

Effect of inbreeding on salinity tolerance in the guppy (*Poecilia reticulata*)

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Abstract

This experiment was designed to study the relationship between different levels of inbreeding and observed inbreeding depression for salinity tolerance, one of the most important tolerances to environmental conditions, in the guppy (*Poecilia reticulata*). Two generations of full-sib mating and six generations of mating in the $n = 10$ produced individuals with an expected level of inbreeding coefficient of 0.375 and 0.265, respectively. A significant decrease in the mean value of salinity tolerance, expressed by survival time in 35-ppt seawater, was observed in both the full-sib mated line and the closed line of $n = 10$, indicating inbreeding depression for salinity tolerance. The mean and coefficient of variation (C.V.) of salinity tolerance decreased linearly with the increase in inbreeding coefficient with a rate of 9.1% and 10.1% per 10% increase in the inbreeding coefficient, respectively. Analyses among seven lines of the full-sib matings indicated that inbreeding caused the larger reduction of the means observed in the lines having higher salinity tolerance at the P generation and decreased both deviations of the means among the lines and C.V. within each line. The linear decrease in salinity tolerance with an increase in inbreeding coefficient suggests that inbreeding depression for salinity tolerance results from additive combination among the loci responsible for inbreeding depression. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Inbreeding depression; Inbreeding coefficient; Full-sib mating; Effective population size; Salinity tolerance; Guppy; *Poecilia reticulata*

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1. Introduction

The genetic consequences of inbreeding are measured by the inbreeding coefficient (F), which is the probability that the two genes at any locus in an individual are identical by descent (Falconer, 1989). In general, inbreeding can be conducted for some specific purposes, e.g., to produce lines for subsequent crossing in order to utilize hybrid vigor and to produce genetically uniform strains for use in bioassay and genetic research. However, inbreeding can occur as a random event due to a limited number of breeding individuals in the population with a rate of increase, $\Delta F = 1/2Ne$, where Ne is the effective population size (Falconer, 1989). Under low Ne , therefore, wild or domestic population is expected to suffer an increase in inbreeding because of bottlenecks at the foundation and through subsequent low average population sizes (Allendorf and Phelps, 1980; Ryman and Ståhl, 1980; Taniguchi et al., 1983; Agnèsè et al., 1995).

Inbreeding reduces the mean phenotypic value of various fitness-related traits (Falconer, 1989). The phenomenon is known as inbreeding depression and has been investigated in most domestic and laboratory animal species including fish. Inbreeding depression has been observed in various characteristics related to fitness in fish (Kincaid, 1976a,b, 1983; Mrakovcic and Haley, 1979; Gjerde et al., 1983; Bondari and Dunham, 1987; Su et al., 1996). Kincaid (1983) reported that one generation of full-sib mating ($F = 0.250$) produced an increase in fry deformities (37.6%) and decreased feed conversion efficiency (5.6%), fry survival (19.0%) and weight at 147 (11.0%) and 364 days of age (23.2%) in rainbow trout. Mrakovcic and Haley (1979) reported that inbreeding ($F = 0.250$) caused a decline in fertility (90.6%), fry survival to 30 days (42.9%) and fry length at 30 days (10.8%) in zebra fish. Bondari and Dunham (1987) reported that inbreeding ($F = 0.250$) caused an increase in the days required for eggs to hatch (21.4%) in channel catfish but did not significantly influence spawning weight or hatchability. To understand the genetic basis of inbreeding depression and the proper design of conservation biology and successful maintenance of domestic populations, it is important to clarify whether fitness components decrease linearly with an increase in the inbreeding coefficient and whether purging of deleterious alleles responsible for inbreeding depression is possible, and at what rate. These analyses may be served by experimental and theoretical studies using different levels of inbreeding and several populations.

Salinity tolerance is one of the most important physiological traits in fish. Although several investigators have reported inbreeding depression for various fitness-related characteristics such as survival, growth and reproductive ability in fish (Kincaid, 1976a,b, 1983; Mrakovcic and Haley, 1979; Gjerde et al., 1983; Bondari and Dunham, 1987; Su et al., 1996), there is little available information on the effect of inbreeding on tolerance to various environmental conditions such as salinity or temperature. As a model organism for genetic analysis in fish, the guppy *Poecilia reticulata* is one of the most useful teleosts because of its short life cycle, ease of breeding and the availability of many strains (Macaranas and Fujio, 1987; Barinova et al., 1997; Shikano and Fujio, 1997). Shikano and Fujio (1994) demonstrated that salinity tolerance significantly differed among the domestic strains, indicating the importance of genetic factors for salinity tolerance. Chiyokubo et al. (1998) also examined salinity tolerance in four wild

populations and 13 domestic strains and reported that all of the wild populations had higher salinity tolerance than the domestic strains.

