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Detection of heat stability variants in GPI isozymes of the guppy, *Poecilia reticulata*

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ABSTRACT

Heat denaturation experiments revealed heat stability differences at a locus encoding glucosephosphate isomerase (GPI) in the guppy. Inheritance experiments indicated that the observed differences in heat stability are controlled by a single incomplete dominant autosomal locus.

1. INTRODUCTION

A combination of electrophoretic and heat denaturation techniques revealed hidden genetic variations of isozymes in *Drosophila* (Bernstein et al., 1973; Singh et al., 1974; Cochrane, 1976; Trippa et al., 1976; Milkman, 1976). Bernstein et al. (1973) discovered different heat stability alleles at xanthine dehydrogenase that revealed 1.74 times as many alleles as did electrophoresis alone in the same population of the virilis group of *Drosophila*. F_2 segregation of heat stability alleles in *Drosophila* were in a Mendelian fashion (Cochrane, 1976; Singh, 1976). In marine molluscs, such heat stability alleles has been reported in phosphoglucomutase in the marine mussel, *Guekensia demissa* (Gosling, 1979), in the scallope, *Patinopecten yessoensis* (Fujino and Matsuya, 1980), and in the Pacific abalone, *Haliotis discus hannai* (Wilkins et al., 1980). Furthermore, in the Pacific abalone, heat stability alleles were also reported in malate dehydrogenase, esterase, and phosphoglucose isomerase (Okumura et al., 1981). In fish, Okumura (1985) revealed the existence of the heat stability alleles in phosphoglucomutase in *Arctoscopus japonics*, *Salvelinus leucomaenis*, and *S. fontinalis*, and also in phosphoglucose isomerase in *Katsuwonus pelamis*. However, there is no evidence on their mode of inheritance.

This paper describes heat-resistant and heat-sensitive alleles at the *Gpi-1* locus (glucosephosphate isomerase) in the guppy, *Poecilia reticulata*, and their mode of inheritance.

2. MATERIALS AND METHODS

Animals

Ten guppy strains, S, S3, S3-HR, S3-HS, S2, F, G, M, T, and T1 were used.

A description of the guppy strains was given in an earlier paper (Macaranas and Fujio, 1987). S, S3, and S2 strains are the standard-type guppies, and the S3 strain is a recent isolate of the S strain. F, G, M, T, and T1 are fancy-types, and the T1 strain is an isolate from the T strain. Two new guppy strains, S3-HR and S3-HS, were originated from the S3 strain; the S3-HR was selected for its resistance to high temperatures, 24 h at 35°C and S3-HS was selected for its sensitivity to high temperatures (Kanda et al., 1992). They were maintained in closed colonies in 60 l aquaria at reasonable densities of 300–500 individuals per aquarium, depending on the average size of the strain. The fish were kept at a temperature of 23±2°C. The heat denaturation studies were performed continuously throughout the year with all available fish in a random fashion.

Cross experiments

Reciprocal crossing of the G strain with the S3-HR strain was performed. The gravid females from each parental stock were selected at random and kept in 2.5 l aquaria in order to prepare the virgin female (P) for crossing. Each virgin female mated with a male from parental stock reciprocally in 2.5 l aquaria. After producing the litters, virgin females were stored for the heat denaturation studies. The litters (F_1) produced were separated from the parents and reared in 2.5 l aquaria. After 60 days, when the sex could usually be distinguished, 1–3 pairs from each litter were separated for producing F_2 offspring, and the remainder were stored for the heat denaturation studies. A maximum of 3 litters of guppies were produced from each F_1 pair. Each litter was raised over 60 days and then used for the heat denaturation studies.

Electrophoresis and heat treatment

Starch gel electrophoresis for glucosephosphate isomerase (GPI) was carried out according to Fujio (1984). Each whole body was homogenized in the same quantity of distilled water with its body weight, and homogenates were frozen overnight, thawed and centrifuged to obtain the supernatant. After the electrophoresis, the gel was subjected to the heat treatment in the water bath. One of the two slice of the gel, the other slice was the control gel, was packed with Saran wrap and then heated at 53±1°C for 10 min. The temperature and time of the heat treatment were decided empirically. Finally, the two gels were stained together for an hour. Heat resistant (R) and sensitive (S) phenotypes were determined by comparison with the control gel.

3. RESULTS AND DISCUSSION

Electrophoretic variants

Activity of glucosephosphate isomerase (GPI) was observed in the zymogram, indicating three banded phenotypes in all the guppy samples from the S, S3,

S3-HR, S3-HS, M, T, and T1 strains. The homodimeric isozymes of GPI-1 subunits migrated more rapidly toward the anode than the homodimeric isozyme of GPI-2 subunits, and their heterodimer (hybrid band) was located between them. It indicated that the GPI-1 and GPI-2 subunits were controlled by the *Gpi-1* and *Gpi-2* locus, respectively (Fig. 1). The extensive survey revealed variant patterns in the S2, F, and G strains. The A, B, and C phenotypes exhibited three isozymes for alternative homozygotes at the *Gpi-2* locus and the AB, BC, and AC phenotypes exhibited six isozymes for heterozygotes at *Gpi-2* locus.

