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Aquaculture 220 (2003) 219–226
Aquaculture
www.elsevier.com/locate/aqua-online

Inbreeding depression and heterosis in various quantitative traits of the guppy, *Poecilia reticulata*

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Received 8 October 2001; received in revised form 10 August 2002; accepted 12 August 2002

Abstract

The present study examines the effects of inbreeding and crossing on various quantitative traits in the guppy, *Poecilia reticulata*. Effects of inbreeding and crossing were examined in six quantitative traits; body length at birth, survival at day 120, undwarf rate at day 120, body length at day 120, salinity tolerance and high temperature tolerance. Full-sib matings revealed that the amount of inbreeding depression varied from − 1.0% to 24.6% among the traits and a significant decrease in survival at day 120 and salinity tolerance was observed. This result indicates that inbreeding reduces the performance for some of the quantitative traits but not all. On the other hand, crosses between genetically different strains showed that the amount of heterosis varied from − 1.3% to 42.2% among the six quantitative traits and a significant increase in survival at day 120 and salinity tolerance was observed. The relationship between the amount of inbreeding depression and heterosis supports the theory that the phenomenon of heterosis is the reverse of inbreeding depression, indicating that the traits which have decreased by inbreeding can be recovered by means of crossing.

Keywords: Inbreeding depression; Heterosis; Quantitative trait; Fitness; Guppy; *Poecilia reticulata*

1. Introduction

Inbreeding is one of the most important factors for genetic evaluation of species and populations. The degree of inbreeding is measured by inbreeding coefficient (F), which is the probability that the two genes at any locus in an individual are identical by descent (Falconer, 1989). Small effective population size leads to increase in the inbreeding

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0044-8486/03/$ - see front matter
doi:10.1016/S0044-8486(02)00432-5

coefficient with a rate of $\Delta F = 1/2Ne$, where Ne is the effective population size. In fish breeding, therefore, domestic populations are expected to suffer an increase in inbreeding because of bottlenecks at the foundation, and through subsequent low average population sizes.

Inbreeding reduces the mean phenotypic value of various fitness-related traits and the phenomenon is known as inbreeding depression (Falconer, 1989). On the other hand, heterosis resulting from crosses between strains or between different races or varieties is theoretically known as the reverse of inbreeding depression, and forms an important means of genetic improvements (Falconer, 1989). In fish, inbreeding depression and heterosis have been observed in various quantitative traits related to fitness (Gall, 1975; Moav et al., 1975; Kincaid, 1976a,b; Mrakovcic and Haley, 1979; Brody et al., 1980; Gjerde et al., 1983; Bondari and Dunham, 1987; Su et al., 1996).

In plants and mammals, inbreeding depression and heterosis have been studied in detail for dozens of decades. However, such studies have not been conducted enough for fish. Because of the further importance of aquaculture in the world, more detailed information on inbreeding depression and heterosis is required for fish at the present time. Furthermore, such information may also be available to conservation programs of fish. Up till now, although several investigators have reported inbreeding depression and heterosis for various fitness-related traits such as survival, growth and reproductive ability in fish, there is little available information on the effect of inbreeding on tolerance to various environmental conditions such as salinity or temperature. Therefore, it is important to investigate the effects of inbreeding and crossing on various quantitative traits, including fish specific traits, and the relationship between the amount of inbreeding depression and heterosis for the various quantitative traits in fish. This information would be useful for fish breeding and production.

Inbreeding depression and heterosis for various quantitative traits should be examined under controlled conditions where environmental variation can be minimized, because the traits are affected by both genetic and environmental factors. As a model organism for genetic analysis, the guppy *Poecilia reticulata* is one of the most useful teleosts because of its short life cycle, ease of breeding and establishment of populations in the laboratory, and the availability of many strains (Macaranas and Fujio, 1987; Barinova et al., 1997a; Shikano and Fujio, 1997). These strains have various characteristics in terms of morphological and physiological traits (Macaranas and Fujio, 1987, 1990; Fujio et al., 1990; Kanda et al., 1991; Fujio and Nakajima, 1993; Nakajima and Fujio, 1993; Shikano and Fujio, 1994; Ando et al., 1995).

The present study examines the effects of inbreeding and crossing on various quantitative traits, including fish specific traits, and the relationship between the amount of inbreeding depression and heterosis for these traits in the guppy.

2. Materials and methods

2.1. Animals

One wild population, N4, and two domestic strains, S3HR and F22, were used in this study. The N4 population was caught in a stream in Okinawa prefecture in Japan in 1999.

