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# Strain Differences at Thermal Resistance in the Guppy, *Poecilia reticulata*

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# Summary

Response to high temperature was measured in 10 different strains of the guppy, *Poecilia reticulata*. The response of each strain was measured as survival rate, which were calculated from the survived fishes after 24 hr at 35°C for fishes acclimated at 23°C. The values of response measurements demonstrated that the survival rates were very different among strains, and further, among body length (or age) and among females and males within each strain.

The 16 sub-populations which were made from an isolated pair of the S3 strain, respectively, showed significantly differences in thermal resistance. The existence of a different response to high temperature among sub-populations suggests the control of, at least, one major gene.

# Introduction

Water temperature is one of the most important limiting factors for growth, reproduction, and survival rate in aquatic species. The genetic studies of thermal resistance have been investigated among two species, Esox lucius and E. masquinongy (1), among sub-species, Micropterus salmoides salmoides and M.s. floridanus (2), among local races within Etheostoma spectabile (3), especially for evolutionary genetics. They found inter- and intra-specific differences from different environments, and suggested that these fishes might be genetically adapted to changing temperatures through natural selection. The inheritance of thermal resistance, however, has not been determined, and it is not known how many genes regulate this process.

The guppy, *Poecilia reticulata*, is useful as an experimental organism for genetic and breeding studies in fishes. The establishment of several strains can provide useful information about the genetic differences among strains. The genetic differences among strains were demonstrated using electrophoretic markers, growth- and reproductive-related traits, and low temperature resistance (4-6).

The purpose of this study is an account of genetic differences among 10 guppy strains in thermal resistance, and presents the suggestion that this thermal resistance is controlled by, at least, one major gene.

# Materials and Methods

# Animals

Ten guppy strains, S, S3, S2, F, M, T, T1, G, SA, and SC are being maintained in closed colonies in this laboratory. A description of the guppy strains, how they were produced and maintained, is given in an early paper (4). S, S3, and S2 strains are the standard-types, and S3 is a recent isolate of the S strain. F, M, T, T1, and G are the fancy-types, and T1 is an isolate from the T strain. SA and SC strains were made from the crosses between the S females and a single albino-like male, and between the S females and a single cobra-type male, respectively. In addition, size differences are more apparent in male body length although the same tendency is observed for female body length. Two size groups are apparently presented, one group composed by F, M, T, T1, and G, which are bigger, and the other group, S, S3, S2, SA, and SC, which are smaller. They are maintained in 60 l aquaria at reasonable densities of 300-500 individuals per aquarium, depending on the average size of the strain. The fishes are kept at a temperature of 23±2°C. The high temperature treatment was performed continuously throughout the year with all available strains in a random fashion.

# Establishment of Sub-populations

Each sub-population was made from a gravid female mated with one male from the parental stock of the S3 strain. The litters produced were kept in 60 l aquaria for more than 3 generations, and offsprings increased at the density of more than 300 individuals per aquarium. The high temperature treatment was performed with all 16 available sub-populations at random.

# High Temperature Treatment

Up to 3 individuals, older than 45 days, from each of the strains and the sub-populations were collected and held in thermally regulated 500 ml conical beakers, initially adjusted at 23°C. The water temperature in conical beakers was increased at the rate of 0.4°C per 10 min to 35°C. This rate minimized cumulative stress on the fish. The detection of death was determined by the complete cessation of opercular movement. After 24 hr, dead and survived fishes were recorded and their body length (standard length) were measured. The mean body length and its range of the used fishes in each strain are presented in Table 1.

