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# Genetic features of salinity tolerance in wild and domestic guppies (*Poecilia reticulata*)

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**Abstract**

To elucidate the genetic features of salinity tolerance in wild and domestic guppies, *Poecilia reticulata*, the present study examined the salinity tolerance in four wild populations and 13 domestic strains. Salinity tolerance was measured as survival time after transfer from fresh water to 35 ppt seawater. In the wild guppies, all four of the wild populations showed significantly higher salinity tolerance than the 13 domestic strains. After domestication of the wild guppies, their salinity tolerance significantly decreased with reductions in salinity tolerant individuals, suggesting inbreeding depression. In the domestic guppies, on the other hand, strain differences were observed during both 1993 and 1997. A significant positive correlation between those in 1993 and 1997 suggests that the genetic constitutions for salinity tolerance have stabilized in each strain as a consequence of long-term maintenance. $F_1$ hybrids between the domestic strains showed significantly higher salinity tolerance with many salinity tolerant individuals which were not observed in their parental strains, thus indicating a heterotic effect. The salinity tolerance in the $F_1$ hybrids reached the same level as that in the wild populations. Salinity tolerance significantly decreased with reductions in the salinity tolerant individuals in the $F_2$. The results of the domestications and the cross experiments suggested that the significant difference in salinity tolerance between the wild and the domestic guppies was caused by heterosis and inbreeding depression. 

*Keywords:* Salinity tolerance; Domestication; Heterosis; Inbreeding depression; Population genetics

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## 1. Introduction

An understanding of the inheritance of physiological traits is of fundamental significance in genetic improvements in fish. Many strains with various characteristics have been established in fish (Burnside et al., 1975; Moav and Hulata, 1975; Broussard and Stickney, 1981; Hyodo-Taguchi and Egami, 1985; Macaranas and Fujio, 1987; Sylvén and Elvingson, 1992). These strains originated from wild populations, which contain a large amount of genetic variations in several traits, and were maintained under artificial conditions. The genetic changes associated with domestication can result from not only artificial selection for a trait but also inbreeding or genetic drift (Allendorf and Phelps, 1980; Ryman and Ståhl, 1980; Taniguchi et al., 1983; Agnèse et al., 1995). After the long-term maintenance, genetic constitutions of each strain will be stabilized at a specific status and genetic differences will occur among the strains. Such strain differences may be useful as a source of genetic material for introgression or hybridization.

Salinity tolerance is one of the most important physiological traits in fish. Genetic analyses of salinity tolerance have recently been performed in teleosts (Staurnes et al., 1992; Shikano and Fujio, 1994; Nakajima et al., 1995; Shikano et al., 1997; Shikano and Fujio, 1998; Shikano et al., 1998). As a model organism for genetic analysis of salinity tolerance, the guppy *Poecila reticulata* is a suitable teleost because of its short life cycle, the establishment of many strains (Macaranas and Fujio, 1987; Barinova et al., 1997a; Shikano and Fujio, 1997) and high euryhalinity similar to salmonids. Shikano and Fujio (1994, 1995) demonstrated that salinity tolerance and seawater adaptability significantly differed among the domestic strains, suggesting the importance of genetic factors for salinity tolerance and seawater adaptability.

To elucidate the genetic features of salinity tolerance in wild and domestic guppies, the present study examined the salinity tolerance of four wild populations and 13 domestic strains.

## 2. Materials and methods

### *2.1. Wild guppies*

Four wild populations, O, O1, O2 and I, were used in this study. The O, O1 and O2 populations were caught in different streams on Okinawa Island in Japan in 1993, 1996 and 1996, respectively. The I population was caught in a stream on Ishigaki Island in Japan in 1996. About 30 individuals were transferred from each point to our laboratory and maintained in 60-l aquaria. To nullify the environmental effects which they had acquired in nature, experiments were performed using their offspring after one or two generations. Each population was maintained at a temperature of $23 \pm 2$°C with lighting for 10 h per day.

### *2.2. Domestication of wild guppies*

The O population was domesticated as a closed colony in a 60-l aquarium in our laboratory from 1993 to 1997. The O1-1, O2-1 and I-1 domestic populations originated

from half the O1, O2 and I base populations, respectively, and were maintained as closed colonies in 60-l aquaria in our laboratory for a half year.