The present study examined the effect of different levels of inbreeding on salinity tolerance of the guppy using a full-sib mated line and a closed line of $n = 10$ and demonstrated the relationship between the inbreeding level and the observed inbreeding depression.

2. Materials and methods

2.1. Animals

Guppies were caught in a stream on Okinawa Island in Japan in 1996. About 30 individuals were transferred to our laboratory and maintained in a 60-l aquarium. This population was named O2. To reduce any environmental effect that they had experienced in nature, experiments were performed using their offspring after one or two generations. Fish were fed twice daily with ground carp pellets and dried *Daphnia* as a supplementary diet. The laboratory for breeding experiments of guppies was maintained at a temperature of $23 \pm 1^\circ\text{C}$ (mean \pm range) using an air conditioner with lighting for 10 h/day. All experiments were performed in this laboratory.

2.2. Full-sib mating

Full-sib mating was performed in seven lines over two generations in the O2 population. Offspring obtained from one pair of the O2 population were reared in 2.5-l aquaria (P generation). As soon as sex can be identified (usually 45–60 days), males were separated from females to obtain virgin females. Seven sib pairs in the P generation were mated in 2.5-l aquaria to obtain their offspring (F_1 generation). F_1 offspring were reared in 2.5-l aquaria with separation of males from females. Sibs in the F_1 generation were mated in 2.5-l aquaria to obtain their offspring (F_2 generation). The F_2 offspring were likewise reared in 2.5-l aquaria. Salinity tolerance was examined in the P, F_1 and F_2 generations.

2.3. Closed line

A closed line of $n = 10$ was established from the O2 population. Base generation (generation 0) was established from five pairs in the O2 population. The closed line was maintained in a 60-l aquarium using five pairs of parental fish per generation over six generations. Salinity tolerance was examined in the base generation and in the first to sixth generations.

2.4. Salinity tolerance

Up to 20 individuals of mature fish, older than about 60 days, were held in a 2.5-l aquarium filled with 35-ppt artificial seawater (Aquasalz, Nissei, Japan) at $23.0 \pm 0.5^\circ\text{C}$. Dead fish were recorded at 30-min intervals after transfer from fresh water to seawater.

2.5. Calculation of inbreeding coefficient

The expected inbreeding coefficient is 0.250 after one generation of full-sib mating and 0.375 after two generations (Falconer, 1989). Inbreeding coefficient in the closed line of $n = 10$ was estimated from the formula given by Falconer (1989) as $F = 1 - (1 - \Delta F)^t$, where $\Delta F = 1/2Ne$ and Ne is the effective population size. Because five pairs of parental fish per generation were used for the closed line, F was estimated from $Ne = 10$ with the assumption of random mating and equal sex ratio.

2.6. Statistical analysis

Statistical comparisons between generations for each line were performed with one-way factorial analysis of variance (ANOVA), followed by the Fisher’s protected least significant difference (PLSD) multiple comparison test. Statistical analyses among

