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Genetic Variation for Thermal Resistance of the Guppy, *Poecilia reticulata*

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Summary

Responses to high temperature were measured in 10 sub-populations, each of which was prepared from a gravid female mated with one male of the S3 strain of the guppy, *Poecilia reticulata*. The responses were measured as the death time, the time at which the fish exposed to 37℃ died, for those acclimated at 23℃. The response measurement demonstrated that the death time of immature fish was later than that of mature females and males within each sub-population. The regression lines of the death time on body length in the mature females and males intersected at the average death time of the immature fish in every sub-population. These indicated that the genetic difference of thermal resistance can be clearly shown by only using immature fish.

Distribution of the death time among the immature fish showed that thermal resistance had continuous variation, and that the class of the greatest frequency was separated into two. The two-way selection for thermal resistance led to significant difference of the death time of immature fish between the high temperature resistant population and the sensitive one. From these results, two alleles, resistant and sensitive, at the locus were presumed for thermal resistance in the guppy.

The previous paper (1) showed a significant difference among the survival rates after 24 hr at 35℃ for fish acclimated at 23℃ among 16 sub-populations, each from an isolated pair of the S3 strain of the guppy, *Poecilia reticulata*. Although this measurement was not so appropriate to clearly present the difference of thermal resistance among individuals because it also fluctuated by the effects of sex and body length (age), we suggested that thermal resistance might be controlled by one major gene. In practice, however, it is not easily known whether the genetic influence on thermal resistance is due to a single gene or polygenes. Thermal resistance is called threshold character. The character has an underlying continuity with threshold which imposes a discontinuity on visible expression, dead or alive. This continuous variation is influenced by more than one locus and also by environment (2).

This paper is an account of the genetic variation for thermal resistance measured as the death time after subjecting the guppies to a temperature of 37℃, and presents the result of a two-way selection for thermal resistance.

Materials and Methods

Animals

The guppy, *Poecilia reticulata*, is useful as a pilot organism for genetic and breeding studies in fish (3–5). All of the populations used here were derived from the original S3 strain and were maintained in closed colonies in our laboratory. S3-A, S3-B and S3-C were the new populations originated from about 5 pairs, respectively, and S3-4, S3-5, S3-6, S3-7, S3-11, S3-12, S3-13, S3-14, S3-15 and S3-16 were the sub-populations originated from one pair, respectively. They were maintained in 60 l aquaria at the reasonable densities of 300–500 individuals per aquarium. The fish were kept at a temperature of 23±2℃. The high temperature treatment was performed continuously throughout the year with all available populations in a random fashion.

Selection Procedure

Selection was carried out at 35℃ for 24 hr for females of the original S3 strain after producing offspring. The high temperature treatment was followed by the method described in the earlier paper (1). The offspring from the 6 surviving females were reared as the high temperature resistant population (S3-HR) and those from the 6 dead females were done as the high temperature sensitive population (S3-HS).

High Temperature Treatment

Up to 10 fish were collected from each population in a random fashion and held in mesh cages (10×10×10 cm) placed in the aquarium initially thermoregulated at 23℃, and the fish were held in the aquarium for 30 min to allow for their adjustment to the new environment. The water temperature was increased at the rate of 1℃ per 12 min to 37℃. This rate minimized cumulative stress on the fish. Aeration created water mixing and supplied oxygen in the aquarium.

Dead fish were recorded at 30 min intervals after reaching a temperature of 37℃, and then their standard body length (mm) was measured. The detection of death was determined by the complete cessation of opercular movement. All tested fish were divided into three categories by their body length—mature females (14–32 mm), mature males (14–19 mm) and immature fish (8–13 mm).

Results

Fig. 1 shows the death time of the guppy exposed to 37℃ against body length in S3, S3-A, S3-B and S3-C populations. Each regression line of mature females and males was calculated including the immature fish because each was the approximation to the regression line calculated except the immature fish. The greater body length the fish had, the faster they died. The longest surviving in every population was the immature fish. Females tended to survive longer than males. These indicate that thermal resistance fluctuates with sex and body length (age). Females' and males' regression lines of the death time on body length intersected at the average death time of the immature fish in every population. This suggests that the expression of the thermal resistant gene could be observed by only using immature fish.