## Results

Fig. 1 shows a comparison of the plotted survival rates after the high tempera-

TABLE 1.	The Mean	Body	Length	(mm)	of	Tested	Fishes
	in	10 Gr	ippy Sti	rains			

Strain		Female	Male			
	N	Mean SD	N	Mean SD		
S	136	$20.0 \pm 3.0 \ (12-29)$	156	$17.0 \pm 2.0 \ (11-20)$		
S3	335	$20.0 \pm 4.0 \ (12 - 29)$	317	$16.0 \pm 2.0 \ (12-20)$		
S2	87	$21.0 \pm 4.0 \ (14-29)$	116	$18.0 \pm 2.0 \ (13-23)$		
SA	25	$22.0 \pm 3.0 \ (14-27)$	26	$18.0 \pm 1.0 \ (15-20)$		
SC	91	$22.0 \pm 4.0 \ (13-29)$	134	$17.0 \pm 2.0 \ (13-20)$		
$\mathbf{F}$	202	$22.0 \pm 4.0 \ (13-31)$	224	$19.0 \pm 3.0 \ (12-25)$		
G	65	$24.0 \pm 4.0 \ (15-32)$	54	$21.0 \pm 2.0 \ (16-25)$		
M	110	$22.0 \pm 3.0 \ (14 - 30)$	118	$18.0 \pm 2.0 \ (14-22)$		
T	39	$23.0 \pm 6.0 \ (16 - 40)$	43	$20.0 \pm 3.0 \ (14-25)$		
<b>T</b> 1	74	$25.0 \pm 5.0 \ (16 - 32)$	93	$20.0 \pm 2.0 \ (15 - 25)$		

Length range of tested fishes is enclosed in ( ).

N: The number of tested fishes.

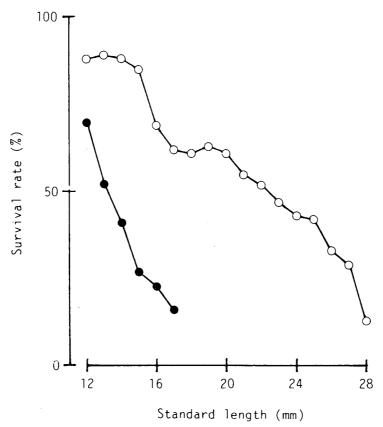


Fig. 1. Change of survival rates after high temperature treatment in relation to body length of the S3 guppy strain.  $\bigcirc$ , Female;  $\bullet$ , Male. Each plot is expressed by moving average with 3 mm units per plot.

		Fen	nale			Ma	le		
Strain	Sn	Small size*		Large size**		Small size*		Large size**	
	N	Survival	N	Survival	N	Survival	N	Survival	
S3	169	$76.3 \pm 3.3$	158	$51.3 \pm 4.0$	129	$54.3 \pm 4.4$	188	$23.4 \pm 3.1$	
SC	51	$72.5 \pm 6.3$	40	$30.0 \pm 7.2$	72	$59.7 \pm 5.8$	62	$22.6 \pm 5.3$	
T	24	$70.8\pm~9.3$	15	$33.3\pm12.2$	24	$70.8 \pm 9.3$	19	$36.8 \pm 11.1$	
SA	14	$64.3\pm12.8$	11	$18.2\pm11.6$	9	$33.3 \pm 15.7$	17	$11.8 \pm 7.8$	
S	70	$62.9\pm~5.8$	66	$39.4\pm~6.0$	76	$32.9\pm 5.4$	81	$29.6 \pm 5.1$	
M	60	$56.7 \pm 6.4$	<b>5</b> 0	$30.0\pm~6.5$	49	$49.0 \pm 7.1$	69	$21.7 \pm 5.0$	
$\mathbf{F}$	92	$56.5\pm~5.2$	95	$23.2\pm~4.3$	92	$37.0 \pm 5.0$	22	$13.1 \pm 7.2$	
<b>T</b> 1	43	$41.9 \pm 7.5$	31	$22.6\pm7.5$	45	$35.6\pm\ 5.0$	48	$16.7\pm 5.4$	
S2	42	$40.5\pm7.6$	44	$6.8 \pm 3.8$	65	$27.7 \pm 5.6$	51	$7.8 \pm 3.8$	
G	36	$25.0 \pm 7.2$	29	$17.2 \pm 7.0$	24	$29.2 \pm 9.3$	30	$3.3 \pm 3.3$	

Table 2. Survival Rates (%) after High Temperature Treatment in 10 Guppy Strains

ture treatment in relation to body length between females and males in the S3 strain of the guppy. The survival rates, in both females and males, decreased linearly from small body size to large body size. It indicates that the survival rates are significantly different among fish ages and among females and males within the strain.

