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Fluctuation of Responses to the Low and High Temperature Treatments in the Sub-populations from One Guppy Population

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Summary

Starch gel electrophoresis was carried out to estimate the random change of the gene frequency in the sub-populations established from an isolated pair of the S3 guppy strain. The gene frequencies at the *Aat-1* and *Pgm-1* loci fluctuated within a wide range among 16 sub-populations, suggesting that the fluctuations are due to the sampling errors of the parents.

Responses to the low and high temperatures were measured in the same 16 sub-populations as the survival rate, which were calculated from the surviving fishes after 24 hr at 12°C or 35°C for fishes acclimated at 23°C. The existence of the different responses to the low and high temperatures among 16 sub-populations suggests the control of, at least, one major gene, respectively.

Introduction

Random change of gene frequency occurring independently in the different sub-populations leads to genetic differentiation between them. Cultured populations are often subdivided into the different strains. Strain differences in the guppy, *Poecilia reticulata*, were demonstrated using electrophoretic markers, growth- and reproductive-related traits and responses to the low and high temperature treatments (1-5). Strain difference of the guppy in response to the low temperature was demonstrated previously (4), and Fujio *et al.* presented evidence that the low temperature resistance was due to a major gene which followed a mode of sex-linked inheritance.

Nakajima *et al.* (6) reported that the *Aat-1* and *Pgm-1* gene frequencies fluctuated within a wide range among sub-populations of the S guppy strain, each was made from one pair. Therefore, the change of the gene frequencies resulting from the sampling is random in the sense that the distribution is unpredictable. The magnitude can be predicted in terms of the variance of the change. If a

certain character is controlled by polygenes, each with relatively small effect, the fluctuation of the incidence expressed on the basis of the threshold effect hypothesis would be inversly proportional to the number of loci contributing to the variance of the character.

This paper is to confirm the fluctuation of the responses to the low and high temperature treatments in relation to that of the gene frequency for the isozyme in the sub-populations established from one experimental population of the guppy.

Materials and Methods

Animal Population

Standard strain (S3) of the guppy, *Poecilia reticulata*, was used as the experimental population. It was maintained as a closed colony in our laboratory. The population size is about 500 individuals which includes about 300 adult fishes. From the S3 original population, 16 sub-populations were prepared from an isolated pair, respectively. Each sub-population was made from a gravid female mated to one male from the original stock. The litters produced were kept in 60 l aquaria for more than 3 generations, and the offspring increased to the density of more than 300 individuals per aquarium. Sampling was done in a random fashion independently for the isozyme analysis, the low temperature treatment and the high temperature treatment.

Detection of Isozyme

Starch gel electrophoresis was performed on the original stock and 16 sub-populations of the S3 strain according to the method of Fujio (7). For the genetic markers, *Aat-1* and *Pgm-1*, which were already known as polymorphic loci (1) were used.

Low and High Temperature Treatments

For the low temperature treatment, 5 individuals, older than 60 days, from the original stock and from each of sub-populations were collected and held in the thermally regulated 500 ml conical beakers initially adjusted at 23°C. The water temperature in the conical beakers was decreased at the rate of 0.5°C per 10 min to 12°C. After 24 hr, the conical beakers were heated up to 23°C in the water. The survival rates after the low temperature treatment were found not to be significantly different among fishes with varing body size and age (8). Dead and surviving fishes were recorded.

For the high temperature treatment, 3 individuals, older than 45 days and smaller than 20.0 mm in females and 16.0 mm in males of standard body length, were collected and held in the same way as the low temperature treatment. The

water temperature was increased at the rate of 4°C per 10 min to 35°C. After 24 hr, dead and surviving fishes were recorded. The survival rates after the high temperature treatment decreased linearly from small body size to large body size in both females and males (5).

Results

Original Stock

Table 1 shows three genotypic distributions at the *Aat-1* and *Pgm-1* loci in the original S3 population of the guppy. The occurrence of genotypes fits the expectations under the Hardy-Weinberg's equilibrium. It indicates that the original S3 population is maintained at random mating. The survival rates after the low and high temperature treatments in the original S3 population are shown

TABLE 1. *Genotypes and gene frequencies at the Aat-1 and Pgm-1 loci in the original S3 population of guppy*

Locus	No. of fishes	Genotype			χ^2	Gene frequency	
		AA	*AB*	*BB*		q*A*	q*B*
Aat-1	98	26 (26.5)	50 (48.9)	22 (22.6)	0.050	0.520	0.480
Pgm-1	98	37 (36.7)	46 (45.6)	15 (14.7)	0.012	0.612	0.388

The expected value under the Hardy-Weinberg's equilibrium is enclosed in ( ).