Fig. 2 shows the distributions of the death time among the immature fish exposed to 37℃ in S3, S3-A, S3-B and S3-C populations. Every population

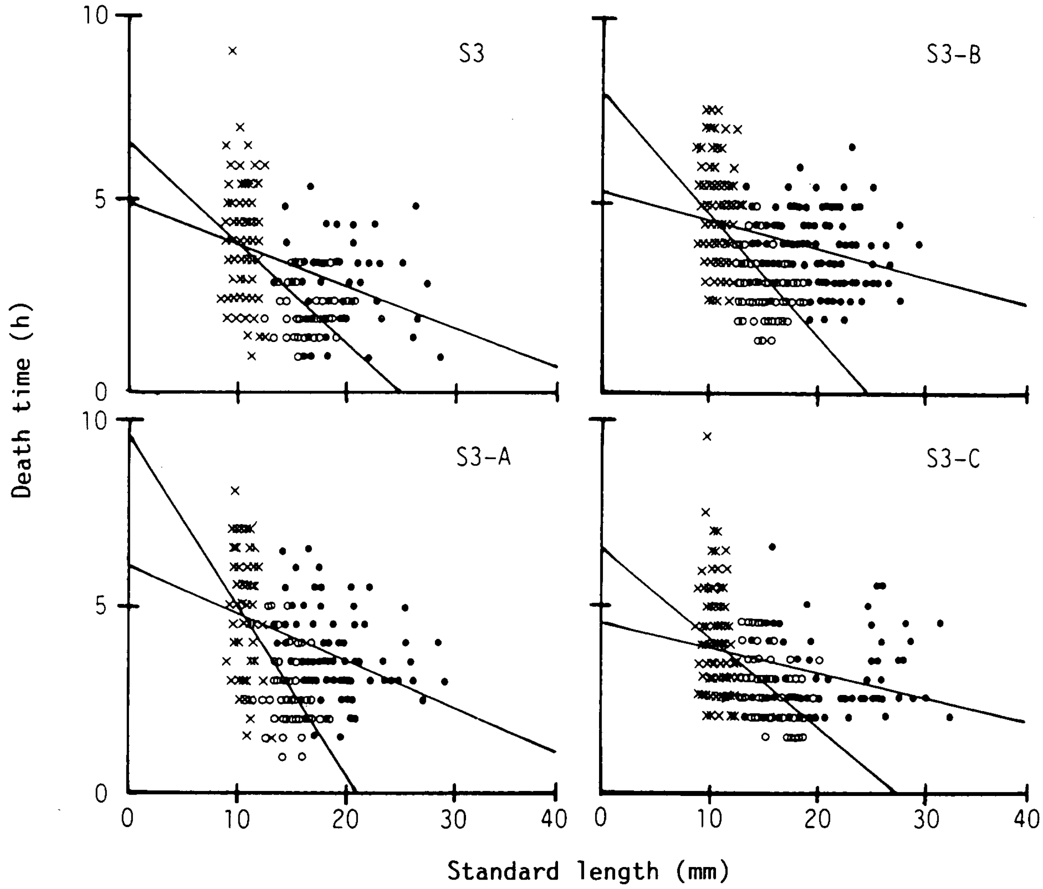

FIG. 1. Death time of the guppy exposed to 37°C against body length in S3, S3-A, S3-B and S3-C populations.
●, Mature female ; ○, Mature male ; ×, Immature fish.
Each regression line is calculated including immature fish.