Table 2 shows the survival rates at small and large body sizes in 10 guppy strains. The small and large body sizes were classified by the mean standard length as shown in Table 1. The survival rates varied among strains as well as between small and large sizes and between females and males, being the rank order S3>SC>T>SA>S>M>F>T1>S2>G. The survival rate of small sizes was higher than that of large ones in each of the strains, and a correlation of survival rates was obtained between small and large sizes in both females and males as shown in Fig. 2. In addition, the survival rate of females was higher than that of males in each of the strains, and the correlation of survival rates was obtained between females and males in both small and large sizes as shown in Fig. 3. Interestingly, the S3 strain isolated from the S strain had a value significantly higher than the S strain, and the T1 strain isolated from the T strain had a value significantly lower than the T strain. It suggests the possibility that thermal resistant gene is polymorphic in each of the strains of the guppy.

Considering the formation of the lines from the base population, each line is formed from a sample of N individuals drawn from the base population. If each individual carries two genes at a locus, the lines would come to differ in gene

<sup>\*:</sup> Smaller than the mean body length.

<sup>\*\*:</sup> Equal to or larger than the mean body length.

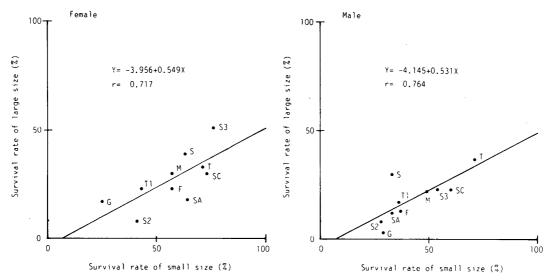


Fig. 2. Correlation of survival rates after high temperature treatment between small body sizes and large body sizes in 10 guppy strains.

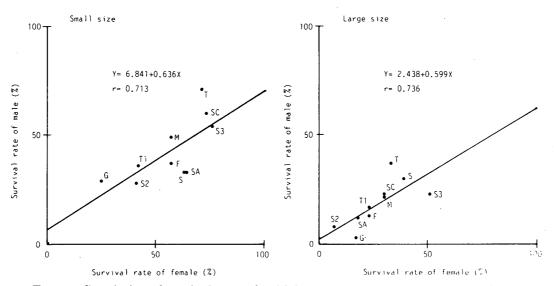


Fig. 3. Correlation of survival rates after high temperature treatment between females and males in 10 guppy strains.

frequency. Table 3 presents the survival rates in the 16 sub-populations from one parents drawn at random from the S3 strain population. The survival rates varied among sub-populations as well as between small and large sizes and between females and males. An average value of the survival rates in these sub-populations was near equal to that in original population. Thus, it suggests that thermal resistance might be controlled by, at least, one major gene.

Table 3. Survival Rates (%) after High Temperature Treatment in 16 Sub-populations from the S3 Guppy Strain

