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## **Thermal Selection of Allozyme Polymorphism in the Guppy *Poecilia reticulata***

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### **Abstract**

Two single strains of the guppy (*Poecilia reticulata*, Peters) – the “Swansea” and the “Lowestoft” – were selected for high temperature tolerance, five generations in the Swansea strain and six generations in the Lowestoft strain. The results obtained were assayed with respect to four polymorphic loci, EST-2, GOT-2, PGM and SOD. In the selected lines of both strains, the faster alleles of EST-2, GOT-2 and SOD were increased in frequency but in PGM, the slow allele in the Swansea strain and faster allele in the Lowestoft strain increased in frequency in the selected lines. The results obtained were discussed in the light of the adaptive nature of polymorphic proteins.

## Introduction

Whether polymorphic proteins are adaptive or neutral to the action of selection is a debatable question in population and evolutionary genetics. It appears that both natural selection and random genetic drift are in part responsible as a force in maintaining protein polymorphism (Powell 1975). However, the relative importance of the two has yet to be resolved.

The problem of the adaptive role of variable proteins has been approached from two main experimental levels in terms of the evolutionary distances between the species being compared. On the one hand, the enzymes of widely different organisms, e.g., mammals and ectothermic species have been compared. On the other hand, efforts

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have been made to determine the selective advantage of different allelic enzyme variants (allozymes) in populations of single species exposed to different environmental conditions, e.g., temperature (Powell 1971; Johnson 1974; Nevo et al. 1977; Hoffman 1981; Graves and Somero 1982; Graves et al. 1983), temperature and other ecological variables (Mitton and Koehn 1975), temperature and food media (Koehn 1969) and pollution (Nevo et al. 1978). An ample review of the molecular mechanisms of temperature adaptation in poikilothermic organisms is available from Hochachka and Somero (1968, 1971).

Nevo et al. (1977, 1978) tested the genetic effects of temperature and pollution on acorn barnacles (*Balanus amphitrite*) and suggested that temperature is a strong selective force in allozymic and size variation in barnacles and that specific alleles are presumably favored in polluted environments.

There are two general approaches to study the effect of temperature on poikilotherms, resistance adaptation and capacity adaptation. In addition to these two approaches, the adaptive properties involved with environmentally induced stress can be studied for three different time periods: direct response; compensatory acclimation for days or weeks; and long-term genetic changes evidenced after generations.

In the present study using guppy (*Poecilia reticulata*, Peters) stocks, selection for thermal adaptation was carried out by exposing fish to high temperatures at their incipient lethal level, thereby killing some of them. This falls under the purview of resistance adaptation (Fry 1971) and in the present case, the genetic basis of adaptation was looked at electrophoretically after a few generations of selection.

### Materials and Methods

Both the Swansea and the Lowestoft strains, which exhibit long established color patterns, had been kept under laboratory conditions for approximately 10 years.

Fish were housed in fiberglass 35-l tanks (60 x 25 cm) with an underground filter bed. The culture water was made 10% saline with "Instant Ocean" Sea Salt.

The time taken between each generation of selection was 130 days for both strains and selection took place when the progenies were about 5-6 weeks old. One control population was maintained for each selected line and cultured under similar conditions.

Heat shocks were administered by putting 15 fish, aged 5-6 weeks, in a 250-ml beaker, containing 200 ml water. These beakers were then placed in a thermostatically controlled hot water bath and raised to 44°C for 8 minutes. After exposure, the fish were transferred to a recovery tank for 2-3 hours, after which their percentage survival was calculated. Selection continued for five generations in the Swansea strain and six generations in the Lowestoft strain.

Electrophoretic methods followed were after Shami and Beardmore (1978). Most of the buffers and straining recipes were obtained from Shaw and Prasad (1970) and Harris and Hopkinson (1976).

## Results

The results of selection were assayed electrophoretically, in terms of the effects of four polymorphic loci, at the terminating generation. The loci were EST-2, GOT-2, PGM and SOD. The effects of selection were assessed, in terms of distribution of the frequency of different genotypes and of the alleles in the selected lines in comparison with the same in the controls. It is apparent from Tables 1 and 2 that, in both strains, EST<sub>2</sub><sup>2</sup>, GOT<sub>2</sub><sup>2</sup> and SOD<sup>2</sup> alleles increased in frequency in the selected lines. While with respect to PGM locus, the PGM<sup>3</sup> allele in the Lowestoft strain and PGM<sup>1</sup> allele in the Swansea strain increased in frequency in the selected line. The contingency  $\chi^2$  test performed on the comparison of the allele frequencies between the control and the selected lines for the individual loci produced significant differences ( $P < 0.005$ ) for all the loci (Tables 3 and 4).