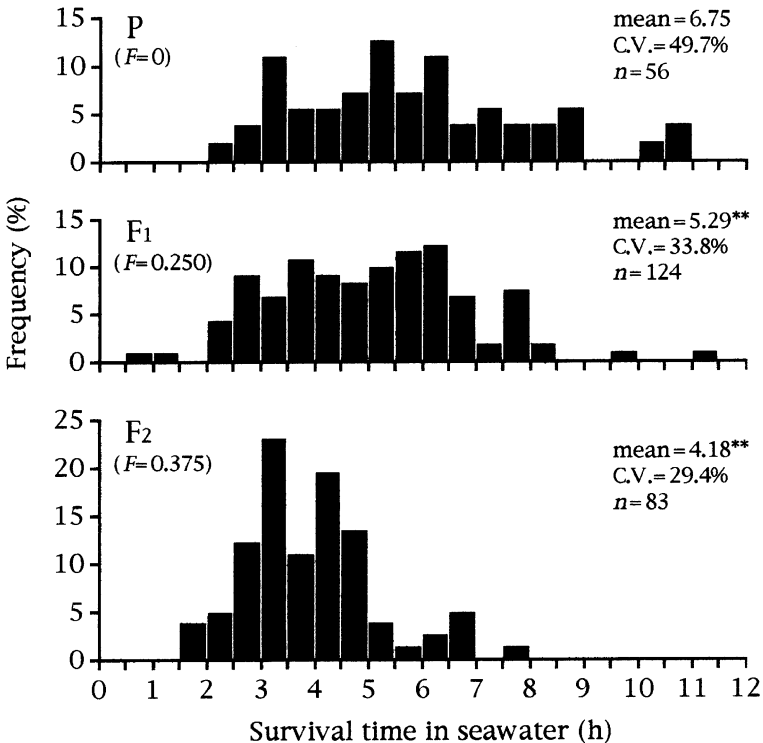


Fig. 1. Frequency distribution of survival time in 35-ppt seawater in full-sib mated line. Frequency distribution at the P generation excludes the data of 1.8% at 13.5 h, 3.6% at 14.0 h and 1.8% at 15.0 and 16.0 h. Significantly different from the P generation with $** P < 0.01$.

different generations in seven lines of full-sib matings were assessed using one-way repeated measures ANOVA.

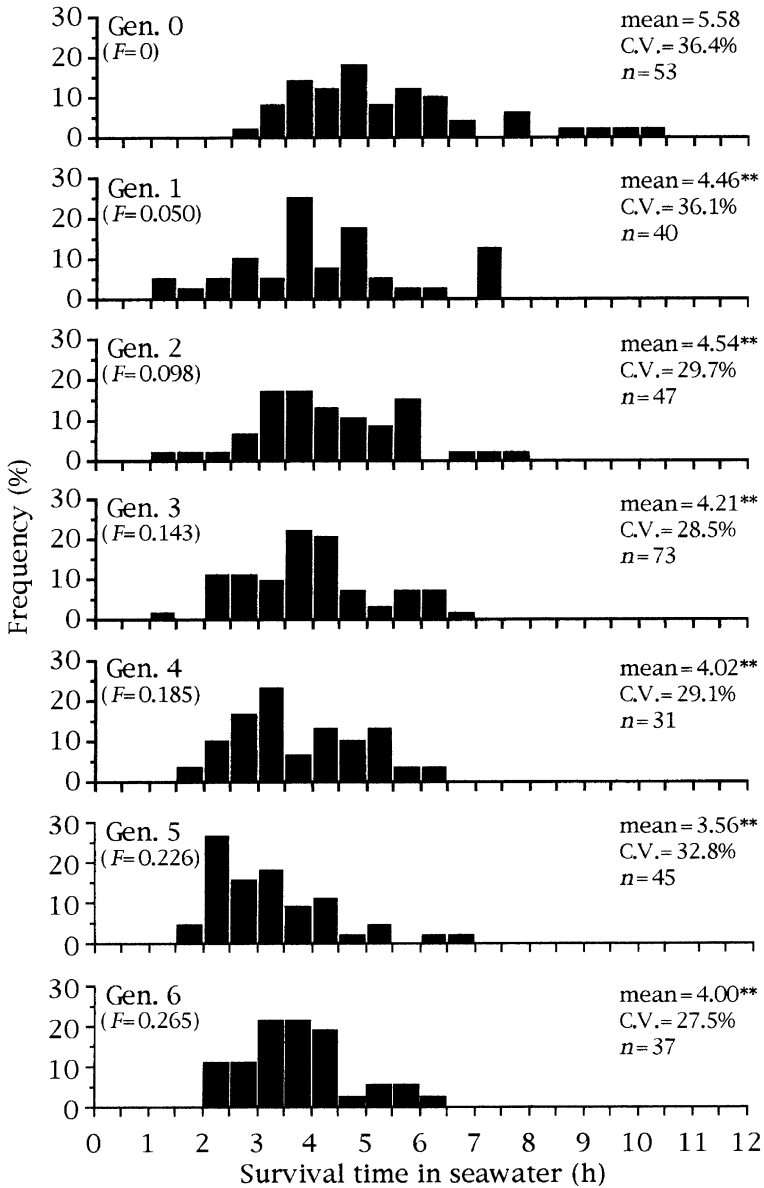


Fig. 2. Frequency distribution of survival time in 35-ppt seawater in closed line of $n=10$. Frequency distribution at the base generation excludes the data of 1.9% at 13.5 h. Significantly different from the base generation with ** $P < 0.01$.