### 2.3. Domestic guppies

Thirteen domestic strains, S, S3, SC, M1, F, F22, T, T1, D, D1, A, B and C, were used in this study. The history of introduction and the kinship are shown in Fig. 1. The S, F, T, D and B strains were purchased from pet shops in 1975, 1982, 1985, 1991 and 1991, respectively. The S3, F22, T1, D1, A and C strains were subdivided from the S strain in 1983, the F strain in 1991, the T strain in 1985, the D strain in 1991, the B strain in 1991 and the B strain in 1991, respectively. The SC and M1 strains originated from hybrids between the S strain and cobra-type fish in 1982 and between the S strain and mosaic-type fish in 1991 respectively. Each strain was maintained in a 60-l aquarium as a closed colony at a temperature of $23 \pm 2$°C with lighting for 10 h per day.

### 2.4. Cross experiment in domestic guppies

A cross experiment was performed between the F22 and S3HR strains. The S3HR strain originated from some individuals in the S3 strain (Kanda et al., 1992). Immature fish in the F22 strain were randomly taken from the parental stock and reared in 2.5-l aquaria in order to obtain virgin females for crossing. Each virgin female in the F22 strain was mated with a male in the S3HR strain in a 2-l aquarium to produce $F_1$. The $F_1$ were reared in 2.5-l aquaria. The $F_1$ individuals were mated together at random to produce $F_2$. The $F_2$ were reared in 2.5-l aquaria. All laboratory-reared fish were fed twice daily with ground carp pellets and dried Daphnia as a supplementary diet.

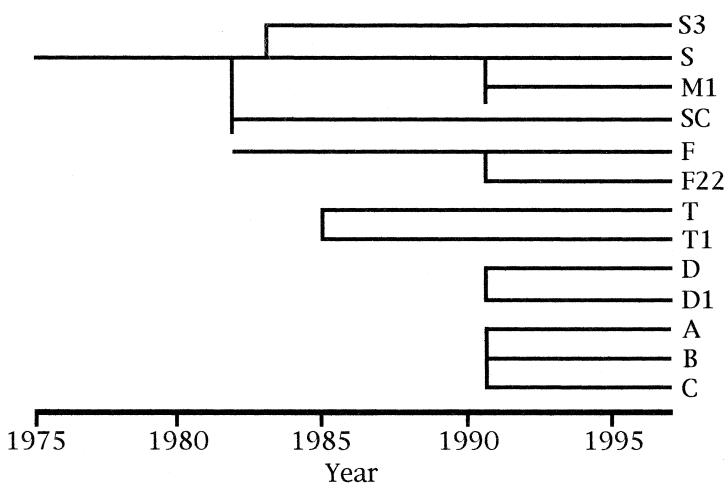


Fig. 1. History of introduction and kinship in the 13 domestic strains. One year is equivalent to 2–3 generations.

Table 1
Survival times in 35 ppt seawater for the four wild populations

| Population | Year | *n* | Mean (h) ± s.e. |
|---|---|---|---|
| O2 | 1997 | 51 | 5.58 ± 0.24 |
| I | 1997 | 50 | 4.98 ± 0.16 |
| O1 | 1997 | 50 | 4.94 ± 0.23 |
| O | 1993 | 83 | 4.90 ± 0.23 |

### *2.5. Seawater challenge test*

Mature fish, older than about 60 days, were collected from each population or strain in a random fashion. Up to 30 individuals were held in a 2.5-l aquarium filled with 35 ppt artificial seawater (Aquasalz, Nissei, Japan) at 23.0 ± 0.5°C. Dead fish were recorded at 30 min intervals after the transfer.

### *2.6. Statistical analysis*

Statistical comparisons between or among populations were assessed using the Student's *t*-test or one-way analysis of variance (ANOVA).

## 3. Results

### *3.1. Wild guppies*

Table 1 shows mean survival times after transfer to 35 ppt seawater for the four wild populations. No significant differences were observed among them ($P > 0.05$).

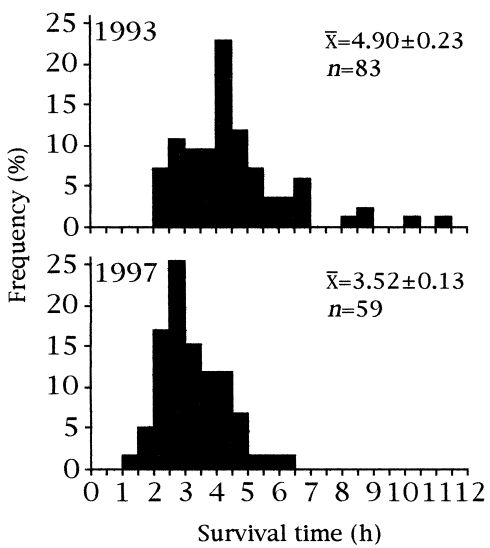


Fig. 2. Frequency distributions of survival time in 35 ppt seawater for the O population in 1993 and 1997. Values represent mean ± s.e.