The  $\chi^2$  tests for the conformity of the genotypic proportions to Hardy-Weinberg expectation (Tables 5-8) showed that in the selected line of the Lowestoft strain, significant deviations were obtained in the PGM locus ( $P < 0.05$  and  $P < 0.001$ ) due to an absence of  $\frac{1}{4}$  homozygotes (Table 6).

The mean heterozygosity values obtained for the individual polymorphic loci did not show a regular trend of increase or decrease in the control or the selected lines of both the strains. In the Lowestoft strain, H values decreased in EST<sub>2</sub> and GOT<sub>2</sub> in the selected line and increased in SOD and PGM. In the Swansea strain, H values at EST<sub>2</sub>, GOT<sub>2</sub> and SOD decreased and the value at PGM increased. However, the overall H values ( $\bar{H}$ ) decreased in the selected lines of both strains (Tables 9 and 10).

Table 1. Gene and genotype frequency distribution in the control and the selected (6th generation) lines of Lowestoft strain of guppy at four polymorphic loci.

Locus	Sample	Genotype frequency								Allele frequency			No. of fish
		$1/1$	$1/2$	$2/2$	$1/3$	$2/3$	$3/3$	1	2	3			
EST-2	Control	0.2669	0.5622	0.0969	-	-	-	-	0.5870	0.4130			46
	Selected	0.0000	0.4680	0.5319	-	-	-	-	0.2340	0.7660			47
GOT-2	Control	0.3061	0.5714	0.1212	-	-	-	-	0.5918	0.4082			49
	Selected	0.0139	0.3194	0.6667	-	-	-	-	0.1736	0.8264			72
SOD	Control	0.4042	0.3617	0.2340	-	-	-	-	0.5851	0.4149			47
	Selected	0.2000	0.4909	0.3091	-	-	-	-	0.4455	0.5545			55
PGM	Control	0.1214	0.2449	0.0408	0.3265	0.1857	0.0816	0.4082	0.2551	0.3367			49
	Selected	0.0000	0.0429	0.0429	0.2571	0.4286	0.0857	0.1500	0.3500	0.5000			72

Table 2. Gene and genotype frequency distribution in the control and the selected (5th generation) lines of Swansea strain of guppy at four polymorphic loci.

Locus	Sample	Genotype frequency						Allele frequency			No. of fish
		$1/1$	$1/2$	$2/2$	$1/3$	$2/3$	$3/3$	1	2	3	
EST-2	Control	0.2600	0.5200	0.2200	-	-	-	0.5200	0.4800	-	50
	Selected	0.0758	0.3182	0.6061	-	-	-	0.2348	0.7652	-	66
GOT-2	Control	0.1000	0.4400	0.4600	-	-	-	0.3200	0.6800	-	50
	Selected	0.0000	0.0455	0.9545	-	-	-	0.0227	0.9773	-	66
SOD	Control	0.5400	0.3400	0.1200	-	-	-	0.7100	0.2900	-	50
	Selected	0.1060	0.3182	0.5758	-	-	-	0.2652	0.7348	-	66
PGM	Control	0.0000	0.0588	0.9608	0.0000	0.0000	0.0000	0.0294	0.9706	0.0000	51
	Selected	0.1061	0.2273	0.6364	0.0000	0.0303	0.0000	0.2197	0.7652	0.0152	66

Table 3. Results of 2 x 2 contingency chi-square test for the comparison of allele frequencies between the control and selected (6th generation) lines of Lowestoft stock of guppy at four polymorphic loci.

Locus	Sample	Alleles			Total	$\chi^2$ (1)	P
		1	2	3			
EST-2	Control	54 (37.59)	28 (54.41)	-	92	23.9681	<0.001
	Selected	22 (38.41)	72 (55.59)	-			
		76	110		186		
GOT-2	Control	58 (33.61)	40 (64.39)	-	98	45.2698	<0.001
	Selected	25 (49.39)	119 (94.61)	-			
		83	159		242		
SOD	Control	55 (47.92)	39 (46.08)	-	94	3.9573	<0.05
	Selected	49 (56.08)	61 (53.92)	-			
		104	100		204	$\chi^2$ (2)	
PGM	Control	40 (25.11)	25 (30.37)	33 (42.52)	98	20.0174	<0.001
	Selected	22 (36.89)	50 (44.63)	72 (62.48)			
		62	75	105	242		

Table 4. Results of 2 x 2 contingency chi-square tests for the comparison of allele frequencies between the control and selected (5th generation) lines of Swansea stock of guppy at four polymorphic loci.