About 100 individuals were transferred to our laboratory and maintained in a 60-l aquarium. To reduce any environmental effects which they had experienced in nature, experiments were performed using their offspring after one or two generations. A description of the S3HR and F22 strains, and how they were produced and maintained, has been given in previous papers (Macaranas and Fujio, 1987; Shikano and Fujio, 1994). These strains have distant kinship on the basis of their introduction and establishment (Macaranas and Fujio, 1987; Shikano et al., 2000).

2.2. Full-sib mating

Full-sib mating was performed in the N4 population as follows. Offspring obtained from each pair of the N4 population were reared in 2.5-l aquaria up to 10 individuals per aquarium (P generation). As soon as sex can be identified, males were separated from females to obtain virgin females. Twelve 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 up to 10 individuals per aquarium. The expected inbreeding coefficient is 0.250 after one generation of full-sib matings.

2.3. Crossing experiment

Reciprocal crosses were performed between the S3HR and F22 strains. Immature fish in each 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 was mated with a male in a 2.5-l aquarium to produce F_1. The F_1 were reared in 2.5-l aquaria up to 10 individuals per aquarium. To distinguish the reciprocal crosses, the F_1 hybrids were designated by letters indicating the female parent followed by the male parent (S3HR × F22 and F22 × S3HR).

2.4. Maintenance conditions

The laboratory for breeding experiments of guppies was maintained at a water temperature of 23 ± 1 °C (mean ± range) using an air conditioner with lighting for 10 h per day. All experiments were performed in this laboratory. All fish were fed twice daily with ground carp pellets and dried *Daphnia* as a supplementary diet.

2.5. Quantitative traits

Six quantitative traits, body length at birth, survival at day 120, undwarf rate at day 120, body length at day 120, salinity tolerance and high temperature tolerance, were examined. Survival and growth experiments were performed in 2.5-l aquaria up to 10 individuals per aquarium. Undwarf individuals were determined on the basis of more than 12 mm of standard body length at day 120 (Fujio and Nakajima, 1993). Salinity tolerance was measured by survival time of adult fish after transfer from fresh water to 35-ppt seawater (Aquasalz, Nissei, Japan) at 23.0 ± 0.5 °C. Dead fish in 35-ppt seawater were recorded at 30-min intervals after the transfer. High temperature tolerance was measured by survival time of adult fish after transfer from 23.0 to 36.5 °C. Dead fish at 36.5 °C were recorded at 15-min intervals after the transfer. Number of family used in this study was 8 for N4, 12 for F_1(full-sib mating) of N4, 10 for S3HR, 9 for F22, 7 for F_1(S3HR × F22) and F_1(S3HR × F22). Standard error for survival rate and undwarf rate was calculated among the family data.

2.6. Amount of inbreeding depression and heterosis

The amount of inbreeding depression was expressed as $(1 - m_f/m) \times 100$, where m_f represents the mean value in the F_1 of full-sib matings, and m represents the mean value of the parent population utilized for the full-sib matings. The amount of heterosis was expressed as $(m_h/m - 1) \times 100$, where m_h represents the mean value in the reciprocal F_1 hybrids, and m represents the mid-parent value of the parent strains utilized for the cross.

2.7. Statistical analysis

Statistical comparisons were assessed using the Student's *t*-test or one-way analysis of variance (ANOVA) followed by the Fisher's protected least significant difference (PLSD) multiple comparison test.

3. Results

3.1. Effects of full-sib mating

Six quantitative traits for N4 population and the F_1 of full-sib matings are shown in Table 1. Because body length and high temperature tolerance differed between sexes as reported in previous papers (Macaranas and Fujio, 1987; Kanda et al., 1991), these data are represented for each sex. In the F_1 of full-sib matings, survival rate at day 120 significantly decreased from 88.1% to 70.7% ($P < 0.05$, *t*-test) and salinity tolerance from

Table 1
Six quantitative traits in P and F_1 generations in full-sib matings of N4 population and the amount of inbreeding depression

Trait	Generation		Amount of inbreeding depression (%)
	P(N4)	F_1(full-sib mating)	
Body length at birth (mm)	6.60 ± 0.05 (78)	6.59 ± 0.04 (110)	0.2
Survival rate at day 120 (%)	88.1 ± 3.3 (78)	70.7 ± 5.8 (110)	24.6
Undwarf rate at day 120 (%)	96.9 ± 2.0 (69)	97.9 ± 1.5 (78)	− 1.0
Body length at day 120 (mm)			
Female	16.48 ± 0.32 (35)	16.49 ± 0.37 (52)	− 0.1
Male	14.51 ± 0.19 (31)	14.22 ± 0.18 (24)	2.0
Salinity tolerance (h)	5.76 ± 0.25 (52)	4.72 ± 0.18 (52)	22.0
High temperature tolerance (h)			
Female	3.37 ± 0.22 (65)	3.29 ± 0.19 (46)	2.4
Male	2.81 ± 0.23 (41)	2.63 ± 0.20 (34)	6.8

The number of individuals is shown in parentheses. Values represent mean ± S.E.

