Heterotic Effects in Salinity Tolerance in Varying Genetic States of Populations in the Guppy Poecilia reticulata

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Heterotic Effects in Salinity Tolerance in Varying Genetic States of Populations in the Guppy *Poecilia reticulata*

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Summary

The relationship between fitness advantage of isozymic genotype and a state of genetic constitution in a closed colony was examined in guppy strains. Salinity tolerance was a suitable index to estimate heterotic effects within a population. Sixteen of the 18 strains examined were initially divided into two groups; one group was comprised of those strains exhibiting heterozygote excess and the other was comprised of those exhibiting homozygote excess. The heterozygote excess group showed no tendency to an increase of survival time in heterozygous individuals, but the homozygote excess group showed a tendency to an increase of survival time in heterozygous individuals could be explained through the result of heterotic effects to be actualized in the inbreeding state.

The fitness advantage of heterozygous individuals at isozymic loci has been reported for some cultured fish species. Danzmann *et al.*(1) demonstrated an advantage of heterozygoutes at the isozymic loci for developmental rate in cultured rainbow trout. Also, an advantage of developmental stability in cultured rainbow trout has been reported (2-4). In the relationship between genetic variability and morphological variability, a positive correlation was observed in cultured stocks of masu salmon (5), but not in cultured stocks of rainbow trout (6). Such a situation might be dependent on the breeding state of stocks.

As a model organism for maintenance of heterozygous individuals with fitness advantage in a population, the guppy *Poecilia reticulata* is a suitable fish because of its short life cycle, reproductive capacity and ease of breeding. Salinity tolerance might be a suitable index to estimate heterotic effects within a population.

Shikano and Fujio (7) demonstrated that survival times in artificial seawater with 35 ppt salinity significantly differed among 14 strains of the guppy. Cross-

ing of the guppy strains has also demonstrated heterosis for seawater tolerance (8).

Nakajima *et al.*(9) selected the guppy population for seawater tolerance, and demonstrated a correlation between high seawater tolerance in the selected population and higher heterozygote excess of the isozymic loci.

In the present work, strain difference for seawater tolerance was examined and the heterotic effect was demonstrated by the correlation between the coefficient of variation in survival time and genetic uniformity of the population.

Materials and Methods

Animals

Eighteen guppy strains (S, S3, S3HL, S3SWS1, S3SWR1, SC, M1, O, A, B, C, D, D1, F, F22, G, T and T1) were used in this work, and they are maintained in a closed colony in our laboratory. A description of the guppy strains, how they were produced and maintained, is given in earlier papers (7, 10). The S3HL, S3SWS1 and S3SWR1 originated from the S3 strain which was separated from the S strain. They are maintained in 60 *l* aquaria at reasonable densities of 300-500

TABLE 1 *Mean survival time after transfer to 35 ppt seawater and the expected and observed heterozygosity in 18 guppy strains.*

Strian	N	Survival time (h) (Mean±S.D)	Heterozygosity	
			Expected (He)	Observed (Ho)
F	99	4.86±1.29	0.017	0.017
O	76	4.65±1.55	0.036	0.033
S3SWS1	100	4.31±0.97	0.030	0.029
S3HL	120	4.13±1.30	0.038	0.037
S	50	3.75±0.78	0.023	0.020
F22	132	3.72±1.11	0.016	0.017
S3SWR1	100	3.45±0.69	0.034	0.032
T	116	3.34±0.82	0.018	0.019
G	58	3.26±0.85	0.006	0.006
B	103	3.08±0.81	0.038	0.043
S3	185	3.05±0.83	0.035	0.039
M1	102	2.96±0.69	0.032	0.031
A	119	2.90±0.84	0.023	0.028
C	97	2.89±0.55	0.033	0.037
T1	104	2.89±0.77	0.021	0.020
SC	107	2.74±0.78	0.044	0.043
D	75	2.71±0.43	0.037	0.042
D1	84	2.31±0.49	0.043	0.050

N : Number of tested fish.

individuals per aquarium, depending on the average body size of the strain. The fish are reared at a temperature of 23±2°C and fed ground carp pellets twice a day.