3. Results

3.1. Effect of inbreeding line on salinity tolerance

Fig. 1 shows the changes in the frequency distributions of survival time in 35-ppt seawater during two generations of full-sib matings. In the P generation, the survival time ranged from 2.5 to 16.0 h with a mean of 6.75 h. The number of individuals that showed longer survival times decreased during the two generations of full-sib matings. The mean survival time significantly decreased to 5.29 and 4.18 h in the F₁ and F₂ generations, respectively ($P < 0.01$).

Fig. 2 shows the changes in frequency distributions of survival time in 35-ppt seawater in the closed line of $n = 10$ over six generations. The mean survival time was 5.58 h in the base generation, significantly decreased after one generation ($P < 0.01$) and became 4.00 h after six generations. The inbreeding coefficient estimated from the

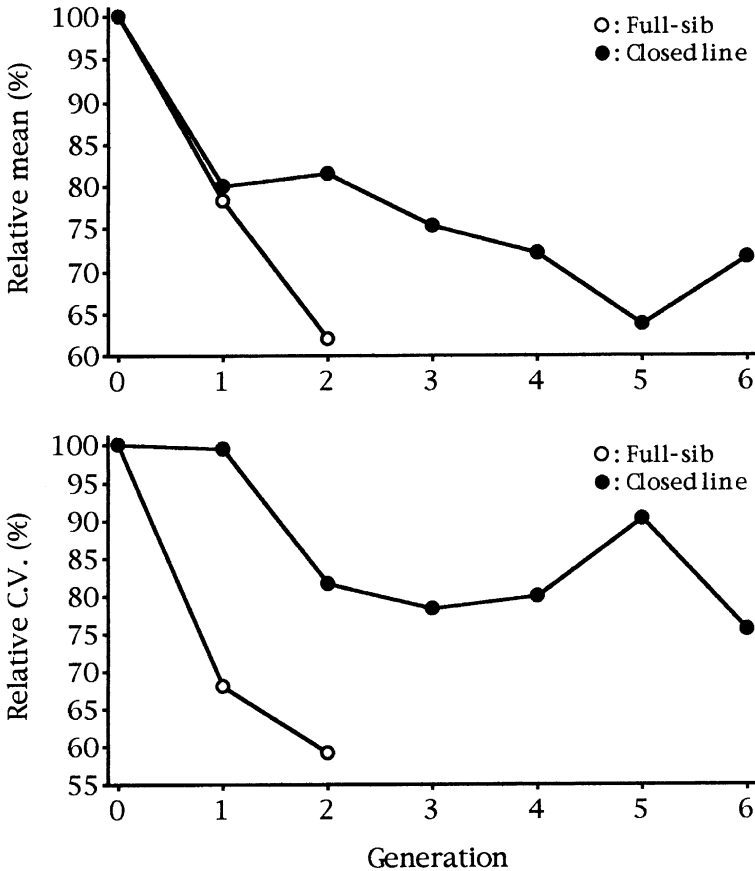


Fig. 3. Changes in relative values in the mean and C.V. to the P or base generations in the full-sib mated line (open circles) and the closed line of $n = 10$ (closed circle).

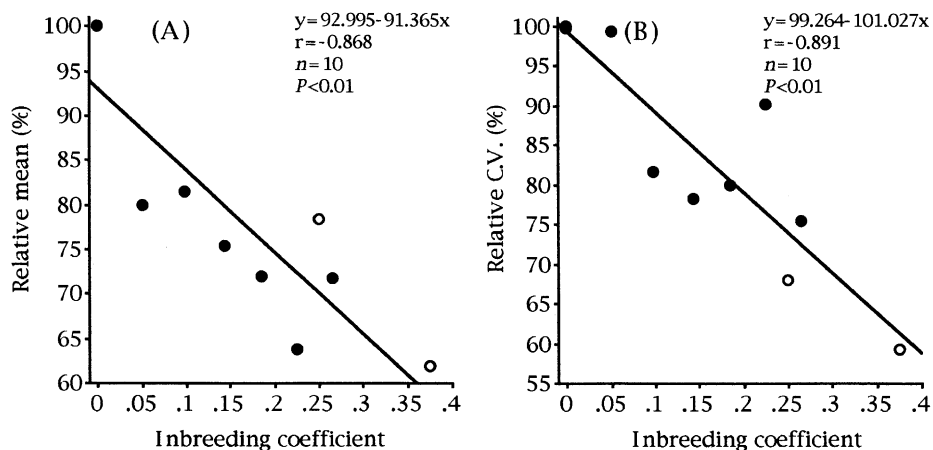


Fig. 4. Relationships between inbreeding coefficient and relative mean (A) or relative C.V. (B). Open circles represent the data from full-sib mated line, whereas closed circles represent the data from closed line of $n = 10$.

