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Genetic Recombination Between The Regulatory And The Structural Elements Of A Gene Coding For The Enzyme Phosphoglucomutase In Rainbow Trout (Salmo gairdneri)

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GENETIC RECOMBINATION BETWEEN THE REGULATORY AND THE STRUCTURAL ELEMENTS OF A GENE CODING FOR THE ENZYME PHOSPHOGLUCOMUTASE IN RAINBOW TROUT (Salmo gairdneri)

Submitted in Partial Fulfillment of the Requirements for Graduation with Honors to the Department of Biology at Carroll College, Helena, Montana

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March 30, 1987
This thesis for honors recognition has been approved for the Department of Biology by:

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March 30, 1987
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"Come, my lad, and drink some beer."

-Samuel Johnson
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I would like to express my deepest appreciation and thanks for the understanding, support, and encouragement that my parents, Ron and Mary Mueller, have provided throughout my education. I extend thanks to my advisor, Dr. Addis, and my readers, Mr. Bugni and Dr. Wood. I also wish to thank Dr. Wood for his help with the statistical analysis used in this thesis.

I am also greatly indebted to Mr. and Mrs. Jerry Lubbers for providing me with room and board during the time this research was conducted. Lastly I wish to thank Angela for putting up with me during both the research for, and the writing of, this thesis.
Rainbow trout doubly heterozygous for both the structural and the regulatory genes (100/90; a/b) of phosphoglucomutase (PGM) were crossed with doubly homozygous individuals (100/100; a/a) to produce the progeny used in the genetic testing. Allelic variants of both the regulatory and the structural elements (represented by allelic isozymes) were separated and identified using starch gel enzyme electrophoresis. The number of recombinant genotypes was then used as an estimate of the frequency of recombination between the structural and the regulatory elements of the pgm gene. Results show that such recombination is a rare event, supporting previous indications that the regulatory gene is cis acting and is also tightly linked to the structural gene.
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INTRODUCTION AND LITERATURE REVIEW

Detection of Genetic Variation by Electrophoresis

The amino acids that comprise a protein possess particular charges dependent upon the pH. As a result, the protein molecule itself usually has a net charge based on the arrangement of the amino acids in its structure. In an electric field this charge on the protein molecule causes it to migrate toward the oppositely charged terminal. The greater the charge on a molecule, the faster its migration will be. This separation of protein molecules in an electric field is called electrophoresis.

Particular proteins can thus be visualized as bands on the gel. These proteins are either the products of single genes or a number of interacting genes. A mutation of a gene may result in a protein product that is structurally different from the common form of the protein. Thus, these two forms of a particular protein possess electrophoretic variation and are represented by bands of differing mobility.

The size and shape of the molecule as well as its charge affects the rate of migration in a starch gel. The matrix nature of the starch
maintains the position of the protein molecule after electrophoresis is terminated. If the protein products of two alleles at a particular locus have different charges or significantly different shapes, their rate of migration will be different and they will appear as separate bands on the gel when it is stained (May 1975). Therefore, allelic variation and thusly allelic isozymes can be detected electrophoretically.

It is possible to visualize the location of a particular enzyme on the starch gel by supplying the appropriate cofactors and substrates and by involving a product of the enzymatic reaction in a color producing reaction. The inheritance of genetic variation can thus be easily documented by specific matings and subsequent electrophoretic analysis of the progeny.

**Genetic Control of Phosphoglucomutase**

Regulation of gene expression results in a series of molecular events that effect the time of appearance and the tissue specific amounts of functional enzymes (Davidson and Britten 1979). The control of gene expression appears to be exerted principally through the initiation of transcription (Edlund et al. 1985). Differences in the regulation of genes
are an important basis for evolutionary changes in morphology and metabolism. It has been proposed that these differences may result in large organismal effects by altering the rate and timing of developmental events (Britten and Davidson 1969). Ayala and McDonald (1981) have suggested that changes in the regulation of enzyme loci may be of more evolutionary importance than structural differences in the enzymes coded by these loci. Research on prokaryotes suggests that adaptation to new environments often involves changes in the regulation of structural loci rather than changes in the enzymes themselves (Lin et al. 1976). Some of these regulatory genes control only the structural gene that they are linked to, that is they exhibit "cis" regulation. Other regulatory genes appear to produce diffusible effector molecules that result in "trans" regulation. Cis-acting regulatory elements have been discovered in several eukaryotes (Mcknight and Kingsbury 1982).

