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Genetics

Adaptive significance of differences in the tissue-specific expression of a phosphoglucomutase gene in rainbow trout

(polymorphism/developmental rate/glycolysis/*Salmo gairdneri*)

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ABSTRACT **We have investigated the phenotypic effects of a mutant allele that results in the expression of a phosphoglucomutase locus (*Pgm1*) in the liver of rainbow trout. Embryos with liver *Pgm1* expression hatch earlier than embryos without liver *Pgm1* expression. These differences apparently result from increased flux through glycolysis in embryos with liver PGM1 activity while they are dependent on the yolk for energy. Fish with liver PGM1 activity are also more developmentally buffered, as indicated by less fluctuating asymmetry of five bilateral meristic traits. The more rapidly developing individuals begin exogenous feeding earlier and achieve a size advantage that is maintained until sexual maturity. This size advantage produces a significant tendency for earlier age of sexual maturity. These results show that different genotypes at this regulatory gene result in important phenotypic differences that are likely to be of important adaptive significance.**

The potential importance of gene regulation in adaptive evolutionary change has been recognized by evolutionary biologists in a wide variety of disciplines. Experiments with prokaryotes have shown that adaptation to new environments often involves changes in regulatory genes (1, 2). A number of authors have suggested that changes at regulatory genes are also important in eukaryotic evolution (3–5). The evidence for this view has remained largely indirect. Differences between the rate of change at structural loci encoding proteins and the rate of change in phenotypes having adaptive importance suggests that significant evolutionary changes may be due to changes in gene regulation (4). Further indirect evidence of the importance of gene regulation has come from paleontology (6, 7) and developmental genetics (8, 9).

The eventual acceptance of these views awaits direct evidence of the amount and adaptive significance of variation at regulatory genes in eukaryotes. Evidence is accumulating that regulatory elements affecting the expression of the structural locus (*Adh*) encoding alcohol dehydrogenase (ADH) in *Drosophila melanogaster* play an important role in adaptive evolution. Regulatory elements have been identified on the third chromosome that modify the amounts of ADH produced by the *Adh* locus on the second chromosome (10, 11). The amount of ADH has been found to play an important role in the adaptation of *D. melanogaster* to high concentrations of environmental alcohol (12, 13).

We have initiated a search for intraspecific variation in the tissue-specific expression of enzyme loci in the rainbow trout (*Salmo gairdneri*) that is directed at ascertaining the amounts and adaptive significance of variation in gene regulation. We have identified a mutant allele, *Pgm1-t(b)*, that results in a >100-fold increase in the expression of a phosphoglucomutase (PGM; α-D-glucose-1,6-bisphosphate:α-D-glucose-1-phosphate phosphotransferase EC 2.7.5.1) locus, *Pgm1*, in liver tissue (14, 15). The results of inheritance experiments are consistent with a single regulatory gene, *Pgm1-t*, with additive inheritance being responsible for the differences in the expression of this locus (15).

We report here that the presence or absence of PGM1 in the liver gives rise to important differences in several phenotypic characteristics of adaptive significance (developmental rate, developmental stability, body size, and age at first maturity). These differences are apparently related to the action of PGM affecting the flux through glycolysis during embryonic development.

MATERIALS AND METHODS

Electrophoresis. The presence or absence of liver PGM1 activity was determined by horizontal starch gel electrophoresis in an aminopropylmorpholine/citric acid buffer, pH 6.6 (16).

Nomenclature. The nomenclature is adapted from Allendorf and Utter (17) and Paigen (18). Multiple structural loci encoding enzymes in salmonids are identified by a sequential numerical designation beginning with the least anodal form. The structural locus encoding the PGM1 enzyme is designated *Pgm1*. The genetic site responsible for differences in tissue expression of *Pgm1* is designated a temporal regulatory locus, *Pgm1-t*. The common allele (*a*) at this locus is associated with no detectable PGM1 in the liver, in contrast with the large amount of activity associated with the alternative allele (*b*).

Culture Methods. Eggs and sperm were collected from mature fish and fertilized after electrophoretic typing of the parents. The fish were raised at 9 ± 1°C in temperature-controlled aquaria or at the Jocko River State Trout Hatchery of the Montana Department of Fish, Wildlife, and Parks. The fish used in these experiments belong to the Arlee strain of rainbow trout. This strain has been maintained with a large effective population size (approximately 500) in isolation at this hatchery for some 12 generations.

RESULTS

Body Size. We began our search for phenotypic or "organismal" effects of the *Pgm1-t* polymorphism by comparing the lengths of full sibs with and without liver PGM1. These two groups were raised in the same environment and differ genetically only at *Pgm1-t* or tightly linked loci. We suspected that the presence of PGM1 in the liver might increase the efficiency of use of carbohydrates in the diet, resulting in increased growth rate.