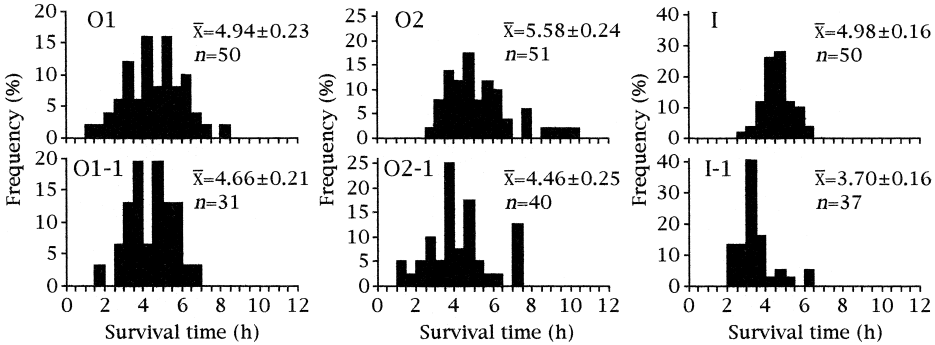


Fig. 3. Frequency distributions of survival time in 35 ppt seawater for the three wild populations and the three domestic populations that originated from the wild populations. Values represent mean ± s.e.

Frequency distributions of survival time in 35 ppt seawater were compared between the O population in 1993 and 4 years after maintenance in the laboratory (Fig. 2). Although the individuals which showed longer survival times were frequently observed in 1993, the number was reduced in 1997 and also the mode decreased from 4.5 h to 3.0 h. The mean survival time significantly decreased from 4.90 h to 3.52 h ($P < 0.01$).

Fig. 3 shows frequency distributions of survival time in 35 ppt seawater for the O1, O2 and I base populations and the O1-1, O2-1 and I-1 domestic populations. Compared with the base populations, their domestic populations showed a reduce in the individuals which showed longer survival times in all of the three populations. Although no significant difference was observed between the mean survival times of the O1 and O1-1 populations ($P > 0.05$), the significant decreases were observed between the O2 and O2-1 populations and between the I and I-1 populations ($P < 0.01$).

Table 2
Survival times in 35 ppt seawater for the 13 domestic strains in 1993 and 1997

| Strain | 1993 | | 1997 | |
|---|---|---|---|---|
| | *n* | Mean (h) ± s.e. | *n* | Mean (h) ± s.e. |
| F | 289 | 3.83 ± 0.08 | 39 | 3.73 ± 0.28 |
| F22 | 218 | 3.47 ± 0.05 | 43 | 4.21 ± 0.24 |
| SC | 321 | 3.40 ± 0.05 | 78 | 3.69 ± 0.22 |
| T | 175 | 3.21 ± 0.06 | 22 | 3.95 ± 0.39 |
| S3 | 280 | 3.16 ± 0.05 | 65 | 3.56 ± 0.11 |
| C | 289 | 3.11 ± 0.04 | 40 | 2.75 ± 0.10 |
| B | 217 | 3.06 ± 0.05 | 75 | 3.31 ± 0.14 |
| A | 125 | 2.91 ± 0.07 | 32 | 2.75 ± 0.24 |
| T1 | 106 | 2.86 ± 0.07 | 64 | 2.91 ± 0.12 |
| M1 | 376 | 2.80 ± 0.04 | 52 | 2.80 ± 0.08 |
| S | 346 | 2.66 ± 0.04 | 63 | 3.01 ± 0.10 |
| D | 153 | 2.60 ± 0.04 | 50 | 2.44 ± 0.06 |
| D1 | 117 | 2.52 ± 0.05 | 64 | 2.51 ± 0.06 |

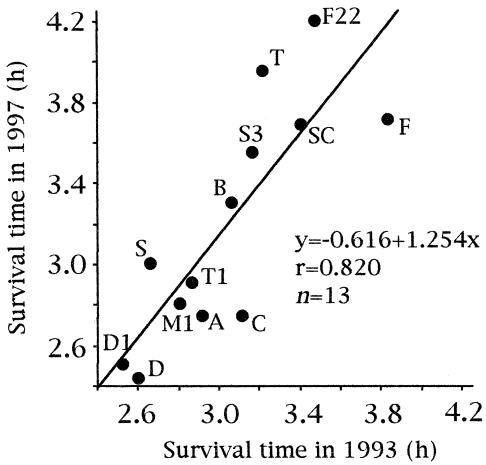


Fig. 4. Correlation between survival times in 35 ppt seawater for the 13 domestic strains in 1993 and 1997.

