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A Factor in Seawater Restricting Strain Differences in Seawater Tolerance of the Guppy *Poecilia reticulata*

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Summary

A factor in seawater restricting strain differences in seawater tolerance was examined in the guppy. Seawater tolerance was measured as LD_{50}, which was estimated from the survival rates 24 h after transfer to serial concentrations of seawater. Seawater tolerance differed from an LD_{50} of 21.4 ppt to 26.9 ppt among the 13 strains. When fish were transferred to several seawater components, all fish survived in 0.029 M Na_2SO_4, 0.026 M $MgCl_2$, 0.009 M KCl or 0.008 M $CaCl_2$ but all fish died in 0.420 M NaCl. NaCl tolerance differed from an LD_{50} of 0.285 M to 0.345 M among the 13 strains. A significant positive correlation was observed between the strain differences in seawater and NaCl tolerances. This indicates that the NaCl in seawater is a factor restricting the strain differences in seawater tolerance.

In aquatic organizations, salinity is one of the most important environmental factors in their survival, metabolism and distribution. Teleosts need to keep internal osmotic pressure at about one third that of seawater. Thus, they need to excrete excess salts into the seawater and uptake ions from fresh water in order to maintain their homeostasis (1).

Recently, some investigators reported that seawater tolerance differed among genetically different strains in freshwater teleosts (2, 3). Genetic analyses for seawater tolerance were also conducted (4, 5). Because seawater contain several ions, it is important to examine the environmental factor restricting the genetic differences in seawater tolerance.

The guppy is one of useful teleosts for the genetic analyses of several traits because of its short life cycle, establishment of many strains (6, 7) and ease of breeding. Shikano and Fujio (3) previously reported that seawater tolerance significantly differed among the strains with no relation to sex and size. The present study examines the factor in seawater restricting the strain differences in seawater tolerance of the guppy.

Materials and Methods

Animals

Thirteen guppy strains, S, S3, SC, M1, O, F22, T, T1, G, D, D1, B and C, were used in this study. A description of the guppy strains, how they were produced and maintained, was given in previous papers (3, 6). 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 carp pellets and dried Daphnia as a supplementary diet.

Tolerance to seawater

Mature guppies, older than about 60 days, were collected from each strain in a random fashion. Up to 5 individuals were transferred to a 1 *l* aquarium filled with 20.0 ppt, 22.5 ppt, 25.0 ppt, 27.5 ppt or 30.0 ppt artificial seawater (Aquasalz, Nissei) at a temperature of 23 ± 1°C. The detection of death was determined by the complete cessetion of opercular movement and the non-reaction against stimulations. The fifty percent lethal dose (LD_{50}) was calculated from the regression line drawn using the survival rate data.

Tolerance to seawater components

Mature guppies of the O, S3 and D1 strains were transferred to fresh water containing 35 ppt seawater components of 0.420 M NaCl, 0.029 M Na_2SO_4, 0.026 M $MgCl_2$, 0.009 M KCl or 0.008 M $CaCl_2$. Up to 5 individuals were held in a 1 *l* aquarium. Survival rates were measured 24 h after the transfer.

Tolerance to NaCl

Mature guppies of the 13 strains were transferred to 0.240 M, 0.270 M, 0.300 M, 0.330 M, 0.360 M or 0.390 M NaCl dissolved in fresh water. Up to 5 individuals were held in a 1 l aquarium. Survival rates were measured 24 h after the transfer. The LD_{50} was calculated from the regression line drawn using the survival rate data.

Results

Table 1 shows the survival rates 24 h after transfer to the serial concentrations of seawater. Most fish in the 13 strains survived in 20.0 ppt seawater. Survival rates decreased with increased salinity and all fish died in 30.0 ppt seawater. Survival rates differed among the strains from 22.2% to 100.0% in 22.5 ppt, from 0% to 62.5% in 25.0 ppt and from 0% to 46.9% in 27.5 ppt. The LD_{50} differed

TABLE 1. *Survival Rates and LD_{50} 24 h after Transfer to Seawater in the 13 Strains*