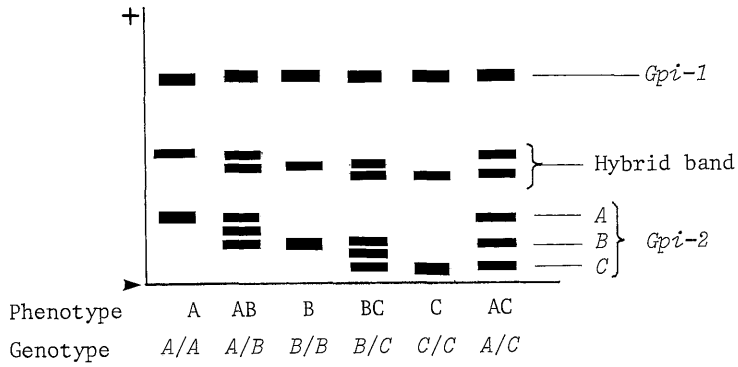

Fig. 1. Zymogram of GPI isozyme in the guppy.

Table 1. Phenotype and allele frequencies at *Gpi-2* locus in 10 strains of the guppy

Strain	No. of tested	Phenotype						Allele frequency		
		A	B	C	AB	AC	BC	q*A*	q*B*	q*C*
S	95	0	95	0	0	0	0	0	1.000	0
S3	65	0	65	0	0	0	0	0	1.000	0
S3-HR	62	0	62	0	0	0	0	0	1.000	0
S3-HS	39	0	39	0	0	0	0	0	1.000	0
S2	97	4	52	2	36	0	3	0.227	0.737	0.036
F	89	2	28	9	12	5	33	0.118	0.567	0.315
G	74	0	34	0	5	0	35	0.034	0.730	0.236
M	79	0	79	0	0	0	0	0	1.000	0
T	108	0	108	0	0	0	0	0	1.000	0
T1	94	0	94	0	0	0	0	0	1.000	0

The observed six phenotypes of dimeric structure are theoretically possible at the *Gpi-2* locus, indicating polymorphism with the three alleles postulated (Fig. 1). The frequencies of occurrence of the phenotypes and alleles for 10 guppy strains were summarized in Table 1.

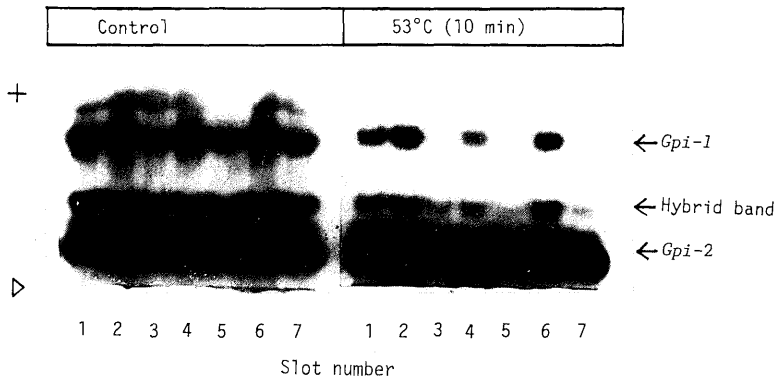

Fig. 2. Zymogram of GPI isozyme.
Heat experiment showed heat-resistant and heat-sensitive types in the GPI-1 isozyme due to *Gpi-1* locus.

Heat stability variants

Fig. 2 shows the zymogram prepared in two separate gels, one of which kept at 4°C and the other heated at 53°C for 10 min after electrophoresis. Activity of the GPI-1 isozyme was observed in slots 1, 2, 4, and 6 but not observed in slots 3, 5, and 7 after heat treatment in comparison with the GPI-2 isozyme. Hybrid band activity was also observed at 53°C for 10 min. The zymogram indicated the heat stability difference among the individual types. Activity of the GPI-1 isozyme and the hybrid band was lost in all the guppy samples after heat treatment at 55°C for 10 min, also that of the GPI-2 isozyme, after heat treatment at 61°C for 10 min. The detection of heat stability variants of GPI was reviewed by Okumura (1985) in aquatic organisms; under the conditions of 28 min at 51°C for abalone in marine molluscs, 36 min at 53°C for skipjap tuna in fish, 14 min at 50°C for Porphyra yezoensis in seaweed, and 10 min at 48°C for *Undaria pinnatifida* in seaweed. It suggests that the heat stability variants are detected by the heat treatment between 48°C and 53°C, and that this heat stability exists in many species.