effective population size with the assumption of random mating increased to 0.265 after six generations. The range of the distributions became narrower with reductions in the individuals, which showed longer survival times.

Relative values in the mean and coefficient of variation (C.V.) of the survival time at the P or the base generation were examined in the full-sib mating and the closed line (Fig. 3). As shown in Fig. 4, significant negative correlations were observed between the inbreeding coefficient and the relative mean and C.V. of the survival time in the full-sib matings and the closed line ($P < 0.01$).

3.2. Analysis of different lines in full-sib mating

Survival times in 35-ppt seawater were compared among seven lines of the full-sib matings (Table 1). The mean survival time significantly differed from 4.63 to 10.93 h among the seven lines at the P generation ($P < 0.01$) and significantly decreased in four

Table 1
Survival times in 35-ppt seawater for P, F₁ and F₂ generations of seven lines in full-sib mating

| Line number | P ($F = 0$) | | | F ₁ ($F = 0.250$) | | | F ₂ ($F = 0.375$) | | |
|-------------|---------------|----------|----------|--------------------------------|----------|----------|--------------------------------|----------|----------|
| | <i>n</i> | mean (h) | C.V. (%) | <i>n</i> | mean (h) | C.V. (%) | <i>n</i> | mean (h) | C.V. (%) |
| 1 | 7 | 10.93 | 30.7 | 28 | 3.82** | 39.0 | 15 | 4.80** | 23.3 |
| 2 | 9 | 7.22 | 29.2 | 26 | 5.56* | 25.2 | 7 | 4.79** | 39.9 |
| 3 | 7 | 7.14 | 62.3 | 34 | 6.07 | 23.7 | 20 | 3.73** | 29.0 |
| 4 | 7 | 6.71 | 37.7 | 12 | 5.13 | 34.5 | 8 | 4.25 | 42.6 |
| 5 | 6 | 6.50 | 58.8 | 7 | 5.86 | 50.7 | 8 | 3.44 | 26.2 |
| 6 | 12 | 5.29 | 25.3 | 13 | 5.96 | 27.5 | 8 | 3.88** | 20.4 |
| 7 | 8 | 4.63 | 29.8 | 4 | 4.63 | 24.0 | 17 | 4.38 | 18.3 |

* Significantly different from the respective P generation with $P < 0.05$.

** Significantly different from the respective P generation with $P < 0.01$.

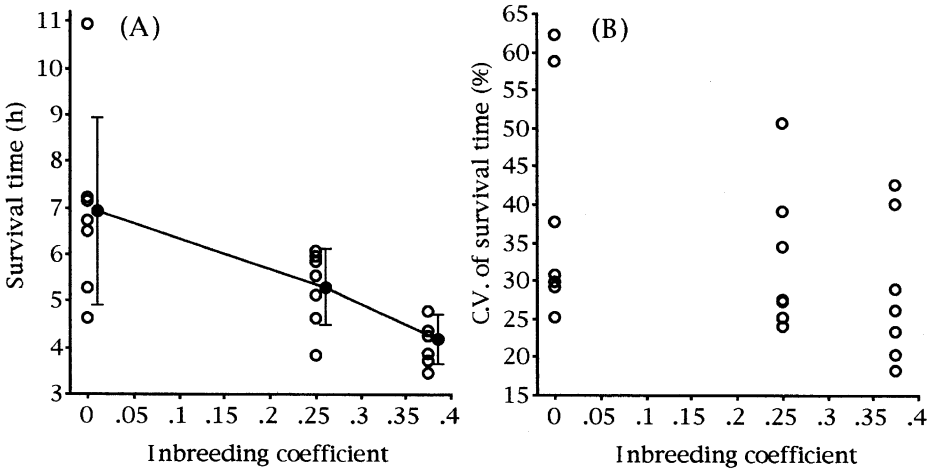


Fig. 5. Distributions of the mean (A) and C.V. (B) of survival time within each line at different levels of inbreeding coefficient in full-sib mating. Open circles represent the mean and C.V. within each line. Closed circles and bars represent the average \pm S.D. among seven lines.

out of the seven lines during two generations of the full-sib matings ($P < 0.01$ or $P < 0.05$). Moreover, a significant difference in the mean survival time was observed among the seven lines in the F_1 generation ($P < 0.01$), but not in the F_2 generation ($P > 0.05$).