Measurement of salinity tolerance

Mature guppies, older than 60 days after birth, from each of the strains were collected randomly and were exposed to artificial seawater (Aquasaltz, Nissei) with 35 ppt salinity. The survival time was examined at 30 minute intervals. Strain differences were analysed by one-way ANOVA. The survival time in artificial seawater did not significantly differ among mature guppies of varying body sizes and sexes, and all guppies died within 24 h in artificial seawater (7). After the measurement of salinity tolerance, the guppies were kept at −50°C until starch gel electropholesis.

Isozymes

For genetic analysis, thirty isozyme loci (*Aat-1, Aat-2, Aat-3, Ak, Ck-1, Ck-2, Fh, αGpd-1, αGpd-2, Gpi-1, Gpi-2, Idh-1, Idh-2, Ldh-1, Ldh-2, Ldh-3, Mdh-1, Mdh-2, Mdh-3, Me, Mpi, Odh-1, Odh-2, 6Pgd, Pgm-1, Pgm-2, Sdh-1, Sdh-2, Sod-1* and

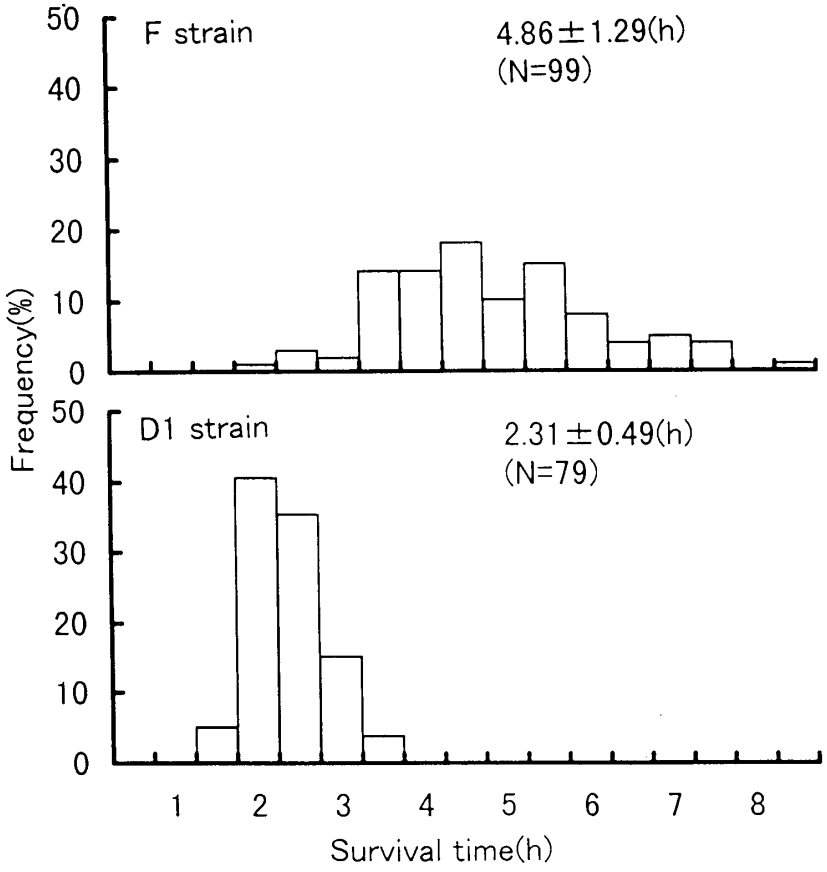

FIG. 1. Distribution of the survival time after transfer to 35 ppt seawater in F and D1 strains of the guppy.

Sod-2) were used. Of the 30 loci scored, *Aat-1*, *Gpi-2*, *Pgm-1*, *Pgm-2* and *Sod-2* were already known as polymorphic loci and the others are monomorphic loci (10). The procedures used to detect isozymes were based on Fujio (11).

Results

Table 1 shows the mean survival time for 18 guppy strains after the guppies had been subjected to 35 ppt artificial seawater. Strain differences were observed. The S3SWS1 strain, which was derived originally from the S3 strain, had a survival time significantly longer than the S3 strain ($P<0.05$).

Fig. 1 shows distributions of the survival time in high salinity-tolerant F strain and low salinity-tolerant D1 strain. The F strain had wide distribution, and the D1 strain had a narrow one. The class of the greatest frequency appeared at 4.5 h in the F strain and at 2.0 h in the D1 strain. As shown in Fig. 2, a positive correlation was noted between the mean survival time and the coefficient of variation in 18 guppy strains.