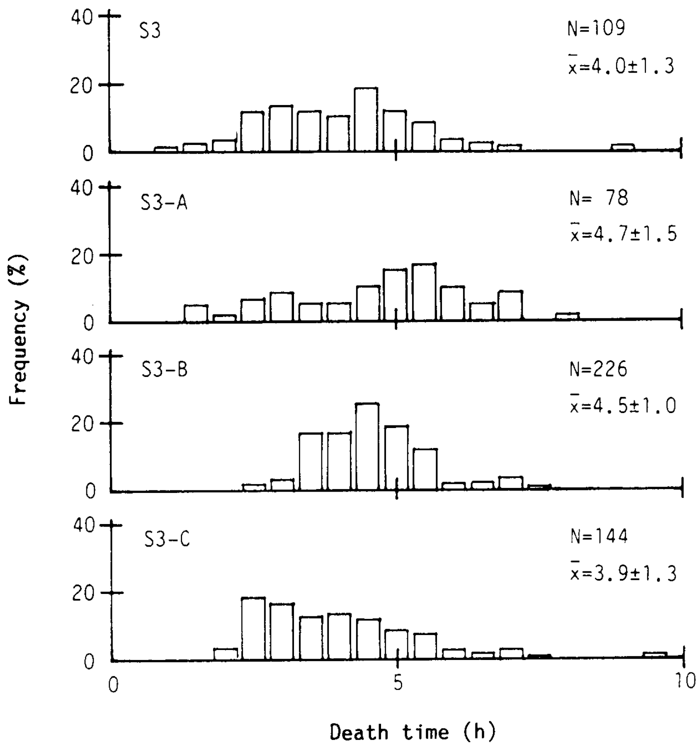

FIG. 2. Distributions of the death time among the immature fish exposed to 37°C in S3, S3-A, S3-B and S3-C populations of the guppy.

represented a continuous variation. S3, S3-A and S3-C had wide variations and S3-B had a narrow one. The class of the greatest frequency appeared at 4.5 hr in S3, at 5.5 hr in S3-A, at 4.5 hr in S3-B and at 2.5 hr in S3-C.

Fig. 3 shows the distributions of the death time among the immature fish exposed to 37℃ in 10 sub-populations. They also represented continuous variations. Some of 10 sub-populations seemed to have a wide variation with two peaks and others had a narrow one with one peak. Thus, the obvious difference in the distributions of the death time among the 10 sub-populations suggests the existence of two alleles at one major gene locus for thermal resistance.

Considering that thermal resistant gene is polymorphic in the population of the guppy, artificial selection for thermal resistance could be designed. Selection for resistance (S3-HR) and sensitivity (S3-HS) to high temperature was performed. Fig. 4 shows the distributions of the death time among the immature fish exposed to 37℃ in the original S3, S3-HR, selected for resistance, and S3-HS, selected for sensitivity, populations. The original S3 had a wide variation from 1.0 hr to 9.0 hr with the mean of 4.0 hr and showed the class of the greatest frequency at 4.5 hr. S3-HR also had a wide variation from 1.5 hr to 9.5 hr with the mean of 4.9 hr and showed the class of the greatest frequency at 4.5–5.0 hrs. On the other hand, S3-HS had a narrow variation from 1.5 hr to 5.0 hr with the mean of 2.6 hr and showed the class of the greatest frequency at 2.5 hr. These results indicate