### 3.2. Domestic guppies

Table 2 shows mean survival times after transfer to 35 ppt seawater for the 13 domestic strains in 1993 and 1997. The mean survival times significantly differed among the strains from 2.52 h to 3.83 h in 1993 and from 2.44 h to 4.21 h in 1997 ($P < 0.01$). In comparison between the mean survival times of each strain in 1993 and 1997, the differences were from 0 h to 0.74 h. A positive correlation was observed between the mean survival times in the 13 domestic strains in 1993 and 1997 (Fig. 4).

A relationship was examined between times from subdivision and differences in the mean survival times among the subdivided domestic strains, which were derived from the same strains. As shown in Fig. 5, a positive correlation was observed between them.

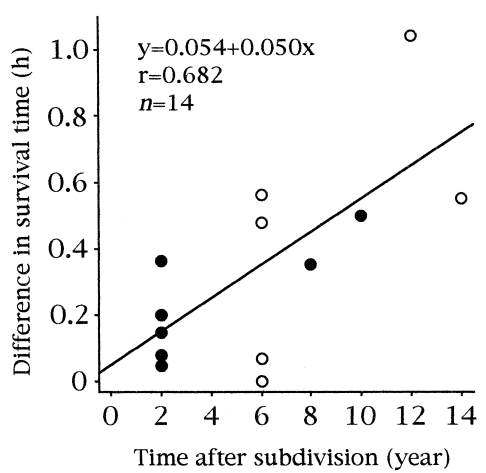


Fig. 5. Correlation between time after subdivision and differences in survival time in 35 ppt seawater in the subdivided domestic strains, which were derived from the same strains. ●, Data from 1993; ○, from 1997.

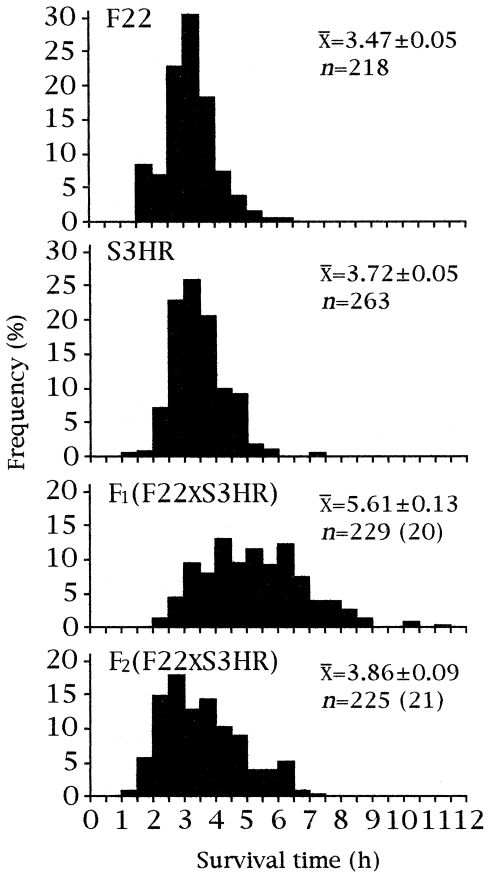


Fig. 6. Frequency distributions of survival time in 35 ppt seawater in the S3HR and F22 strains, the $F_1$ and the $F_2$. Values represent mean ± s.e. The number of crosses is included in parentheses.

### *3.3. Cross experiments in domestic guppies*

Fig. 6 shows the frequency distributions of survival times in 35 ppt seawater for the F22 and S3HR domestic strains, the $F_1$ and the $F_2$. The frequency distributions showed that the $F_1$ hybrids included individuals which showed the longer survival times that were not observed in their parental strains. Mean survival time in the $F_1$ hybrids (5.61 h) was significantly longer than that of the F22 strain (3.47) and the S3HR strain (3.62 h) ($P < 0.01$). In $F_2$, the mean survival time significantly decreased to 3.86 h ($P < 0.01$) with reduction of the individuals which showed longer survival times.