In preliminary data in our laboratory, a positive correlation between morphological variance of standard body length and genetic variability (Ho) was

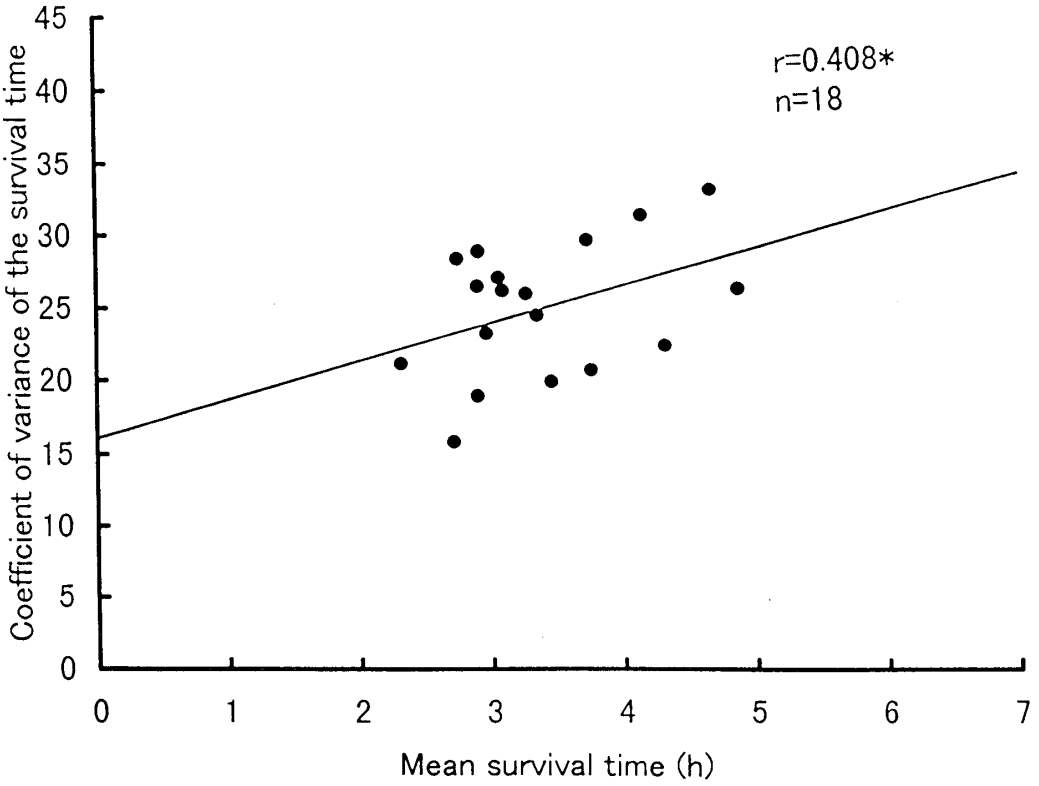

FIG. 2. Relationship between mean survival time (h) and the coefficient of variance in 18 guppy strains.
* Significant at $P<0.10$

observed in 60-days-old males of the guppy strains. Generally, positive correlations would be observed when the traits are related to the variations of additive variance in genetic components. In order to estimate the relationship between variance of salinity tolerance and genetic variability in guppy strains, the coefficient of variance of salinity tolerance was plotted against the average heterozygosity (Ho) estimated by direct counting of heterozygous individuals at the 30 isozyme loci mentioned before. No correlation was observed between them as shown in Fig. 3.

The observation of no correlation between them might be caused by a different state of genetic constitution of strains. The state of genetic constitution can be described by genetic variability and by deviation from Hardy-Weinberg's equilibrium (homozygote or heterozygote excess). The deviation from Hardy-Weinberg's equilibrium was measured by (Ho-He)/He, where He is the expected heterozygosity. The observed heterozygosity (Ho) is calculated by direct counting of the observed heterozygotes. The He is defined as (Σ he)/n. The he is defined as 1-Σ qi^2, where qi is the frequency of the i-th allele for a given locus, and n is the number of loci examined. A negative value indicates homozygote excess and a positive value heterozygote excess. As shown in Fig. 4, the 18 strains were distributed in a linear manner, suggesting a colleration of inbreeding with the