Fig. 5 shows the distributions of the mean and C.V. of the survival time in each line at different levels of inbreeding coefficient. Deviations of the mean and C.V. of the survival time in each line decreased with the increase in inbreeding coefficient. The average values in the mean decreased linearly with the increase in inbreeding coefficient ($P = 0.01$).

4. Discussion

The fitness function expected for progressive inbreeding depends on the mode of gene action considered. According to additive models of gene action, fitness components are expected to decrease linearly with the inbreeding coefficient (Falconer, 1989). The linear relationship between inbreeding coefficient and a fitness-related trait has been reported in the height and yield of seed in plants (Falconer, 1989; Hauser and Loeschcke, 1995), litter size and body weight in mammals (Falconer, 1989; Analla et al., 1999), and fertility in *Drosophila* (Falconer, 1989), but has been poorly documented in fish. The present study on the guppy examined the relationship between different levels of inbreeding and inbreeding depression for salinity tolerance, one of the most important tolerances to environmental conditions in fish. A linear decline in the salinity tolerance with increased inbreeding coefficient was observed in both the full-sib mated line and the closed line. The result is in accordance with the assumption of additive combination among the loci responsible for inbreeding depression. The mean value of

salinity tolerance decreased at 9.1% per 10% increase in the inbreeding coefficient. This rate of inbreeding depression is one of the highest values observed in various quantitative traits in several fish species (Kincaid, 1976a,b, 1983; Mrakovcic and Haley, 1979; Gjerde et al., 1983; Bondari and Dunham, 1987; Su et al., 1996), indicating that salinity tolerance in the guppy is strongly sensitive to inbreeding.

While the mean value of salinity tolerance decreased linearly with an increase in inbreeding coefficient in both the full-sib mating and the closed line, the decreased rate was somewhat higher in the experiment with the closed line. A possible explanation for this is that the actual inbreeding coefficient in the closed line is higher than that estimated from the number of breeding individuals because effective population size is influenced by different numbers of males and females or non-random distribution of family size (Falconer, 1989).

One of the most important characterizations observed in the present study is that inbreeding was accompanied by reductions in both the mean value and the C.V. of salinity tolerance. The high C.V. of salinity tolerance within the wild population was shown from both the significant deviation of the means among lines and the large C.V. within each line. This significant difference in salinity tolerance might be caused by the different levels of actual inbreeding coefficient among lines and/or the different genotypes at the loci responsible for inbreeding depression, even though the wild population had been maintained under a large effective population size. Analyses of different lines in the full-sib matings showed that the amount of inbreeding depression was larger in the lines having higher salinity tolerance at the P generation, indicating that the rate of inbreeding depression differs among the lines within the population. The difference may be due to any of the genetic differences existing among the lines at the P generation, for example, discrepancies in the level of inbreeding accumulated and/or the genotypes at the loci responsible for inbreeding depression. The results obtained from the different lines of the full-sib matings indicate that decrease in salinity tolerance due to inbreeding consists of two processes. One is the larger reduction of the means in the lines having higher salinity tolerance at the P generation, with equalizing of the means at a lower level. The other is the decrease in the C.V. within each line. These characterizations indicate the way inbreeding gradually decreases both the mean and C.V. of salinity tolerance within a population, and therefore, a positive correlation may be constructed between the mean and C.V. during inbreeding (Shikano and Fujio, 1994).

Selection is almost impossible to avoid during inbreeding even in a laboratory experiment because deliberate inbreeding is usually accompanied by some artificial selection for characteristics subject to inbreeding depression (Falconer, 1989). Individuals of high inbreeding levels will likely represent a selected proportion of the original gene pool, especially if inbreeding depression is severe for survival components in the preceding generation such as survival rate and fertility. However, the present study could represent detailed relationships between different levels of inbreeding and observed inbreeding depression. This is due to the facts that the trait examined is strongly sensitive to inbreeding and has directly no effect on survival in the preceding generation under the laboratory condition.