5.76 to 4.72 h ($P<0.01$, t-test). Significant differences between the P and F_1 generations were not observed in body length at birth, undwarf rate at day 120, body length at day 120 and high temperature tolerance ($P>0.05$, t-test). The amount of inbreeding depression ranged from − 0.1% of body length of female at day 120 to 24.6% of survival rate at day 120.

3.2. Effects of crossing

Six quantitative traits for S3HR and F22 strains and their reciprocal F_1 hybrids are shown in Table 2. Strain differences were observed in body length at birth, body length of male at day 120 and salinity tolerance ($P<0.01$, t-test). Significant differences among the P and F_1 generations were observed in body length at birth ($P<0.01$, ANOVA), survival rate at day 120 ($P<0.01$, ANOVA), body length of male at day 120 ($P<0.01$, ANOVA) and salinity tolerance ($P<0.01$, ANOVA) but not in undwarf rate at day 120, body length of female at day 120 and high temperature tolerance ($P>0.05$, ANOVA). A significant difference between the reciprocal crosses of the F_1 hybrids was observed in body length at birth ($P<0.01$, t-test) but not in other traits ($P>0.05$, t-test). Body length at birth of the F_1 hybrids significantly differed from that of the male parents ($P<0.01$ or $P<0.05$, ANOVA and Fisher's PLSD) but not from the female parents ($P>0.05$, ANOVA and Fisher's PLSD). Although significant differences were observed among the P and F_1 generations ($P<0.01$, ANOVA), body length of males at day 120 in the F_1 hybrids ranged within those of their parental strains. Survival rate at day 120 and salinity tolerance of the reciprocal F_1 hybrids were higher than those of their parental strains ($P<0.01$, ANOVA

Table 2
Six quantitative traits in P and F_1 generations in reciprocal crosses between S3HR and F22 strains and the amount of heterosis

Trait	Generation				Amount of heterosis (%)
	P(S3HR)	P(F22)	F_1(S3HR × F22)	F_1(F22 × S3HR)	
Body length at birth (mm)	6.87 ± 0.05 (98)	6.57 ± 0.04 (87)	6.79 ± 0.08 (54)	6.47 ± 0.09 (56)	− 1.3
Survival rate at day 120 (%)	72.0 ± 4.9 (98)	67.0 ± 6.5 (87)	90.0 ± 5.3 (54)	96.4 ± 2.4 (56)	34.1
Undwarf rate at day 120 (%)	89.4 ± 4.6 (70)	91.9 ± 6.6 (59)	91.9 ± 2.9 (47)	90.6 ± 4.8 (54)	0.7
Body length at day 120 (mm)					
Female	17.30 ± 0.38 (36)	17.65 ± 0.49 (26)	18.38 ± 0.64 (23)	18.13 ± 0.51 (26)	4.5
Male	13.61 ± 0.17 (26)	16.48 ± 0.28 (29)	14.84 ± 0.22 (20)	15.24 ± 0.23 (23)	− 0.1
Salinity tolerance (h)	4.13 ± 0.12 (120)	3.18 ± 0.10 (60)	5.06 ± 0.15 (174)	5.35 ± 0.11 (319)	42.2
High temperature tolerance (h)					
Female	4.77 ± 0.41 (42)	5.09 ± 0.50 (22)	4.94 ± 0.42 (41)	4.92 ± 0.37 (49)	0.0
Male	3.04 ± 0.42 (23)	3.67 ± 0.25 (30)	3.57 ± 0.29 (27)	3.31 ± 0.18 (38)	2.4

F_1 hybrids are designated by letters indicating the female parent followed by the male parent.
The number of individuals is shown in parentheses. Values represent mean ± S.E.

and Fisher's PLSD). The amount of heterosis ranged from − 1.3% for body length at birth to 42.2% for salinity tolerance.