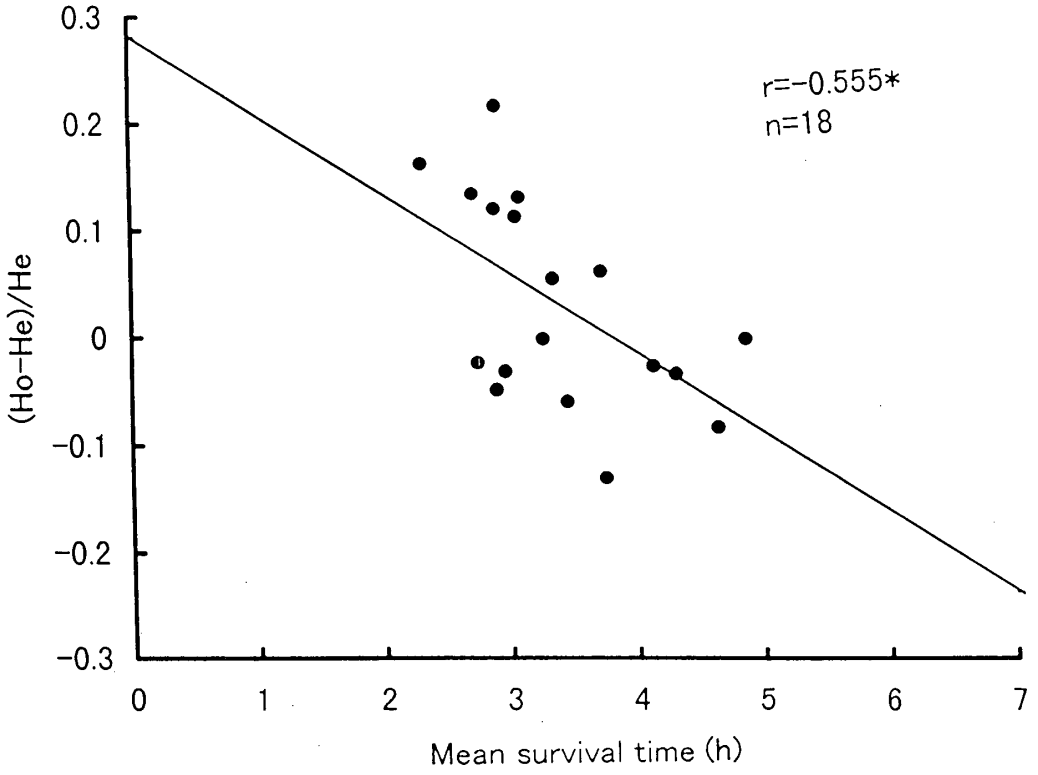

FIG. 3. Relationship between coefficient of variance of survival time after transfer to 35 ppt seawater and average heterozygosity (Ho).
* Significant at $P<0.05$