Allendorf et al. (1982) have reported a gene that regulates the tissue expression of a phosphoglucomutase (PGM; EC 2.7.5.1) locus in rainbow trout. The gene regulates the tissue-specific expression at a single structural locus, pgm1. Most rainbow trout have no detectable activity of PGM1 in the liver. These fish have been shown to be
homozygous at the regulatory locus, pgm1-t(a/a). Individuals with one
copy of the variant allele, pgm1-t(a/b), show a greater than 100-fold
increase in the amount of PGM1 enzyme in the liver (Allendorf et al. 1982).
Homozygotes for the variant allele, pgm1-t(b/b), have approximately
twice as much PGM1 activity in liver tissue as heterozygotes. The
observed intermediate expression in heterozygotes is consistent with
cis-regulation resulting in two independently regulated structural genes.
There are no apparent differences between these types in the expression
of PGM1 in other tissues (skeletal muscle, eye, heart, brain, stomach, and
pyloric cecum).

The presence of PGM1 activity in the liver is inherited as a simple
Mendelian dominant trait. The results of inheritance experiments are
consistent with a single regulatory gene, pgm1-t, with additive
inheritance being responsible for the large differences in expression of
the pgm1 locus in liver tissue (Allendorf et al. 1982).

The pgm1-t(b) allele is apparently a recent mutation. Other
species examined that are closely related to rainbow trout show no
expression of pgm1 in liver tissue. Ohno states that fish of the family
Salmonidae underwent a tetraploid event an estimated 100 million years
ago (cited in Allendorf et al. 1984). Multilocus isozyme loci in the salmonids show considerable regulatory divergence between the duplicated loci produced by polyploidy (Ferris and Whitt 1979). In addition, the pgm1-t(b) allele is rare even among rainbow trout. It has been found in only four out of thirty strains of rainbow trout that have been screened. The phenotype showing expression of pgm1 in the liver was present in 57 out of 602 fish examined from the Arlee strain of rainbow trout. The frequency of the variant allele in this hatchery stock is estimated to be .049. The expected frequency of the heterozygous (a/b) and homozygous (b/b) genotypes are .094 and .002, respectively (Allendorf et al. 1983a). Thus only one out of every 39 fish possessing PGM activity in the liver is expected to be homozygous (b/b) for the variant allele.

Phenotypic Effects of pgm1-t

The use of glycogen during glycolysis is an important source of energy for all aerobic organisms. Blaxter has found that glycogen is synthesized during the development of the trout embryo and is present exclusively in the liver (cited in Allendorf 1985). Glycogen enters the glycolytic sequence after being converted to glucose-1-phosphate (G1P) by
Figure 1—Metabolic pathways connected to the reaction catalyzed by PGM.
glycogen phosphorylase. Phosphoglucomutase then catalyses the reversible reaction between G1P and G6P (Figure 1). PGM thus possesses a dual role functioning in the catabolic pathway of glycogen degradation and the anabolic pathway of glycogen synthesis. PGM expression in the liver affects the flux through the pathways of glycogen metabolism. This is the biochemical basis for the phenotypic effects of PGM expression. Fish with liver PGM activity should be able to better use carbohydrates in the diet as well as both store more glycogen in the liver and utilize the stored glycogen faster than fish lacking liver PGM (Allendorf, 1985). Aronson (1985) has verified this by demonstrating that fish with liver PGM activity stored more glycogen in the liver during normal hatchery feeding and when fed a diet with high amounts of glucose. In addition, fish with liver PGM activity showed a greater decline in liver glycogen levels shortly after the initiation of starvation.

All of the phenotypic effects of PGM are related to developmental rate. Fish with liver PGM activity develop at a faster rate, as measured by mean time of hatching, than their full sibs lacking activity. Approximately 78% of the early hatching fish (compared to 30% of the late
hatching fish) possessed liver PGM activity (Allendorf et al. 1983a). Allendorf concluded that the presence of PGM enzyme activity in the liver results in a more rapid developmental rate in the period between liver organogenesis (15 days) and hatching (33 days).

The more rapidly developing fish begin exogenous feeding earlier and obtain a size advantage over their siblings lacking the regulatory (b) allele (Allendorf et al. 1983b). Fish with liver PGM activity were 3.6% longer than their full sibs lacking activity, averaged over all of the families sampled. This resulted in an average difference of approximately 11% in weights.