Locus	Sample	Alleles			Total	$\chi^2$ (1)	P
		1	2	3			
EST-2	Control	52 (35.78)	48 (64.22)	-	100	20.1244	<0.001
	Selected	31 (47.22)	101 (84.78)	-	132		
GOT-2	Control	52 (23.71)	48 (76.29)	-	100	77.7697	<0.001
	Selected	3 (31.29)	129 (100.71)	-	132		
SOD	Control	71 (45.69)	29 (54.31)	-	100	45.3790	<0.001
	Selected	35 (60.31)	97 (71.69)	-	132		
PGM	Control	106	126	-	232	$\chi^2$ (2)	19.6187
	Selected	3 (13.95)	99 (87.18)	0 (0.87)	102		
		29 (18.05)	101 (112.82)	2 (1.13)	234		
		32	200	2			



Table 5. Distribution of genotypes and  $\chi^2$  test for conformity with Hardy-Weinberg proportions for four polymorphic loci in the control line of Lowestoft stock of the guppy.

Locus	Genotypes				$\chi^2$	d.f.	P	Allele frequency			Number of fish
	$1/1$	$1/2$	$2/2$					1	2	3	
EST-2	12 (16.01)	30 (22.25)	4 (7.73)		5.5037	2	>0.05	0.5870	0.4130	-	46
GOT-2	15 (17.06)	28 (23.71)	6 (8.24)		1.6339	2	>0.50	0.5918	0.4082	-	49
SOD	19 (16.86)	17 (22.74)	11 (7.90)		3.0914	2	>0.20	0.5851	0.4149	-	47
PGM	$1/1$ 6 (8.24)	$1/2$ 12 (10.44)	$2/2$ 2 (3.31)	$1/2$ 9 (8.66)	$1/2$ 16 (13.66)	$2/2$ 4 (5.66)		0.4082	0.2551	0.3367	49

Table 6. Distribution of genotypes and  $\chi^2$  test for conformity with Hardy-Weinberg proportions for four polymorphic loci in the selected (6th generation) line of Lowestoft stock of the guppy.

Locus	Genotypes			$\chi^2$	d.f.	P	Allele frequency			Number of fish
	$1/1$	$1/2$	$2/2$				1	2	3	
EST-2	0 (2.49)	22 (16.65)	25 (27.87)	4.5046	2	>0.10	0.2340	0.7660	-	47
GOT-2	1 (2.08)	23 (20.32)	48 (49.60)	0.9658	2	>0.50	0.1736	0.8264	-	72
SOD	11 (11.14)	27 (27.23)	17 (16.64)	0.0115	2	>0.99	0.4455	0.5545	-	55
PGM	$1/1$	$1/2$	$2/2$	$1/3$	$2/3$	$2/3$				
	0 (1.62)	3 (7.56)	3 (8.82)	41 (25.50)	19 (10.80)	6 (18.00)	0.1500	0.3500	0.5000	72
				31.8584	5	<0.001				

Table 7. Distribution of genotypes and  $\chi^2$  test for conformity with Hardy-Weinberg proportions for four polymorphic loci in the control line of Swansea stock of guppy.

Locus	Genotypes			$\chi^2$	d.f.	P	Allele frequency			Number of fish
	$1/1$	$1/2$	$2/2$				.1	.2	.3	
EST-2	13 (13.52)	26 (24.96)	11 (11.52)	0.0868	2	>0.95	0.5200	0.4800	-	50
GOT-2	5 (5.12)	22 (21.76)	23 (23.12)	0.0061	2	>0.99	0.3200	0.6800	-	50
SOD	27 (25.21)	17 (20.59)	6 (4.21)	1.5141	2	>0.20	0.7100	0.2900	-	50
PGM	0 (0.05)	49 (47.99)	0 (2.97)	0.0716	2	>0.95	0.0300	0.9700	0.0000	51

Table 8. Distribution of genotypes and  $\chi^2$  test for conformity with Hardy-Weinberg proportions for four polymorphic loci in the selected (5th generation) line of Swansea stock of guppy.