Table 2 shows the frequencies of heat-resistant (R) and heat-sensitive (S) phenotypes in the GPI-1 isozymes in 10 guppy strains. The frequencies of R and S phenotypes varied among strains. There were no differences between females and males. Seven of 10 strains showed a segregation of R and S phenotypes but three of 10 strains did not. One strain (G) showed only R phenotype and two strains (S and S3-HR) showed only S phenotype. The S3-HR from which the guppies were selected for resistance to high temperatures (Kanda et al., 1992), was heat sensitive in the GPI-1 isozyme, indicating to be contrary to expectations.

Mode of inheritance

The mode of inheritance of the heat stability variants was examined in F_2 progenies, from the crosses of the G strain with the S3-HR strain. The results

Table 2. Heat stability of GPI-1 isozyme among 10 strains of the guppy

Strain	Female			Male		
	No. of fishes tested	Heat stability		No. of fishes tested	Heat stability	
		R	S		R	S
S	41	0	41	42	0	42
S3	31	6	25	30	11	19
S3-HR	40	0	40	53	0	53
S3-HS	19	9	10	24	13	11
S2	33	33	0	39	38	1
F	29	24	5	29	26	3
G	37	37	0	37	37	0
M	38	27	11	38	30	8
T	14	12	2	14	9	5
T1	17	8	9	17	9	8

Table 3. F_2 segregation of heat stability of GPI-1 isozyme in the crosses, G×S3-HR

Strain	No. of crosses	Sex	Heat stability			Suppose the segregation in F_2 progenies is 3:1		
			R	S	Total	χ^2	D.F.	P
G		Female	37	0	37			
		Male	37	0	37			
S3-HR		Female	0	40	40			
		Male	0	53	53			
F_1 (G×S3-HR)	9	Female	29	0	29			
		Male	20	0	20			
F_1 (S3-HR×G)	7	Female	11	0	11			
		Male	11	0	11			
F_2 (G×S3-HR)	15	Female	107	47	154	2.502	1	$0.10<P<0.25$
		Male	83	26	109	0.083	1	$0.75<P<0.80$
F_2 (S3-HR×G)	5	Female	47	13	60	0.356	1	$0.50<P<0.70$
		Male	42	15	57	0.046	1	$0.80<P<0.90$

Number represents the number of fishes whose isozyme was resistant or sensitive to heat treatment.

are shown in Table 3. All F_1 offsprings in the reciprocal crosses were R phenotype. F_2 progenies were classified into R and S phenotypes. The ratio between R and S phenotypes was according to 3:1 ratio. As a result, R phenotype was controlled by a single dominant gene. This factor was not sex-linked like the result obtained from the reciprocal crosses.

Another interesting fact was found in the R phenotypes of F_2 progenies; they clearly classified into two types as shown in Fig. 2. The GPI-1 activities of slots 1

and 4 was reduced relatively to the slots 2 and 6. R/R and R/S genotypes are assumed to be the strong and weak staining bands, respectively, which were designated as R phenotype before. The strong and the weak staining bands in F_2 progenies were densitometrically measured in each gel. The ratio between the strong and the weak staining bands was an average of 1:0.45. It indicates that R/S genotype reduces the activity to half of the R/R genotype. The F_2 progenies were again classified into three types, R/R, R/S, and S/S, on the basis of the above point (Table 4). The ratio was accordingly to 1:2:1.

Table 4. F_2 segregation of heat stability of GPI-1 isozyme in the crosses, G×S3-HR

Strain	Presumed genotype				Suppose the segregation in F_2 progenies is 1:2:1		
	R/R	*R/S*	*S/S*	Total	χ^2	D.F.	P
F_2 (G×S3-HR)	60	130	73	263	1.314	2	$0.50<P<0.70$
F_2 (S3-HR×G)	39	50	28	117	4.477	2	$0.10<P<0.20$

Number represents the number of fishes whose isozyme activity after heat treatment was strong (*R/R*), weak (*R/S*), or none (*S/S*).

The reduction of the GPI-1 activity in R/S genotype might be interpreted by the gene dosage effect. The reduction of the activity in the heterodimer (hybrid band) between the GPI-1 and GPI-2 subunits in the S/S genotype supports the fact that the heterodimer between R and S subunits in the R/S genotype reduces the activity to half of the homodimer of R subunits. Since the band expressed by the R/S genotype is the sum of 1R homodimer, 2RS heterodimer, and 1S homodimer; the activity of the R/S genotype will be half of the R/R genotype. This suggests that the heat stability difference resides at the *Gpi-1* structural locus, and not at a separate heat stability locus.

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