This size advantage is maintained until sexual maturity and also produces a significant decrease in the age of first sexual maturity. Two thirds of the males sampled in one family (G4) were sexually mature seventeen months after fertilization. All of the pgm1-t(a/b) males were mature whereas only 46% of the pgm1-t(a/a) males were matured (Allendorf et al. 1983a).

Waddington, Mather, Van Valen and Soule have proposed (cited in Allendorf et al. 1983a) that fluctuating asymmetry reflects accidents during development and that genetically superior individuals will be more
buffered during development and, therefore, will be less asymmetrical. The developmental stability between individuals showing liver PGM activity and those lacking activity was estimated using the asymmetry of bilateral morphological traits (Allendorf et al. 1983a). Allendorf used meristic counts of five bilateral meristic traits: gill rakers on both the upper and the lower portions of the first gill arch, mandibular pores, rays in the pectoral fin, and rays in the pelvic fin. Fish possessing liver PGM activity showed a significantly lower mean number of asymmetrical traits than fish lacking PGM liver activity. Thus, fish with liver PGM activity are apparently more developmentally buffered.
MATERIALS AND METHODS

Sample Preparation

Young fish (4-6 cm) were removed from live tanks and anesthetized with 2-phenoxyethanol. Incisions were then made and the heart and liver were excised. A portion of muscle above the lateral line was also removed. All tissue samples were then stored in 12X75 mm Borosilicate disposable culture tubes. Eventually the excision of the heart was terminated as a step because the muscle gels could be scored satisfactorily. All of the fish bodies were retained for reruns in anticipation of the event that a recombinant was detected. The samples were stored by one of the following methods:

Method 1: Samples were frozen overnight and were then thawed the following day. Imidazole buffer (Table 1) was then added to the culture tubes using 120-140 μl for muscle tissue and 60-100 μl for heart and liver tissue. All samples were then sonicated to release the cellular enzymes and were then centrifuged at 1000Xg for five minutes.
Method 2: Tissue samples were frozen overnight in Imidazole buffer using the aliquots described in method 1.

Method 3: In this method the appropriate samples were simply refrigerated overnight in the Imidazole buffer, again using the amounts specified in method 1. This method as well as method 2 eliminate the time consuming steps of sonication and centrifugation. All methods produced similar banding patterns for the same sample. Method 3 was eventually used on a majority of the samples tested because it was quick and produced scoreable gels.

**Gel Preparation**

The gels were prepared using 25.4 grams of hydrolyzed potato starch mixed with 212 ml of AC gel buffer (Table 1). The starch was suspended in one quarter of the buffer solution which was contained in a one liter erlenmyer flask. The remainder of the buffer was heated to boiling and then added to the starch-buffer mixture while swirling the flask. The mixture was then vigorously swirled and heated to boiling. It was then degassed for approximately one minute to remove any air bubbles. A frame for the gel was made using a 10 1/2" X 7" X 1/4" glass
plate with four 1/4" thick plexiglass strips clamped on to it. The strips were positioned so that the gel would measure 4" X 8 1/2". The degassed starch gel was then poured into the frame, covered with plastic wrap to prevent excess drying, and left to cool to room temperature (Figure 2).

Electrophoresis

Horizontal starch gel electrophoresis was used to differentiate between allelic variants. The cooled gel was cut into two half gel sections approximately 2" X 8 1/2". Both sections were then placed on their own glass plates and another cut was made on both gels approximately 1cm from the edge along the long side. This small slice was then pulled back to allow application of the tissue wicks. Tissue samples were drawn onto filter paper wicks measuring 1/8" X 1/2". These wicks were placed vertically alongside each other in the gel cut (Figure 3). In this manner, 40 samples could be tested on a single gel. The smaller section was then placed firmly against the larger section and the inserts. This smaller section marks the anodal end of the gel. The plastic wrap was then replaced and folded back to expose about 1cm of gel on each end.

Approximately 200 ml of AC electrode buffer (Table 1) were added
Figure 2--Frame for starch gel.

A - The plexiglass strips are held firmly in place with clamps.

B - Plexiglass strips positioned so the gel measures 4" X 8 1/2".