Locus	Genotypes			$\chi^2$	d.f.	P	Allele frequency			Number of fish
	$1/1$	$1/2$	$2/2$				1	2	3	
EST-2	5 (3.49)	21 (23.38)	40 (39.13)	0.9149	2	>0.50	0.2348	0.7652	-	66
GOT-2	0 (0.05)	3 (3.84)	63 (62.10)	0.2468	2	>0.80	0.0227	0.9773	-	66
SOD	7 (4.81)	21 (26.02)	38 (35.71)	2.1933	2	>0.10	0.2652	0.7348	-	66
PGM	$1/1$ 7 (3.19)	$2/2$ 42 (39.13)	$1/3$ 0 (0.0066)	$2/3$ 15 (22.36)	5	>0.10	0.2197	0.7652	0.0152	66

Table 9. Heterozygosity (H) at individual polymorphic loci and overall (18 loci) ( $\bar{H}$ ) in the control and selected (6th generations) lines of Lowestoft stock of guppy.

Loci	Control H	Selected H
EST-2	0.65	0.47
GOT-2	0.57	0.32
SOD	0.36	0.49
PGM	0.76	0.88
$\bar{H}$	0.13	0.12

Table 10. Heterozygosity (H) at individual polymorphic loci and overall (18 loci) ( $\bar{H}$ ) in the control and selected (5th generations) lines of Swansea stock of guppy.

Loci	Control H	Selected H
EST-2	0.52	0.32
GOT-2	0.44	0.05
SOD	0.34	0.32
PGM	0.06	0.26
$\bar{H}$	0.08	0.05

Table 11. Tests of conformity to Hardy-Weinberg expectations for classes of multiple heterozygotes at four variable loci of control and selected (6th generation) lines of Lowestoft stock of guppy. (Expected values in the brackets).

Sample	Heterozygosity class					$\chi^2$ (4)	P
	0	1	2	3	4		
Control	2 (1.13)	6 (9.21)	21 (19.12)	18 (16.82)	2 (4.97)	3.84	>0.20
Selected	5 (1.72)	26 (15.51)	27 (29.74)	13 (20.96)	1 (4.81)	19.64	<0.001

Table 12. Tests of conformity to Hardy-Weinberg expectations for classes of multiple heterozygotes at four variable loci of control and selected (5th generation) lines of Swansea stock of guppy. (Expected values in the brackets).

Sample	Heterozygosity class					$\chi^2$ (4)	P
	0	1	2	3	4		
Control	8 (8.50)	19 (20.55)	22 (16.72)	2 (4.71)	0 (0.24)	3.6129	>0.30
Selected	24 (21.45)	24 (28.86)	16 (13.31)	2 (2.29)	0 (0.09)	1.7920	>0.70

Table 13. Results of 2x5 contingency chi-square tests on the distribution of the heterozygosity class in the control and the selected (6th generation) lines of Lowestoft stock of guppy for 4 loci.

Sample	Heterozygosity class					Total	$\chi^2(4)$	P
	0	1	2	3	4			
Control	2 (2.83)	6 (12.96)	21 (19.44)	18 (12.55)	2 (1.21)	49	11.74	<0.05
Selected	5 (4.17)	26 (19.04)	27 (28.56)	13 (18.45)	1 (1.79)	72		
	7	32	48	37	3	121		

Table 14. Results of 2 x 4 contingency chi-square tests on the distribution of the heterozygosity class in the control and the selected (5th generation) lines of Swansea stock of guppy for 4 loci.

Sample	Heterozygosity class				Total	$\chi^2(3)$	P
	0	1	2	3			
Control	8 (13.95)	19 (18.74)	22 (16.56)	2 (1.74)	51	5.2292	>0.20
Selected	24 (18.05)	24 (24.26)	16 (21.44)	2 (2.26)	66		
	32	43	38	4	117		

The distribution of the multiple heterozygote classes in the selected lines when tested for conformity to Hardy-Weinberg expectation showed a significant deviation in the selected line of the Lowestoft strain (Table 11:  $\chi^2(4) = 19.64$ ;  $P < 0.001$ ). In the Swansea strain the distribution accorded with all  $\chi^2$  values nonsignificant (Table 12). A heterogeneity  $\chi^2$  test on the distribution of the multiple heterozygosity classes between the control and the selected lines showed a significant difference in the Lowestoft strain (Table 13:  $\chi^2/(4) = 11.74$ ;  $P < 0.05$ ). In the Swansea strain, however, a similar test did not show any significant difference (Table 14).