Heterosis is complementary to the phenomenon of inbreeding depression and is thought to be an effect of it (Falconer, 1989). Shikano et al. (1997) have reported that by

using crosses between domestic strains, significant heterosis was observed in salinity tolerance of the guppy. Because salinity tolerance is highly sensitive to inbreeding and shows significant heterosis in the guppy, the trait will be one of the most useful characteristics for the further understanding of the genetic basis of inbreeding depression and heterosis and the expressional mode of inbreeding depression within a population.

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References

- Agnès, J.F., Otémé, Z.J., Gilles, S., 1995. Effects of domestication on genetic variability, fertility, survival and growth rate in a tropical siluriform: *Heterobranchus longifilis* Valenciennes 1840. *Aquaculture* 131, 197–204.
- Allendorf, F.W., Phelps, S.R., 1980. Loss of genetic variation in a hatchery stock of cutthroat trout. *Trans. Am. Fish. Soc.* 109, 537–543.
- Analla, M., Montilla, J.M., Serradilla, J.M., 1999. Study of the variability of the response to inbreeding for meat production in Merino sheep. *J. Anim. Breed. Genet.* 116, 481–488.
- Barinova, A.A., Nakajima, M., Fujio, Y., 1997. Genetic differentiation of laboratory populations in the guppy *Poecilia reticulata*. *Fish Genet. Breed. Sci.* 25, 19–26.
- Bondari, K., Dunham, R.A., 1987. Effects of inbreeding on economic traits of channel catfish. *Theor. Appl. Genet.* 74, 1–9.
- Chiyokubo, T., Shikano, T., Nakajima, M., Fujio, Y., 1998. Genetic features of salinity tolerance in wild and domestic guppies (*Poecilia reticulata*). *Aquaculture* 167, 339–348.
- Falconer, D.S., 1989. *Introduction to Quantitative Genetics*. 3rd edn. Longman, New York.
- Gjerde, B., Gunnes, K., Gjedrem, T., 1983. Effects of inbreeding on survival and growth in rainbow trout. *Aquaculture* 34, 327–332.
- Hauser, T.P., Loeschke, V., 1995. Inbreeding depression in *Lychnis flos-cuculi* (Caryophyllaceae): effects of different levels of inbreeding. *J. Evol. Biol.* 8, 589–600.
- Kincaid, H.L., 1976a. Effects of inbreeding on rainbow trout populations. *Trans. Am. Fish. Soc.* 105, 273–280.
- Kincaid, H.L., 1976b. Inbreeding in rainbow trout (*Salmo Gairdneri*). *J. Fish. Res. Board Can.* 33, 2420–2426.
- Kincaid, H.L., 1983. Inbreeding in fish populations used for aquaculture. *Aquaculture* 33, 215–227.
- Macaranas, J.M., Fujio, Y., 1987. Genetic differences among strains of the guppy, *Poecilia reticulata*. *Tohoku J. Agric. Res.* 37, 75–85.
- Mrakovcic, M., Haley, L.E., 1979. Inbreeding depression in the Zebra fish *Brachydanio rerio* (Hamilton Buchanan). *J. Fish Biol.* 15, 323–327.
- Ryman, N., Ståhl, G., 1980. Genetic changes in hatchery stocks of brown trout (*Salmo trutta*). *Can. J. Aquat. Sci.* 37, 82–87.
- Shikano, T., Fujio, Y., 1994. Strain differences at salinity resistance in the guppy, *Poecilia reticulata*. *Fish Genet. Breed. Sci.* 20, 47–53.
- Shikano, T., Fujio, Y., 1997. Successful propagation in seawater of the guppy *Poecilia reticulata* with reference to high salinity tolerance at birth. *Fish. Sci.* 63, 573–575.

- Shikano, T., Nakadate, M., Nakajima, M., Fujio, Y., 1997. Heterosis and maternal effects in salinity tolerance of the guppy *Poecilia reticulata*. Fish. Sci. 63, 893–896.
- Su, G.-S., Liljedahl, L.-E., Gall, G.A.E., 1996. Effects of inbreeding on growth and reproductive traits in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 142, 139–148.
- Taniguchi, N., Sumantadinata, K., Iyama, S., 1983. Genetic change in the first and second generations of hatchery stock of black seabream. Aquaculture 35, 309–320.