C - Glass plate-10 1/2" X 7" X 1/4".
Figure 3--Placement of samples on starch gel

A - Forceps.

B - Filter paper wicks are saturated with test samples and inserted into the cut. The wicks should be separated by approximately 1mm.

C - Gel cut marking the origin.
Table 1

Preparation of buffers

**Imidazole buffer**

- Imidazole - 2.72 g/l
- MgCl$_2$ - 0.61 g/l
- EDTA (Ethylenediaminetetraacetic acid) - 0.5 g/l
- Adjust pH to 7.8 with HCl

**AC gel buffer**

- 800 ml AC electrode buffer
- 15,200 ml distilled water
- Adjust pH to 6.4 with N-(3-Aminopropyl)-morpholine

**AC electrode buffer**

- 33.6 g of citric acid
- 4 liters of distilled water
- 35-40 ml of N-(3-Aminopropyl)-morpholine
- Adjust pH to 6.1 with N-(3-Aminopropyl)-morpholine
to the buffer trays. The electric current was conducted from the tray buffer to the ends of the gel by disposable utility cloths. The electrophoresis was run by applying 50 milliamps of current and 200 volts. After ten minutes of preliminary electrophoresis the tissue wicks were removed and the gel sections were placed firmly together. Ice packs were then placed on top of the gel to prevent denaturation of the enzyme, phosphoglucomutase. The electrophoresis was continued for a total of three hours.

**Staining procedure**

After the electrophoresis was completed the gels were sliced horizontally into four sections. This was done by sequentially placing 1/16th inch plastic strips on each side of the gel and pulling a monofilament thread through the gel at that level. The top layer was discarded and the others were used for staining. The gel slices were placed individually in trays and stained for phosphoglucomutase (Table 2).

The mechanism of the NBT-PMS staining method has been discussed elsewhere (May, 1975). This PGM stain utilizes the NBT-PMS dehydrogenase method. First of all, PGM catalyzes the conversion of
Table 2--**PGM stain**

Each gel slice requires the following:

- 20 ml Imidazole buffer-pH 7.8 (Table 1)
- 100 mg Glucose-1-phosphate
- 1 ml Glucose-6-phosphate dehydrogenase (20 µg/ml)
- 1 ml NADP (2.5 mg/ml)
- 1 ml MgCl$_2$ (2% solution)
- 0.5 ml Nitro-blue tetrazolium NBT (2.5 mg/ml)
- 0.5 ml Phanazine methosulphate PMS (5 mg/ml)
glucose-1-phosphate (G-1-P) to glucose-6-phosphate (G-6-P) (Figure 4).

Glucose-6-phosphate dehydrogenase (G6PDH) then converts glucose-6-phosphate to 6-phosphogluconate (6-PG). NADP is reduced during this step, releasing a hydrogen ion into solution. This hydrogen ion is picked up by the intermediary PMS, which is then reduced. PMS then passes on the hydrogen to NBT, a yellow soluble salt, converting it to an insoluble blue formazen dye. This blue dye is visible only where the enzyme PGM is present (May, 1975) (Figure 5).

**Nomenclature and Gel Scoring**

Nomenclature follows that given by Allendorf and Utter (1976). An abbreviation is chosen to designate the protein in question. In this case phosphoglucomutase is represented by PGM. These abbreviations, when in lower case letters, represent the loci coding for these proteins. When multiple forms of the same enzyme are present a numeral is included at the end of the locus abbreviation. The form with the greatest cathodal migration is designated one, the next two, and so on. The locus in question here is designated pgm1. Allelic variations are designated according to
Figure 4--PGM catalyzed conversion of Glucose-1-Phosphate to Glucose-6-Phosphate

Glucose-1-Phosphate $\xrightarrow{\text{PGM}}$ Glucose-6-Phosphate

Figure 5--NBT-PMS dehydrogenase staining system for PGM.

G-1-P $\xrightarrow{\text{G6PDH}}$ G-6-P $\xrightarrow{\text{NADP}}$ Formazan

G6PDH $\xrightarrow{\text{NADPH}}$ PMS $\xrightarrow{\text{PMS}}$ NBT
their electrophoretic mobility. The most common allele is designated 100, which represents the distance of migration of the isozyme coded for by this allele. Other allozymes (allelic isozymes) are then assigned a value representing their mobility relative to this unit distance. The two structural variants, present in muscle tissue, that are in question here are pgm1(100) and pgm1(90). Thus the enzyme coded for by the alternate allele migrates 90% of the distance of the protein coded for by the common allele. There are also two elements to the gene in question, a regulatory element and a structural element. The regulatory region is designated pgm1-t and the structural region is designated pgm1-s.