We took a chronological series of samples from 14 families segregating at *Pgm1-t* to detect any size differences. The mean

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Abbreviations: ADH, alcohol dehydrogenase; PGM, phosphoglucomutase.

length of fish with liver PGM1 activity was significantly greater than that of their full sibs lacking activity in most families. The mean lengths in these families, sampled on or near 120 days after fertilization, are given in Table 1. In 12 of these families, the fish with liver PGM1 activity were longer than those without; this difference is statistically significant in 9 families. On the average, fish with liver PGM1 activity were 3.6% longer. There were no differences in the length/weight ratio between fish having or not having liver PGM1. We routinely used lengths rather than weights because weights are more variable due to differences in the time since the last feeding and other reasons. The 3.6% difference in lengths represents an average difference of approximately 11% in weights.

Surprisingly, we could not detect any differences in growth rate between *Pgm1-t* types. Fish at the earliest age that we could remove livers and score *Pgm1-t* phenotypes showed approximately the same size differential as older fish. *Pgm1-t(b)* fish apparently achieved a greater size early in development and maintained that advantage. The oldest fish showing this constant size difference was a sample from family G4 collected at 17 months of age, when most of the males were sexually mature. None of the females were sexually mature at this age and those females with liver activity still maintained their size advantage.

Developmental Rate. We considered the most likely explanation for the size differences between *Pgm1-t* genotypes to be a difference in embryonic developmental rates. At 9°C, liver organogenesis in rainbow trout begins approximately 15 days after fertilization (19). The eggs hatched in ≈33 days and most of the yolk mass was still present at that time. The yolk was not completely absorbed until ≈60 days after hatching. The larvae began exogenous feeding 7–10 days before complete absorption of the yolk. If *Pgm1-t(b)* fish did develop more rapidly, they could begin exogenous feeding earlier and gain an early size advantage.

At the stage in which we suspected differences in developmental rate, the fish are too small to remove livers and score *Pgm1-t* phenotypes. We circumvented this problem by taking advantage of the one developmental stage at which the embryos can be objectively classified into slow or fast developers: hatching. Three families were created by crossing males heterozygous (*a/b*) at *Pgm1-t* having liver PGM1 activity with females (*a/a*) that did not. These families were expected to segregate 1:1 for liver PGM1 activity. We removed the first 50% of each family to hatch and placed them in separate tanks. These six groups of fish were then raised until the time of complete yolk absorption when they were sampled and scored for liver PGM1.

The results of these experiments support our hypothesis that fish having liver PGM1 develop at a faster rate (Table 2). The fish with liver PGM1 activity hatched significantly earlier in all three families. Overall, ≈78% of the early-hatching fish had liver PGM1, in comparison with 30% of the late-hatching fish. We conclude that the presence of PGM1 activity in the liver of embryos results in a more rapid developmental rate in the period between liver organogenesis (15 days) and hatching (33 days), and perhaps longer.

Developmental Stability. Because of the differences in developmental rates detected between fish with and without liver PGM1 activity, we decided to search for differences in developmental stability between these types as estimated by the asymmetry of bilateral morphological traits. It has been proposed that fluctuating asymmetry reflects accidents during development and that developmentally superior individuals will be more buffered and, therefore, will be less asymmetrical (20–23).

We used counts of five bilateral meristic traits to measure developmental stability: gill rakers on the upper portion of the first gill arch, gill rakers on the lower portion of the first gill arch, mandibular pores, rays in the pectoral fin, and rays in the pelvic fin. These counts were carried out on 50 individuals chosen from the Arlee stock to be used as parents in our inheritance studies in December 1981. Thirty-six of these individuals did not have liver PGM1 activity. Fish with liver PGM1 activity were asymmetrical at a significantly smaller mean number of traits than fish lacking liver PGM1 (1.36 and 1.97; $t = 2.05$, $P < 0.02$). Thus, fish with liver PGM1 activity are apparently more developmentally buffered.

Age at First Reproduction. Rainbow trout become sexually mature once a year—in the fall, winter, or spring. Many studies with salmonids have shown that larger individuals tend to mature at an earlier age (24–26). There appears to be a critical period during which each individual makes a "decision," based on size, whether to mature that year or not (26). Therefore, we would expect the larger *Pgm1-t(b)* fish to mature at an earlier age. We were able to test this prediction with the males in family G4. Two-thirds of the males in this family were mature when sampled 17 months after fertilization (Table 3). All of the *Pgm1-t(a/b)* males were mature, in comparison with only 46% of the *Pgm1-t(a/a)* males ($\chi^2 = 6.24$; d.f. = 1; $P < 0.01$).