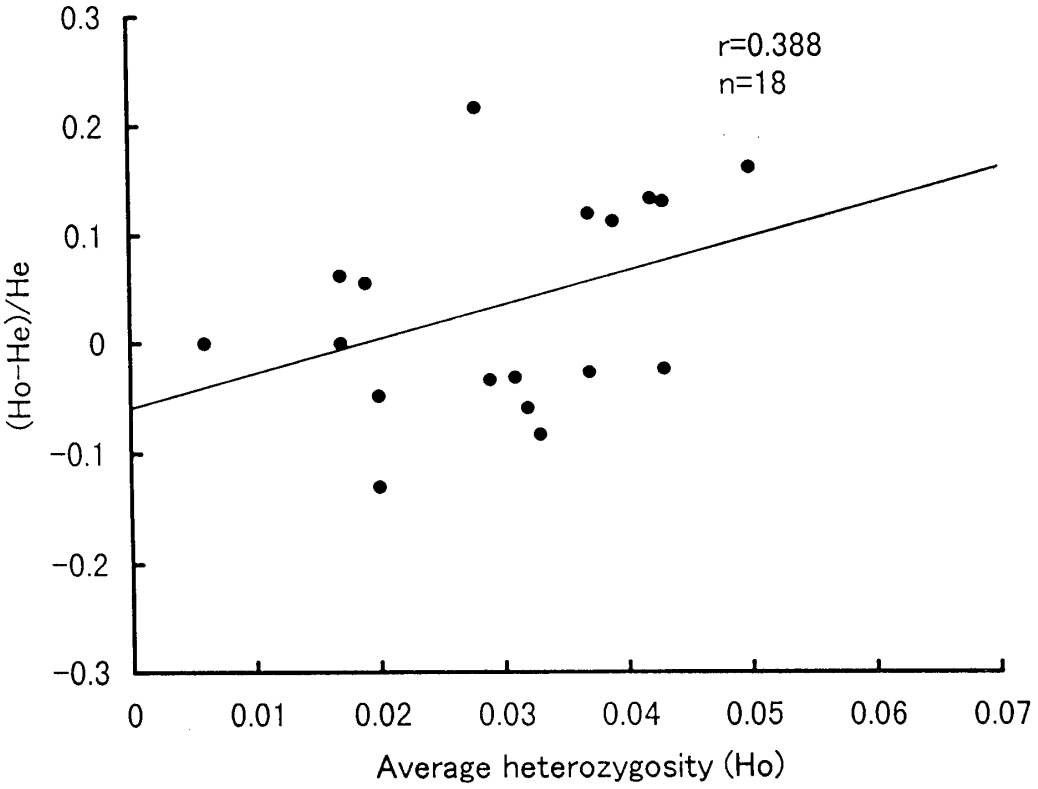

FIG. 4. Relationship between average heterozygosity (Ho) and (Ho-He)/He.

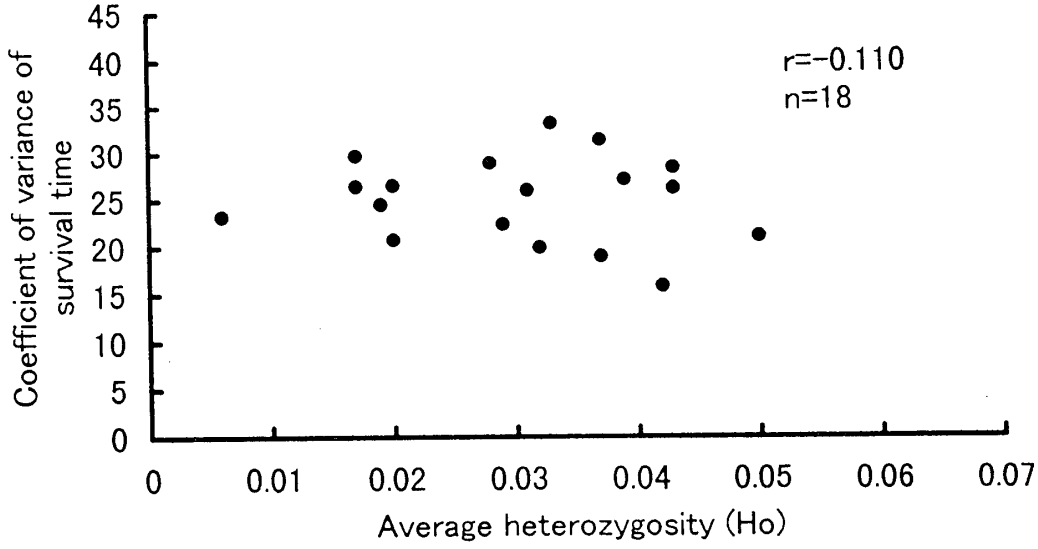

FIG. 5. Relationship between the mean survival time after transfer to 35 ppt seawater and the deviation for Hardy-Weinberg's equilibrium.

state of the genetic constitution of the population. In order to analyze the relationship between the states of genetic constitution of strains and fitness, the mean survival time after transfer to 35 ppt seawater was plotted against the deviation from Hardy-Weinberg's equilibrium (Fig. 5). A negative correlation was observed between them. This suggests that an increase of inbreeding is reflected in an increase of variance within a population, since there is a positive correlation between the mean survival time and the coefficient of variation.

In order to analyze the relationship between the state of genetic constitution of strains and advantage of genotypes, 16 strains (F and G strains having been omitted) were initially divided into two groups as shown in Table 2. Using the five isozymic loci, genetic variation was observed in 18 strains at at least one locus. F and G strains were fitted to Hardy-Weinberg's equilibrium. Eight strains out of 16 strains exhibited a state of heterozygote excess and the remaining eight strains exhibited homozygote excess.