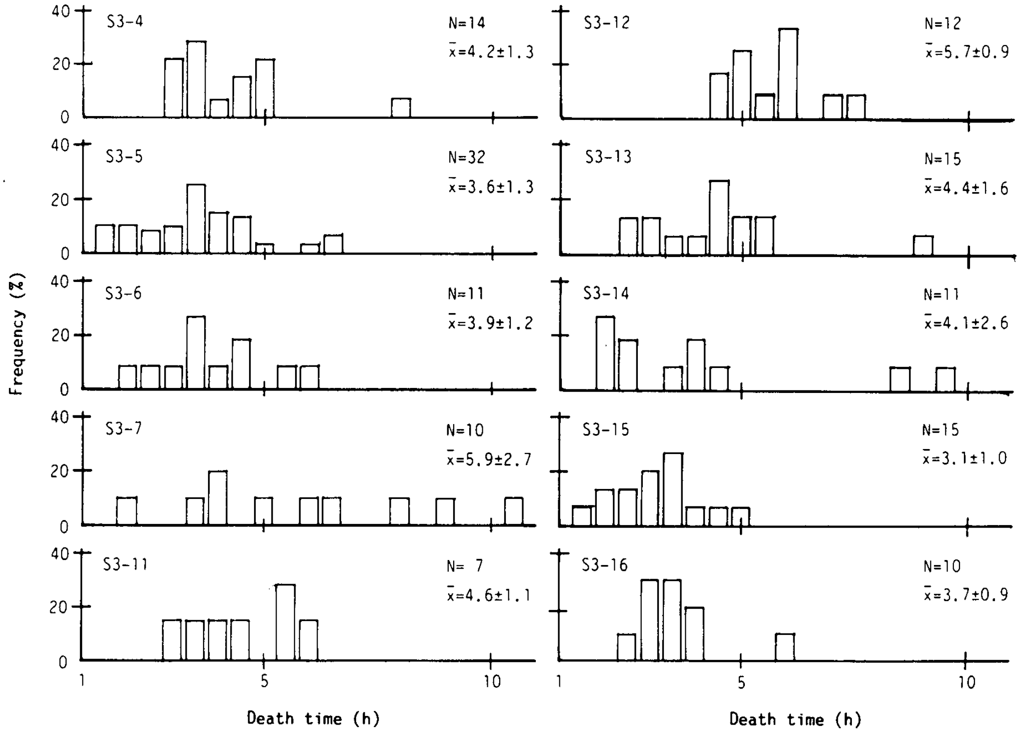

FIG. 3. Distributions of the death time among the immature fish exposed to 37°C in 10 sub-populations of the guppy.

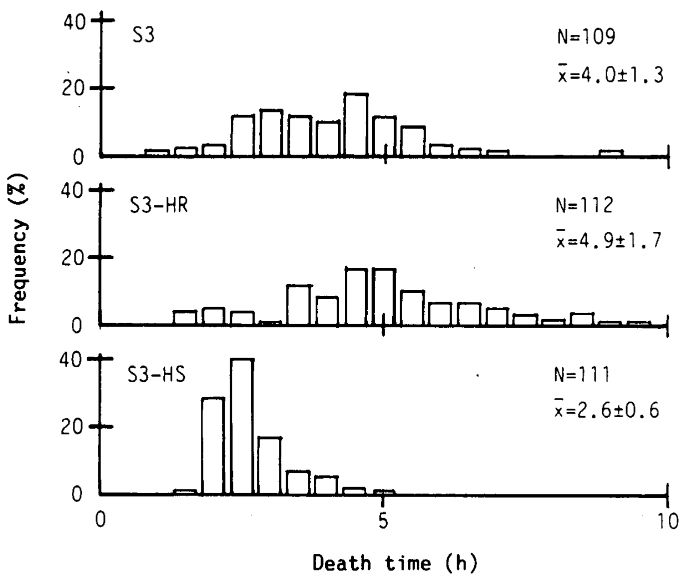

FIG. 4. Distributions of the death time among the immature fish exposed to 37°C in the populations selected for resistance and sensitivity to high temperature.
S3-HR : Population selected for resistance to high temperature.
S3-HS : Population selected for sensitivity to high temperature.
S3 : Original S3 strain.

that the death time is about 4.5-5.0 hrs for the resistant fish and is about 2.5 hr for the sensitive fish. The distribution of the two classes suggests the control of two alleles at the locus.

Discussion

The present study is a second demonstration of the genetic variation for thermal resistance in the guppy populations. The first study (1) revealed that the survival rates after high temperature treatment fluctuated in a wide range among 16 sub-populations and suggested that thermal resistance might be controlled by one major gene. There were, however, other factors associated with sex and body length to affect thermal resistance. The present study focused on the distributions of the death time among the immature fish exposed to 37℃. We found that females' and males' regression lines of the death time on body length intersected at the average death time of the immature fish. Thus, this study proposes that the genetic difference of thermal resistance is determined by only using immature fish.

Quantitative characters show continuous variations, and genes cannot be properly classed as major or as minor (2). Sub-population differences, each of which is made from an isolated pair, give us useful information to resolve this clue. If the phenotype is expressed by a major gene, its distribution would be classified by a peak. The two classes of the greatest frequency in the distributions of the death time among the immature fish exposed to 37℃ suggest two alleles, one is sensitive to high temperature and the other is resistant to high temperature. From that narrow variation arround 2.5 hr in the death time was obtained by the selection for sensitivity, while wide variation arround 4.5-5.0 hrs was obtained by the selection for the resistance, it is assumed that the sensitive allele might be recessive. To prove this possibility, it would be necessary to establish the high temperature resistant and sensitive strains by further selection for cross experiment.

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