4. Discussion

The present study focused on the effects of inbreeding and crossing on various quantitative traits, including fish specific traits, and the relationship between the amount of inbreeding depression and heterosis for the various quantitative traits in the guppy. The wild population used in the present study was revealed to have significantly higher genetic variation than domestic populations (Shikano and Taniguchi, 2002a). We examined effects of inbreeding on various quantitative traits using full-sib matings in the wild population. The present study showed that the amount of inbreeding depression resulted from one generation of full-sib matings ($F = 0.25$) differed from − 1.0% to 24.6% among the quantitative traits, and significant decreases were observed in survival at day 120 (24.6%) and salinity tolerance (22.0%) but not for other traits. Inbreeding depression has been observed in various characteristics in fish (Kincaid, 1976a,b; Mrakovcic and Haley, 1979; Kincaid, 1983; Gjerde et al., 1983; Bondari and Dunham, 1987; Su et al., 1996). Kincaid (1983) reported that one generation of full-sib mating ($F = 0.25$) produced an increase in fry deformities (37.6%) and decreased feed conversion efficiency (5.6%), fry survival (19.0%) and weight at 147 days of age (11.0%) and 364 days of age (23.2%) in rainbow trout. The inbreeding depression for survival at day 120 and salinity tolerance of the guppy was relatively large in comparison with that observed in various quantitative traits of other fish. The present result shows that the effects of inbreeding differ among quantitative traits of a teleost as reported in plants and mammals (Falconer, 1989), indicating that inbreeding can decrease the performance of some quantitative traits but not all.

Heterosis, or hybrid vigour, is resulted from the crossing between inbred lines, or between genetically diverged populations (Falconer, 1989). Previous studies have demonstrated that the strains used for crossing in the present study (S3HR and F22) have lower genetic variation than wild populations due to bottlenecks at their foundation and through subsequent low effective population sizes (Barinova et al., 1997b; Fujio et al., 1999; Shikano and Taniguchi, 2002a). In addition, microsatellite analysis demonstrated that the Nei's genetic distance between these strains is 0.622, indicating the significant genetic differentiation (Shikano and Taniguchi, 2002b). We examined effects of crossing on various quantitative traits using reciprocal crosses between these strains. The crossing experiment indicated that the amount of heterosis differed from − 1.3% to 42.2% among the six quantitative traits. The reciprocal F_1 hybrids showed significant heterosis for survival rate at day 120 and salinity tolerance, indicating that crossing can increase the performance of some quantitative traits but not all.

In general, inbreeding depression and heterosis are associated with changes in heterozygosity and homozygosity for several reasons (Falconer, 1989). First, homozygotes may have reduced fitness value for traits which are controlled by directionally dominant alleles, and second, increasing homozygosity increases the chances of the expression of deleterious recessive alleles. Based on microsatellite allele frequencies (Shikano and Taniguchi, 2002a,b), full-sib matings would decrease mean heterozygosity in the wild population (N4) from 0.462 to 0.347 (− 25.0%). On the other hand, crossing between the S3HR and F22 strains would increase mean heterozygosity from 0.359 and 0.258 to 0.599 (+ 94.2% in the mean value). This suggests that the significant decrease and increase in general level of heterozygosity, which is closely related to inbreeding coefficient, is important for expressions of inbreeding depression and heterosis for survival at day 120 and salinity tolerance of the guppy. In this context, Shikano et al. (2001) reported a linear decrease in salinity tolerance of the guppy with an increase in inbreeding coefficient, suggesting that inbreeding depression for salinity tolerance results from directionally dominant alleles. The relationship between the amount of inbreeding depression and heterosis as defined in the present study is in accordance with the theory that the phenomenon of heterosis is the reverse of inbreeding depression (Falconer, 1989), indicating that the traits which have decreased by inbreeding can be recovered by crosses. The different degree of inbreeding depression and heterosis per mean heterozygosity might result from different genetic background between the wild population and the domestic strains. No effect of inbreeding and crossbreeding on the other traits implies that the general level of heterozygosity had no direct effect on these characters.

Because body length at birth of the F_1 hybrids differed from that of the male parents but not from the female parents, body length at birth may be genetically controlled by maternal effects in the guppy. No maternal effects for body length at 120 days suggest that additive maternal effects are important for body length at birth and the effects decrease with advancing age.

The present study experimentally indicated two important points for fish breeding. One is that effects of inbreeding and crossing differ among quantitative traits. The other is that, when inbreeding depression has occurred for some quantitative traits, a cross between genetically different individuals is an available means to recover the traits.

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