Strain	Salinity (ppt)					LD_{50} (ppt)
	20.0	22.5	25.0	27.5	30.0	
O	100.0 (22)	89.7 (39)	62.5 (56)	46.9 (49)	0 (11)	26.9
F22	100.0 (14)	100.0 (11)	44.4 (9)	0 (7)	0 (7)	24.6
SC	100.0 (13)	88.0 (25)	28.6 (14)	10.3 (39)	0 (30)	24.5
B	100.0 (16)	85.7 (14)	23.5 (17)	0 (14)	0 (8)	24.2
C	93.1 (29)	73.0 (37)	37.5 (24)	4.0 (25)	0 (22)	24.1
T	97.9 (47)	74.0 (50)	35.0 (40)	13.3 (15)	0 (11)	24.0
T1	92.9 (14)	52.6 (19)	38.5 (13)	5.9 (17)	0 (10)	23.5
M1	94.7 (38)	68.6 (51)	27.5 (51)	6.7 (15)	0 (11)	23.5
S3	88.6 (35)	69.8 (43)	15.4 (39)	1.6 (64)	0 (31)	23.0
G	92.3 (13)	66.7 (15)	5.6 (18)	0 (9)	0 (8)	22.8
S	100.0 (11)	47.1 (17)	0 (9)	0 (36)	0 (23)	22.5
D	100.0 (11)	23.1 (13)	0 (10)	0 (7)	0 (8)	22.1
D1	75.9 (29)	22.2 (36)	0 (25)	0 (7)	0 (7)	21.4

Number of tested fish is included in parentheses.

TABLE 2. *Survival Rates 24 h after Trasfer to Several Seawater Components in the O, S3 and D1 Strains*

Strain	Seawater components				
	0.420 M NaCl	0.029 M Na_2SO_4	0.026 M $MgCl_2$	0.009 M KCl	0.008 M $CaCl_2$
O	0 (9)	100.0 (9)	100.0 (9)	100.0 (9)	100.0 (9)
S3	0 (9)	100.0 (9)	100.0 (9)	100.0 (9)	100.0 (9)
D1	0 (9)	100.0 (9)	100.0 (9)	100.0 (9)	100.0 (9)

Number of tested fish is included in parentheses.

from 21.4 ppt to 26.9 ppt among the strains (Table 1).

Table 2 shows the survival rates 24 h after transfer to 0.420 M NaCl, 0.029 M Na_2SO_4, 0.026 M $MgCl_2$, 0.009 M KCl and 0.008 M $CaCl_2$ in the O, S3 and D1 strains. In all three strains, all fish survived in 0.029 M Na_2SO_4, 0.026 M $MgCl_2$, 0.009 M KCl and 0.008 M $CaCl_2$ but all fish died in 0.420 M NaCl.

Table 3 shows the survival rates 24 h after transfer to the serial concentrations of NaCl. Survival rates of the 13 strains decreased with an increase in the NaCl concentration. Survival rates differed among the strains from 66.7% to 100.0% in 0.270 M, from 33.3% to 100.0% in 0.300 M, from 0% to 66.7% in 0.330 M and from 0% to 33.3% in 0.360 M. The LD_{50} differed from 0.285 M to 0.345 M among the

TABLE 3. *Survival Rates and LD_{50} 24 h after Transfer to NaCl in the 13 Strains*

Strain	NaCl (M)						LD_{50} (M)
	0.240	0.270	0.300	0.330	0.360	0.390	
O	100.0 (9)	100.0 (9)	100.0 (9)	66.7 (9)	33.3 (9)	0 (9)	0.345
F22	100.0 (5)	100.0 (5)	100.0 (5)	60.0 (5)	20.0 (5)	0 (5)	0.338
SC	100.0 (9)	100.0 (9)	88.9 (9)	66.7 (9)	11.1 (9)	0 (9)	0.334
S3	100.0 (9)	100.0 (9)	77.8 (9)	66.7 (9)	11.1 (9)	0 (9)	0.332
T	100.0 (9)	100.0 (9)	77.8 (9)	44.4 (9)	11.1 (9)	0 (9)	0.325
T1	100.0 (9)	100.0 (9)	77.8 (9)	44.4 (9)	0 (9)	0 (9)	0.323
C	100.0 (9)	88.9 (9)	66.7 (9)	44.4 (9)	0 (9)	0 (9)	0.318
G	100.0 (9)	88.9 (9)	66.7 (9)	44.9 (9)	0 (9)	0 (9)	0.318
B	100.0 (9)	100.0 (9)	55.6 (9)	55.6 (9)	0 (9)	0 (9)	0.316
M1	100.0 (9)	88.9 (9)	66.7 (9)	33.3 (9)	0 (9)	0 (9)	0.315
S	100.0 (9)	88.9 (9)	44.4 (9)	22.2 (9)	0 (9)	0 (9)	0.292
D	100.0 (9)	66.7 (9)	55.6 (9)	0 (9)	0 (9)	0 (9)	0.292
D1	100.0 (9)	66.7 (9)	33.3 (9)	0 (9)	0 (9)	0 (9)	0.285