Distribution of *Pgm1-t(b)*. The *Pgm1-t(b)* allele is apparently a recent mutation. None of the species closely related to rainbow trout that we have examined show expression of *Pgm1* in

Table 1. Mean lengths of sibs with [t(−/b)] and without [t(a/a)] PGM1 activity in the liver at ≈4 months of age

Family	Age, days	Mean length, mm t(a/a)	Mean length, mm t(−/b)	Relative length [t(−/b)]	*P*
G1	120	41.31	44.42	1.075	0.013
G2	120	40.28	42.37	1.052	0.002
G4	109	44.71	46.24	1.034	0.008
G7	120	43.64	44.68	1.024	NS
G10	120	46.42	47.95	1.033	0.018
G11	120	39.64	39.73	1.002	NS
H1	124	45.15	49.70	1.101	0.002
H2	114	43.23	44.56	1.031	0.008
H3	124	49.00	49.00	1.000	NS
H4	124	51.83	51.10	0.986	NS
H6	114	47.29	49.18	1.040	0.023
H8	124	49.09	51.90	1.057	0.027
H15	104	42.04	43.37	1.034	NS
H16	124	46.94	48.70	1.037	0.028

P was determined by *t* test. NS, not significant.

Table 2. Frequencies of *Pgm1-t(a/a)* and *Pgm1-t(a/b)* genotypes in early- and late-hatching groups

Family	Hatching group	*Pgm1-t* genotype (a/a)	*Pgm1-t* genotype (a/b)	Proportion [*Pgm1-t(a/b)*]	χ^2 (1 d.f.)*
I16	Early	31	81	0.723	
	Late	54	26	0.325	29.99
	Total	85	107	0.557	
I17	Early	17	63	0.788	
	Late	49	30	0.380	27.22
	Total	66	93	0.585	
I18	Early	13	67	0.838	
	Late	64	16	0.200	65.12
	Total	77	83	0.519	

* 2×2 contingency.

Table 3. Proportion of males from family G4 with (*a/b*) and without (*a/a*) liver PGM1 activity that matured at 17 months of age

Pgm1-t genotype	Immature	Mature	Total
a/a	6 (360.8)	7 (299.4)	13 (327.7)
a/b	0	10 (320.0)	10 (320.0)

Numbers in parentheses represent mean length in mm.

liver tissue (15). This allele is also rare in rainbow trout. We have found it in only 4 out of more than 30 rainbow trout hatchery strains that we have examined (15). We have also never seen the *Pgm1-t(b)* allele in any natural population of rainbow trout sampled from Washington, Oregon, or Montana. However, we have recently detected this allele in three out of four natural populations of rainbow trout sampled from the extreme southwestern corner of Idaho.

DISCUSSION

Biochemical Basis of Action. Glycolysis using glycogen as the source of glucose is an important source of energy in fish embryos (27). Terner (28) has shown that rainbow trout embryos generate energy through glycolysis by metabolizing glycogen to lactate. Glycogen is synthesized during development of the trout embryo and is present exclusively in the liver of the embryo (29). The D-glucose units of glycogen enter the glycolytic sequence through the action of glycogen phosphorylase (α-1,4-phosphorylases), which converts glycogen to glucose 1-phosphate (30). The next enzyme in this pathway is PGM, which converts glucose 1-phosphate to glucose 6-phosphate. The presence of PGM1 in the liver apparently affects the flux through the glycolysis pathway, resulting in an accelerated rate of development. It is of interest that Leigh Brown (31) has reported differences in field mice (*Apodemus sylvaticus*) between PGM allozymes in their ability to mobilize liver glycogen.

Glycolysis should be of greater metabolic importance at higher temperatures, when there is less oxygen available and anaerobic metabolism becomes more important (30). The difference in developmental rates between fish with and without liver PGM1 is therefore expected to increase with temperature. Our data are in agreement with this prediction. Families I16, I17, and I18 were all held in the same tank, which was separated into three compartments. The families were placed into the tank in numerical order, with the lowest numbered family being closest to the head of the tank and the refrigeration unit. Temperatures were slightly warmer in each compartment (<0.5°C). As predicted, the proportion of late hatchers with liver PGM1 activity was least in the warmest compartments (Table 2); a contingency χ^2 of these differences is significant ($\chi^2 = 6.42$; d.f. $= 2$; $P < 0.05$).

In a previous quantitative genetic analysis of hatching time in rainbow trout (32), a higher heritability was found for hatching time at a higher temperature (0.00 at 6°C versus 0.23 at 11°C). This result suggests that genetically based biochemical differences may result in metabolic differences that produce greater differences in developmental rate at higher temperatures. Presumably, at higher temperatures, the presence of certain substrates or oxygen may be more rate limiting.