The heterozygote excess group showed a tendency of heterozygote excess at each polymorphic locus. On the other hand, the homozygote excess group showed a tendency of homozygote excess at each polymorphic locus. Thus, it suggests that homozygote excess is reflected in an increase of the coefficient of inbreeding.

TABLE 2 *The state of genetic consitiution of the 18 guppy strains.*

	Total	*Aat-1*	*Gpi-2*	*Pgm-1*	*Pgm-2*	*Sod-2*
Strain	(Ho-He)/He	(ho-he)/he	(ho-he)/he	(ho-he)/he	(ho-he)/he	(ho-he)/he
A	+0.217	+0.261	+0.208	—	—	—
D1	+0.163	+0.087	+0.431	—	−0.065	—
D	+0.135	+0.103	+0.176	—	+0.119	—
B	+0.132	+0.201	+0.049	—	+0.118	—
C	+0.121	+0.119	+0.075	—	+0.129	—
S3	+0.114	+0.115	—	+0.108	+0.087	—
F22	+0.063	—	−0.116	—	—	+0.137
T	+0.056	—	—	+0.164	−0.010	—
F	0.000	—	+0.025	—	—	−0.023
G	0.000	+0.040	—	—	—	+0.045
SC	−0.023	−0.008	−0.025	−0.030	—	+0.044
S3HL	−0.026	−0.004	—	−0.055	—	−0.009
M1	−0.031	+0.047	—	−0.096	—	—
S3SWS1	−0.033	+0.015	—	−0.037	—	—
T1	−0.048	—	—	—	−0.058	+0.080
S3SWR1	−0.059	−0.220	—	+0.084	+0.039	—
O	−0.083	−0.032	+0.011	−0.268	−0.010	−0.212
S	−0.130	−0.078	—	−0.175	—	—

TABLE 3 *Comparison of mean survival time after transfer to 35 ppt seawater between homozygotes and heterozygotes in each isozyme locus.*

	Strain	Locus	Homozygotes	Heterozygotes
Heterozygote excess				
	A	*Aat-1*	2.90±0.89(65)	2.91±0.78(54)*
		Gpi-2	2.92±0.80(72)*	2.88±0.90(47)
	D1	*Aat-1*	2.36±0.46(42)*	2.30±0.50(40)
		Gpi-2	2.57±0.50(28)*	2.18±0.44(56)
		Pgm-2	2.28±0.50(55)	2.36±0.48(29)*
	D	*Aat-1*	2.72±0.39(39)*	2.71±0.47(36)
		Gpi-2	2.70±0.42(40)	2.73±0.44(35)*
		Pgm-2	2.60±0.32(51)	2.92±0.53(24)*
	B	*Aat-1*	3.18±0.83(44)*	3.01±0.79(59)
		Gpi-2	3.05±0.78(72)	3.16±0.87(31)*
		Pgm-2	3.08±0.84(60)	3.09±0.77(43)*
	C	*Aat-1*	2.90±0.58(51)*	2.86±0.53(47)
		Gpi-2	2.89±0.52(79)	2.89±0.68(18)
		Pgm-2	2.92±0.56(56)*	2.83±0.54(42)
	S3	*Aat-1*	3.16±0.98(92)*	2.94±0.88(93)
		Pgm-1	3.04±0.81(92)	3.06±1.05(93)*
		Pgm-2	3.04±0.95(155)	3.08±0.89(30)*
	F22	*Gpi-2*	3.77±1.13(122)*	3.10±0.46(10)
		Sod-2	3.63±1.15(73)	3.83±1.06(59)*
	T	*Pgm-1*	3.31±0.79(83)	3.42±0.89(33)*
		Pgm-2	3.41±0.90(82)*	3.18±0.54(34)
Homozygote excess				
	SC	*Aat-1*	2.73±0.83(67)	2.75±0.71(40)*
		Gpi-2	2.63±0.77(70)	2.95±0.78(37)*
		Pgm-1	2.82±0.79(56)*	2.65±0.78(51)
		Sod-2	2.74±0.79(102)	2.80±0.67(5)*
	S3HL	*Aat-1*	4.27±1.31(64)*	3.96±1.27(56)
		Pgm-1	3.95±1.17(81)	4.49±1.47(39)*
		Sod-2	4.22±1.29(82)*	3.92±1.31(38)
	M1	*Aat-1*	2.99±0.77(52)*	2.93±0.60(50)
		Pgm-1	2.90±0.77(56)	3.03±0.57(46)*
	S3SWS1	*Aat-1*	4.18±0.98(60)	4.51±0.94(40)*
		Pgm-1	4.19±0.92(52)	4.44±1.01(48)*
	T1	*Pgm-2*	2.76±0.62(55)	3.02±0.90(49)*
		Sod-2	2.90±0.79(90)*	2.89±0.81(14)
	S3SWR1	*Aat-1*	3.37±0.67(61)	3.56±0.71(39)*
		Pgm-1	3.49±0.72(51)*	3.40±0.66(49)
		Pgm-2	3.41±0.70(92)	3.81±0.53(8)*
	O	*Aat-1*	4.78±1.60(44)	4.85±1.46(33)*
		Gpi-2	4.60±1.57(63)	4.82±1.45(14)*
		Pgm-1	4.71±1.63(65)*	4.27±0.88(11)
		Pgm-2	4.61±1.53(61)	4.80±1.68(15)*
		Sod-2	4.59±1.43(71)	5.17±2.66(6)*
	S	*Aat-1*	3.70±0.78(37)	3.89±0.80(13)*
		Pgm-1	3.70±0.72(33)	3.85±0.90(17)*