Number of tested fish is included in parentheses.

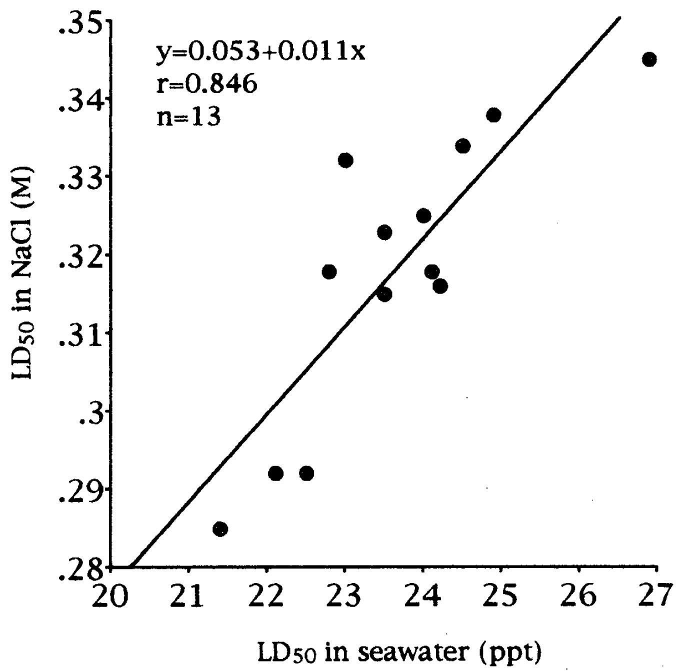

FIG. 1. Relationship between LD_{50} in seawater and NaCl in the 13 strains.

strains (Table 3).

Figure 1 shows a relationship between the LD_{50} in seawater and NaCl in the 13 strains. A significant positive correlation was observed between them.

Discussion

It was reported that seawater tolerance significantly differed among the genetically different guppy strains (3). The present study focused on a factor in seawater restricting the strain differences in seawater tolerance. When fish were transferred to several seawater components, all the fish survived in 0.029 M Na_2SO_4, 0.026 M $MgCl_2$, 0.009 M KCl and 0.008 M $CaCl_2$ but all the fish died in 0.420 M NaCl. This suggests no influence by 0.029 M Na_2SO_4, 0.026 M $MgCl_2$, 0.009 M KCl or 0.008 M $CaCl_2$ on their survival. The present study showed that NaCl tolerance significantly differed among the 13 guppy strains. A intimate positive correlation between the strain differences in seawater and NaCl tolerances indicates that NaCl in seawater is a factor restricting the strain differences in seawater tolerance.

Shikano *et al.* (8) reported that the direct transfer of guppies from fresh water to seawater caused a significant increase in blood osmotic pressure and death. They demonstrated that the hypoosmoregulatory ability in seawater significantly differed among the guppy strains, suggesting genetic differences in it. Judging from the present results, the hypoosmoregulatory differences may be concerned with the excretory ability of excess sodium and chloride.

Concerning the excretion of NaCl in seawater, Na^+, K^+-ATPase plays an important role in the ion excretory mechanisms in the gills (9, 10). The enzyme is mainly located in the basolateral membrane of the chloride cells (11, 12). Many investigators have reported that the gill Na^+, K^+-ATPase activity significantly increased during seawater adaptation (13–15) with proliferation and hypertrophy of the chloride cells (15–18). Therefore, strain differences in seawater tolerance might be caused by the differences in the Na^+, K^+-ATPase activity in the chloride cells.

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