## 4. Discussion

In the wild guppies, all four of the populations showed significantly higher salinity tolerance than the 13 domestic strains. Although the wild guppies may have originated

from native or introduced fish at each point, no significant difference in salinity tolerance was observed among the populations. These wild guppies were thought to have obtained or maintained the high salinity tolerance during successive generations in nature. However, a significant reduction in salinity tolerance was observed in the O wild population after the 4 years of domestication (about 10 generations) in the laboratory. Two possibilities for these results are worth consideration. One is that inbreeding depression caused the decrease in salinity tolerance. Many investigators have reported that domestication caused a decrease in heterozygosities in teleosts (Allendorf and Phelps, 1980; Ryman and Ståhl, 1980; Taniguchi et al., 1983; Agnèse et al., 1995). Another is that genetic drift resulted in the increase in salinity sensitive individuals.

To examine the genetic influence of domestication of the wild guppies on their salinity tolerance, the domestic populations which reduced population size were maintained as closed colonies for one or two generations. In all of the three domestic populations, salinity tolerant individuals, which existed in the base populations, were reduced in number as observed after the domestication of the O wild population. The mean survival times in all of the three domestic populations decreased from those in their respective base populations. The significant reductions suggest that domestication of the wild guppies reduced their salinity tolerance as a consequence of inbreeding. Inbreeding depression has been reported in the traits related to fitness such as survival, growth or hatchability (Kincaid, 1976; Kincaid, 1983; Sasaki and Fujio, 1984; Agnèse et al., 1995).

In the domestic guppies, on the other hand, strain differences in salinity tolerance were observed in both 1993 and 1997. Although salinity tolerance changed during the 4 years' maintenance (about 10 generations) in some strains, these changes were significantly smaller than that of the O population which originated from a wild population. A significant positive correlation between the mean survival times in 1993 and 1997 suggests that the genetic constitutions for salinity tolerance have stabilized in each domestic strain at the present time as a consequence of long-term maintenance.

In the cross experiments between the domestic strains, $F_1$ hybrids showed a significantly longer mean survival time with many salinity tolerant individuals which were not observed in their parental strains, indicating a heterotic effect on salinity tolerance. The mean survival time of the $F_1$ hybrids reached the same level as that of the wild guppies. Significant increases in salinity tolerance of the $F_1$ hybrids of the domestic guppies were also observed in other strain combinations (Shikano et al., 1997). In the $F_2$, although genetic changes will not theoretically occur in gene frequencies but in genotype frequencies under random matings, the mean survival time significantly decreased along with reductions in the salinity tolerant individuals. This phenomenon was similar to the result after the domestication of the wild guppies. Falconer (1989) described that heterosis can be explained as the reverse of inbreeding depression and that the changes in the mean from $F_1$ to $F_2$ may be regarded as inbreeding depression. The significant heterotic effect and the significant reduction in the mean survival time from $F_1$ to $F_2$ suggest the existence of significant inbreeding depression of salinity tolerance in the domestic guppies. Therefore, differences in heterozygosities might cause significant differences in salinity tolerance such as those between the $F_1$ hybrids and their parental domestic strains and also between the wild populations and the domestic strains. The

latter is supported by the fact that average heterozygosities estimated from 30 isozyme loci were $0.048 \pm 0.006$ (mean $\pm$ s.e.) in the wild populations but $0.025 \pm 0.003$ in the domestic strains (unpublished data).

In the domestic strains, a positive correlation was observed between times from subdivision and differences in the mean survival times in seawater among the subdivided domestic strains, which were derived from the same strains. Barinova et al. (1997a) reported a positive correlation between the times from subdivision and Nei's genetic distance among the strains, suggesting that gene frequencies gradually differentiated during the maintenance. The two positive correlations suggest genetic differentiations in salinity tolerance in the domestic strains. Under such a circumstance that the heterozygosities had still been reduced during the long-term maintenance for the domestic strains (Barinova et al., 1997b), the effects of the changes in gene frequencies might appear to some extent. However, the effects might be smaller than that of the heterozygosities because the differences in salinity tolerance among the domestic strains were smaller than those between the $F_1$ hybrids and their parental domestic strains and also between the wild populations and the domestic strains.

In conclusion, the present study revealed that wild guppies had significantly higher salinity tolerance than the domestic guppies. The results of the domestications in the wild populations and the cross experiments in the domestic strains suggested that the significant difference resulted from heterosis and inbreeding depression. The trait of salinity tolerance will be a good model for the examinations of the influence of inbreeding depression and heterosis on a physiological trait during domestication of wild populations.

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