TABLE 2. *Gene frequencies at the isozyme loci and survival rates after low and high temperature treatments in original S3 population*

Characters	No. of fishes	Frequency
Isozyme locus		
A allele at *Aat-1*	98	0.520
A allele at *Pgm-1*	98	0.612
Survival rate after low temperature treatment		
Female	186	0.640
Male	226	0.460
Survival rate after high temperature treatment		
Female	169	0.763
Male	129	0.543

in Table 2. The survival rate of females was higher than that of males in both the low and high temperature treatments.

Differences among Sub-populations

The gene frequencies at the *Aat-1* and *Pgm-1* loci and the survival rates after the low and high temperature treatments in each sub-population are shown in Table 3. The gene frequencies of *A* allele at the *Aat-1* and *Pgm-1* loci were distributed from 0.160 to 1.000 and from 0.131 to 0.934, respectively, while those in the original population were 0.520 and 0.612. The *A* gene frequency calculated from an overall mean of 16 sub-populations was 0.577 at *Aat-1* and 0.632 at *Pgm-1*. These values are near equal to those from the original population, and are an indication of a wide fluctuation among 16 sub-populations, each was made

TABLE 3. *Gene frequencies at the isozyme loci and survival rates after low and high temperature teatments in 16 sub-populations from the S3 guppy strain*

Sub population	*Aat-1*	*Pgm-1*	Survival rate after treatment of			
			Low temperature		High temperature	
	p*A*	q*A*	Female	Male	Female	Male
S3-1	0.545 (66)	0.682 (66)	0.822 (73)	0.627 (75)	0.880 (50)	0.679 (53)
S3-2	0.738 (61)	0.607 (61)	0.828 (93)	0.506 (79)	0.957 (46)	0.973 (37)
S3-3	0.377 (61)	0.131 (61)	0.329 (73)	0.152 (66)	0.857 (35)	0.833 (24)
S3-4	0.939 (57)	0.939 (57)	0.312 (35)	0.108 (37)	0.939 (33)	0.724 (29)
S3-5	0.662 (77)	0.604 (77)	0.952 (83)	0.732 (82)	0.946 (37)	0.808 (26)
S3-6	0.510 (52)	0.529 (52)	0.707 (41)	0.341 (41)	0.848 (33)	0.680 (25)
S3-7	0.967 (45)	0.840 (47)	0.588 (17)	0.300 (10)	0.806 (31)	0.731 (26)
S3-8	0.152 (33)	0.232 (32)	0.720 (25)	0.444 (27)	0.478 (23)	0.125 (24)
S3-9	0.646 (48)	0.531 (48)	0.766 (47)	0.456 (44)	0.400 (30)	0.192 (26)
S3-10	0.848 (46)	0.587 (46)	0.529 (17)	0.263 (19)	0.679 (28)	0.667 (24)
S3-11	0.717 (53)	0.934 (53)	0.472 (36)	0.233 (43)	0.794 (34)	0.818 (22)
S3-12	0.310 (58)	0.537 (54)	0.441 (59)	0.185 (54)	0.879 (33)	0.538 (26)
S3-13	1.000 (66)	0.795 (66)	0.471 (68)	0.206 (68)	0.902 (41)	0.750 (28)
S3-14	0.270 (50)	0.860 (50)	0.519 (27)	0.219 (32)	0.643 (28)	0.733 (30)
S3-15	0.388 (58)	0.723 (56)	0.392 (51)	0.149 (47)	0.912 (34)	0.542 (24)
S3-16	0.160 (50)	0.585 (53)	0.182 (33)	0.097 (31)	0.632 (38)	0.576 (33)
Overall Mean	0.577	0.632	0.564	0.314	0.785	0.648
Degree of Fluctuation	0.328	0.219	0.190	0.166	0.171	0.215

The number of individuals tested is enclosed in ( ).