Phenotypic Effects. At 9°C, rainbow trout larvae become active swimmers and begin exogenous feeding approximately 50 days after fertilization. Fish with liver PGM1 activity may continue to develop more rapidly than their full sibs lacking liver PGM1. The more rapidly developing fish having liver PGM1 activity would begin feeding earlier. Studies with the closely related sockeye salmon (*Oncorhynchus nerka*) have shown that a size advantage gained by earlier exogenous feeding is maintained throughout the life of the fish (33). The more rapid development of the *Pgm1-t(b)* fish apparently results in a difference in length of the young fish that is maintained until sexual maturity.

Several authors have suggested that small differences in the timing of development could cause large differences in adult morphology and life history (34, 35). Our results support these ideas. The more rapid development of *Pgm1-t(b)* fish during this short period of development results in increased body size and earlier sexual maturity. Previous developmental studies with fish have also shown that differences in developmental rate usually produce differences in counts of meristic characteristics (e.g., number of vertebrae) (36–38). Longer developmental periods generally result in higher counts of meristic structures. We thus predict that *Pgm1-t(b)* fish should have different meristic counts for those structures whose sensitive period occurs during the interval in which *Pgm1-t(b)* fish develop more rapidly.

Ayala and McDonald (39) have suggested that the continuous variation observed in metric traits and in fitness may be due to one or very few regulatory genes. Our results are in agreement with this view in that a single regulatory gene (*Pgm1-t*) is responsible for a large proportion of the variation in these rainbow trout for at least three important traits: developmental rate, size, and time of sexual maturity. Other studies have also found that variation in continuous traits is not necessarily determined by many genes, each with a small effect. Wehrhahn and Allard (40) have reported results similar to ours in this regard; 80% of the variation between two lines of wheat (*Triticum aestivum*) in heading time was controlled by a single locus.

Adaptive Significance. In recent studies with killifish (*Fundulus heteroclitus*), DiMichele and Powers (41) have found results similar to ours; they report a significant correlation between different lactate dehydrogenase allozymes and hatching times. One class of homozygotes had a mean hatching time of 11.9 days in comparison with a mean of 12.8 for the other homozygous class; heterozygotes were intermediate (12.4 days). These authors suggest that this developmental polymorphism may have important adaptive significance in killifish.

Costantino (42) has emphasized the potential adaptive significance of differences in developmental rate. The traits found to be affected by *Pgm1-t* have obvious potential effects on fitness in natural populations of rainbow trout. Differences in developmental time will produce differences in the time of emergence from the gravel of the young fry. The timing of emergence has been found to have an important effect on the survival and success of young salmonids (43). If fry emerging at different times are equally successful, these differences in developmental rate will still produce size differences, as found in the hatchery fish. The larger size of the early-emerging fish may have an important effect on relative survival. Predation on salmonids is generally size specific (44). Size differences are also expected to produce differences in age of sexual maturity.

The relative success of fish with and without liver PGM1 activity will depend on environmental conditions. Selection for later emergence or later sexual maturity would favor the absence of liver PGM1 and vice versa. However, it is difficult to imagine conditions under which the phenotypic differences between these types would not affect fitness. Under hatchery conditions, we would expect the larger and earlier maturing fish with liver PGM1 to have an advantage. The frequency of the *Pgm1-t(b)* allele is apparently increasing in the Arlee hatchery strain (45); its frequency increased from 0.030 to 0.074 in one generation ($\chi^2 = 4.96$, $P < 0.05$).

We cannot be certain whether the phenotypic effects associated with the *Pgm1-t* locus are caused by that locus or by a

closely linked locus in linkage disequilibrium with *Pgm1-t*. Such linkage disequilibrium could produce a significant difference between *Pgm1-t* types in this population. However, comparing differences between full sibs provides a test of this possibility. Unless there was complete linkage disequilibrium, we would expect to see the same effects sometimes associated with the *Pgm1-t(a)* allele. However, we have never found a family in which the *Pgm1-t(a/a)* fish were significantly larger in a total of 23 families tested (Table 1 and unpublished data). Thus, the phenotypic effects are due to either the *Pgm1-t* locus or a tightly linked locus that is in nearly complete linkage disequilibrium with this locus. We consider the latter possibility unlikely, but we cannot exclude it. Further elaboration of the mechanism producing these phenotypic effects would further support the hypothesis that these differences are caused by *Pgm1-t*.

These phenotypic effects of *Pgm1-t* are in contrast to the general lack of success in finding phenotypic differences between individuals having different structural alleles at enzyme loci. A great deal of genetic variation exists at structural gene loci. However, it has been somewhat difficult to find evidence for the adaptive significance of allelic variants at individual loci. It has proven more difficult to identify regulatory genes in eukaryotes. Nevertheless, efforts to identify important phenotypic differences between alternative genetic forms at regulatory genes will allow further testing of the hypothesis that regulatory genes play an important role in adaptation.

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