influenced by the mother's genotypes and environmental salinity through the medium of the yolk sac.

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Table 3. Survival rates of newborn guppies in 35 ppt seawater and fresh water in backcross progeny

Cross (Female × Male)	35 ppt seawater					Fresh water (control)				
	N ₁	N ₂	Survived	Died	Survival (%)	N ₁	N ₂	Survived	Died	Survival (%)
B ₁ [(A × S) × S]	18	299	3	296	1.0	5	79	79	0	100.0
B ₁ [(S × A) × A]	14	228	0	228	0	9	99	99	0	100.0

N₁: Number of cross.

N₂: Number of tested newborn guppies.

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