TABLE 4. *Correlation coefficient between every pair of characters in 16 sub-populations of S3 guppy*

	(1)	(2)	(3)	(4)	(5)	(6)
(1) Isozyme *Aat-1*						
(2) Isozyme *Pgm-1*	0.535*					
(3) Low temp. Female	0.161	−0.129				
(4) Low temp. Male	0.094	−0.170	0.955*			
(5) High temp. Female	0.372	0.296	−0.029	−0.002		
(6) High temp. Male	0.428	0.372	−0.061	−0.042	0.771*	

$P<0.05$

from one parents.

The survival rates after the low and high temperature treatments also varied among 16 sub-populations. Futhermoree, there is a significant difference between females and males within a sub-population. Each average value of the survival rates in 16 sub-populations was near equal to that in the original population. It suggests that the temperature resistant gene(s) is polymorphic in the original S3 population of the guppy.

Degree of the fluctuation among sub-populations was measured by $\sigma^2/p\ (1-p)$, where σ^2 is the observed variance of the gene or phenotypic frequency among sub-populations and p is the average gene or phenotypic frequency of all sub-populations. The fluctuation of the gene frequency at the isozyme loci was observed among 16 sub-populations ; the degree of the fluctuation was 0.328 in the *Aat-1* and 0.219 in the *Pgm-1*, and the average was 0.274. Similarly, the degree of the fluctuation obtained from the survival rates after the low temperature treatment was 0.190 in females and 0.166 in males, and the average was 0.178. Also, the value from the high temperature treatment was 0.171 in females and 0.215 in males, and the average was 0.193. These values are almost the same levels as those from the isozyme loci.

Correlation among the Allele Frequencies at the Isozyme and the Temperature Resistant Loci

Assuming that distributions in the allele frequencies, not only at the isozyme loci but also at the loci controlling temperature resistance, vary from sub-population to sub-population, the correlation coefficients among these frequencies could be calculated. Table 4 shows that there is a significant correlation between the *Aat-1* and *Pgm-1*, suggesting the linkage between them. Correlations between females and males in the low and high temperature resistances were also observed, respectively. No correlations between the isozyme loci, the low temperature resistant locus and the high temperature resistant locus suggest that three

loci are independently inherited, that is, no linkage group.

Discussion

Differentiation of strains or populations has been judged from gene frequencies at the individual locus detected by gel electrophoresis. On the other hand, the differences between strains in quantitative characters have far different probability of being demonstrated statistically than the differences in gene frequencies, even for many minor genes whose variation is reflected in quantitative characters. Unfortunately, the differences in quantitative characters between strains cannot be directly compared with the differences in gene frequencies at the individual loci, because the power of statistical tests to discriminate strains is vastly different for the two kind of characters (9, 3). The additive effects of contributory alleles exceeding a critical value are often used to explain many all-or-non phenomena with a polygenic mode of inheritance. In the threshold effect hypothesis, the individuals can have only two possible values which might be designated 0 for normal and 1 for affected. This is referred to the incidence as a simple description of the population. For genetic analysis, therefore, the incidences must mean phenotypic frequency. The clue to estimate the gene frequencies at the random set of loci influencing quantitative characters lies in the idea that the character has an underlying continuity with a threshold which imposes a discontinuity on the visible expression. In point of this view, the present study was focused on the fluctuations of the survival rates after the low and high temperature treatments among sub-populations. Considering the formation of sub-populations in a random fashion from the original population, each sub-population is formed from a sample of 2 individuals and represents 4 genes at a locus. We found that the gene frequencies at the *Aat-1* and *Pgm-1* loci fluctuated within a wide range among 16 sub-populations and that in each locus the average value was near equal to the value of the original population. Thus, it can be said that the fluctuation occurred due to the sampling errors of the parents.

Fluctuations of the survival rates might be also interpreted in similar way as the gene frequencies at the isozyme loci and this suggests that temperature resistance might be controlled by, at least, one major gene. Since the low temperature resistance was due to a major gene which followed a mode of sex-linked inheritance (4), the survival rate of males was equal to the resistant gene frequency. Degree of the fluctuation presented from every character suggests that the random segregation of alleles at the high temperature resistance is the same level at the low temperature resistance. Thus, it suggests that the high temperature resistance might be controlled by, at least, one major gene as the low temperature resistance.

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