## Discussion

The idea that genetic variations are related to environmental variation has roots going back to Dobzhansky and other proponents of the balance view of natural selection. This view of natural selection was theorized by Levins (1968) and was proven by many workers in the field

of population biology and evolution (Koehn 1969; Powell 1971; Mitton and Koehn 1975; Nevo et al. 1977).

The results obtained in the present study, assayed with respect to four polymorphic loci involving the same alleles in both strains, show that the allele frequencies of the selected lines differ significantly from those of the control lines at all loci. In both strains, the faster alleles of EST<sub>2</sub>, GOT<sub>2</sub> and SOD were increased significantly in the selected lines over the control; in the case of the PGM locus, the faster allele is favored in the Lowestoft strain, but in the Swansea strain, the slower allele is increased significantly over the control. The mean heterozygosities in both strains were reduced marginally.

It is apparent from the results that there may be a relation between the tolerance of high thermal stress and genetic variation at the loci mentioned, although the possibility exists of 'hitchhiking' effects of neutral genes which cannot be unmasked without having ideas on the effects of various disciplines including genetics, physiology, biochemistry and ecology of the loci concerned.

Depending upon the role that an enzyme performs in the metabolic systems, Johnson (1974) classified enzymes as regulatory, nonregulatory and variable substrate enzymes. The regulatory enzymes are the most sensitive in the site of action of selection; the variable-substrate enzyme may affect metabolic rates only. According to Somero (1969) the evolutionary changes in enzyme-substrate affinity are directed primarily towards maintenance of controlling functions. Thus, the regulatory enzymes would be expected to select mainly at higher temperatures to increase thermal tolerance. It is notable with the guppy strains, except for the GOT enzyme, that the enzymes showing increased frequencies in the selected lines over the controls are either regulatory or variable substrate enzymes, which might give some clues on the occurrence of selection for adaptation to temperature.

Thermal selection of allozyme variation has been well documented by Nevo et al. (1977) with acorn barnacles (*Balanus amphitrite*). A comparative test for adaptation to cool and warmer canals of an electric plant cooling system in barnacles produced significant differences in frequencies in seven out of eight loci in one year; the alleles selected were either the medium or faster in each case, and together with the evidence that the average size and number of individuals decreased significantly in the warmer canal, they provided evidence of a strong directional selection in this crustacean. With the guppy, temperature selection revealed an apparent correlation between survival and average

size of the fish selected, in that the average size decreased in successive generations as the percentage survival increased (Shah and Beardmore 1986). If, in the present study, the trend for increase in frequencies of the faster alleles is considered in the light of the general decrease of the average length of the fish in the selected lines, it might be indicative of a presumed directional selection as well.

Adaptation of polymorphic proteins to thermal characteristics is also supported by the study of Mitton and Koehn (1975) with killifish *Fundulus heteroclitus*. Fishes living in a heated electric plant cooling pond were found to have a variability pattern different from those living in normal coastal habitats and resembled the variation encountered in populations from warmer latitudes. They also found significant differences in allele frequencies in 10 out of 12 enzyme loci with respect to environments, sexes and/or age classes.

Nevo et al. (1970) obtained the relative fitnesses of thermally favored alleles in the barnacles by comparing the number in the genotypes selected for in the warmer *vs.* cooler canal. However, as fitness is a function of a single generation being compounded in every successive generation and as the assessment on the distribution of the genotypes and alleles have not been done in each generation, a similar test for fitness of the thermally favored alleles could not have been done in the present study.

The mean heterozygosities ( $\bar{H}$ ) decreased marginally in both strains. These reductions may be explained in the light of the Levins theory of fitnesses (Levins 1968) that relates heterozygosity as an adaptation to environmental heterogeneity and uncertainty; the less divergence and uncertainty in the environment, the lower the heterozygosity. In the present situation, in selecting fishes for thermal resistance, the method adopted is likely to subject them to a homogeneous and predictable environment in every generation, thus providing selection for a more homogeneous strategy. Although involving a noncommercial species, the present concept of the genetic effects of temperature could be useful in planning experiments on thermal selection in commercial species where high thermal characteristics could prove to be an important and economic character.

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