		Fen	nale		Male				
Sub-pop. Si		mall size L		Large size	Small size		Large size		
-	N	Survival	N	Survival	N	Survival	N	Survival	
S3- 2	46	$95.7 \pm 3.0$	7	$28.6 \pm 17.1$	37	$97.3 \pm \ 2.7$	14	$57.1 \pm 13.2$	
S3- 5	37	$94.6 \pm 3.7$	16	$62.5\pm12.1$	26	$80.8 \pm 7.7$	21	$61.9 \pm 10.6$	
S3- 4	33	$93.9 \pm 4.2$	9	$33.3 \pm 15.7$	29	$72.4 \pm 8.3$	16	$12.5 \pm 8.3$	
S3-15	34	$91.2 \pm 4.9$	11	100	24	$54.2 \pm 10.2$	13	$23.1\pm11.7$	
S3-13	41	$90.2 \pm 4.6$	14	$92.9 \pm 6.9$	28	$75.0\pm8.2$	13	$15.4\pm10.0$	
S3- 1	<b>5</b> 0	$88.0 \pm 4.6$	7	100	53	$67.9 \pm 6.4$	6	$33.3 \pm 19.2$	
S3-12	33	$87.9\pm5.7$	13	$69.2 \pm 12.8$	26	$53.8\pm~9.8$	14	$14.3\pm 9.4$	
S3- 3	35	$85.7 \pm 5.9$	6	$66.7 \pm 19.2$	24	$83.3\pm7.6$	10	$70.0\pm14.5$	
S3- 6	33	$84.8\pm~6.2$	12	$58.3\pm14.2$	25	$68.0 \pm 9.3$	14	$14.3 \pm 9.4$	
S3- 7	31	$80.6 \pm 7.1$	11	$63.6\pm14.5$	26	$73.1\pm~8.7$	10	$20.0\pm12.6$	
S3-11	34	$79.4 \pm 6.9$	13	$61.5\pm13.5$	22	$81.8\pm~8.2$	17	$29.4\pm11.0$	
S3-10	28	$67.9 \pm 8.8$	16	$81.3 \pm 9.7$	24	$66.7 \pm 9.6$	13	$15.4\pm10.0$	
S3-14	28	$64.3\pm~9.1$	14	$50.0\pm13.4$	30	$73.3\pm~8.1$	13	$38.5\pm13.5$	
S3-16	38	$63.2\pm7.8$	7	$71.4\pm17.1$	33	$57.6\pm8.6$	<b>2</b>	0	
S3- 8	23	$47.8 \pm 10.4$	16	$25.0\pm10.8$	24	$12.5\pm6.8$	9	$11.1\pm10.5$	
S3- 9	30	$40.0 \pm 8.9$	8	$37.5 \pm 17.1$	26	$19.2 \pm 7.7$	6	0	
Average		78.5		62.6		64.8		26.0	

Size classes are the same as that of the S3 strain in Table 2.

## Discussion

The strain difference of the guppy in response to a low temperature treatment was demonstrated by Fujio et al. (6), who presented evidence that the low temperature resistance was due to a major gene which follows a mode of sex-linked inheritance. The present study is a second demonstration of the existence of physiological differences in guppy strains that are genetically different. The genetic differences of the guppy strains used here were demonstrated by electrophoretic markers (4). The present study was focused on the strain differences in response to a high temperature treatment. We found that thermal resistance exihibited a significant difference among strains and among sub-populations, each made from one parents. The genetic difference of the guppy sub-populations used here was demonstrated by two isozyme loci, Aat-1 and Pgm-1 (7), and the gene frequency fluctuated in a wide range among sub-populations. Nakajima et al. (7) explained large fluctuation among sub-populations to result from sampling errors of the parents. The existence of polymorphism of a thermal resistant gene was

suggested by the fluctuation of survival rates among strains and among sub-populations.

The values of response measurement, however, were very different among ages and among females and males within each strain. These differences might be explained by two possibilities. One of them is an incomplete penetrance or expressivity. The interpretation is probably that the balance between effects of the gene and of other physiological conditions to promote the phenotypic expression is so delicate that in some individuals the threshold is not reached. Therefore, a deeper study about the response measurement used here remain for future undertaking.

The other explanation is that such variations may be influenced by an undetermined number of interacting genes associated with age and sex. To prove the genetic inconsistencies in the above mentioned possibilities, it would be necessary to make the population in which a thermal resistant gene was fixed by breeding.

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