*represents a larger mean survival time than anther zygote. Number within the parentheses represents the number of individuals.

The mean survival time of homozygous individuals was compared with that of individuals being heterozygous at each locus. As shown in Table 3, no tendency to an increase of survival time in heterozygous individuals was observed in the heterozygote excess group. But a tendency to an increase of survival time in heterozygous individuals was observed in the homozygote excess group.

Discussion

Strain differences and heterosis might be clear in controlled populations where environmental variation could be minimized. Shikano *et al.*(8) demonstrated heterosis for salinity responses in crosses of the guppy strain and proposed the salinity responses as one of the traits related to fitness. Nakajima *et al.*(9) selected the guppy population for salinity tolerance, and demonstrated a correlation between higher salinity tolerance in selected populations and higher heterozygote excess of the isozyme loci.

The present work was focused on salinity tolerance as the trait related to fitness. The strain differences of the guppy in salinity responses and a correlation between mean survival time and the coefficient of variance of survival time was demonstrated in the present work. This result is similar to that reported by Shikano and Fujio (7). A correlation indicates that variance in strains with low salinity tolerance is small, but variance in strains with salinity tolerance is large.

A greater quantitative variance with low genetic variability was reported by Taniguchi *et al.*(13) based on artificial production of the gynogenetic diploid in Ayu. Increasing the coefficient of inbreeding (F) would be reflected in an increase of morphological variance within a population. Thus, a correlation of inbreeding brings a change of the state of the genetic constitution of populations. The observation of no correlation between the coefficient of variance of salinity tolerance and average heterozygosity (Ho) might be caused by a different state of genetic constitution of guppy strains. The state of genetic constitution of guppy strains can be described by deviation from Hardy-Weinberg's equilibrium (homozygote excess or heterozygote excess). A linear relationship between deviation from Hardy-Weinberg's equilibrium and Ho indicates that homozygote excess suggests an inbreeding structure.

From a state of genetic constitution of the guppy strain, 16 of the 18 strains were divided into two groups, the heterozygote excess group and the homozygote excess group. The homozygote excess group showed a tendency to an increase of survival time in heterozygous individual. Therefore, an appearance of a longer survival time in heterozygous individuals could be explained by the result of heterotic effects to be actualized in an inbreeding structure. In this case, heterozygote advantage is brought by heterotic effects of isozymes themselves; an advantage of heterozygots should be observed in certain isozyme loci, and in all

strains. Such a result was not observed in the present work. The explanation in which the advantage of heterozygots is observed against the low fitness of homozygots in an inbreeding population is more useful than the explanation in which the existence of a super gene is postulated. However, it is necessary to elucidate this, through various states of genetic constitution in the same strain, because the strain difference of salinity tolerance was also reflected by a few genes and heterotic effects (9).

Acknowledgement

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