Because of the regulatory element that affects the liver-specific activity of pgm1, there is either the presence or absence of activity depending on whether the regulatory element is expressed or not. As stated earlier the regulatory element is cis-acting. Therefore only the structural allele on the same chromosome is expressed and only one band is seen (either the 100 or the 90). Thus if activity is seen, by the presence of blue dye in the gel at the point in question, then there is activity of the pgm1 locus in the liver of the individual being examined. If activity is present, that individual was designated with a "b", or pgm1-t(b). In the absence of liver
activity an individual was scored with an "a", as pgm1-t(a). (Fig. 6)

Muscle gels: Due to the non-expression of a regulatory element in the gene coding for PGM in muscle tissue both structural alleles will be expressed. Thus a common homozygote for the structural allele was represented electrophoretically by a single band at the 100 point and was scored as pgm1-s (100/100). A heterozygote was represented by bands at both the 90 and 100 points and was scored as pgm1-s(100/90) (Fig. 7)

**Matings**

Fish for the experiments were from the full sibling M-families (Table 3) which segregate 1:1 (a/a : a/b) for the presence or absence of liver PGM1 activity. The male parents of the M-families were heterozygous at both the structural and the regulatory loci, pgm1-s(100/90)-t(a/b), and thus possessed liver PGM1 activity and expressed both structural alleles. The female parents were common homozygotes for both loci, pgm1-s(100/100) -t(a/a), and thus showed no liver PGM1 activity and in muscle tissue only expressed the 100 structural allele.

The original cross was a hybrid cross between a rainbow trout and a cutthroat trout. The reason for this is that the regulatory variant (b) is
Figure 6—Photograph of gel showing regulatory variants pgm1-t(a) and pgm1-t(b)

The vertical columns represent different tissue samples from different fish. The PGM2 row is used to identify which individual possesses liver PGM1 activity (all individuals show PGM2 expression and thus this can serve as a counter). The presence of dye underneath the PGM2 counter indicates that that individual possesses the regulatory (b) gene and thus will exhibit liver PGM1 expression. Absence of dye indicates an individual without liver PGM1 expression.
Figure 7—Photograph of gel showing structural variants pgm1-s(100/90) and pgm1-s(100/100)

A single band underneath the PGM2 counter indicates that that individual expresses only the common structural allele (100). This 100 allele produces a structural enzyme product that possesses the greatest cathodal migration. The presence of two bands (one at the 100 position and one at the 90 position) indicates that that individual possesses both the common and the variant structural alleles.
Table 3—Creation of the M-families

<table>
<thead>
<tr>
<th>Female I.D. number</th>
<th>Male I.D. number</th>
</tr>
</thead>
<tbody>
<tr>
<td>M39</td>
<td>1</td>
</tr>
<tr>
<td>M40</td>
<td>1</td>
</tr>
<tr>
<td>M41</td>
<td>1</td>
</tr>
<tr>
<td>M45</td>
<td>3</td>
</tr>
<tr>
<td>M46</td>
<td>3</td>
</tr>
<tr>
<td>M47</td>
<td>3</td>
</tr>
</tbody>
</table>

only found in the Arlee strain of rainbow trout and that the structural variant (90) is only found in Coastal strains of cutthroat trout. This allows us to backcross the doubly heterozygous progeny with a doubly homozygous individual. The progeny of this mating can then be tested for the amount of recombination between the structural and the regulatory elements.

Males were used as the doubly heterozygous individuals and females were used as the doubly homozygous individuals. Recombination will only be detected if it occurred in the male individual. This is because the females are doubly homozygous and a recombinant would not show up in the progeny. This indicates that the M-families with the same male
parent should statistically show the same amount of recombination (i.e. M39 and M45, both having male number 7 as their parent, should show equal numbers of recombinants). The complete lineage for the M-families is shown in figure 8 (Allendorf, 1985).
Figure 8—Outline of experimental matings

\[
\begin{align*}
\frac{100}{90} & \times \frac{100}{100} \\
\frac{1}{4} & \quad \frac{100}{100} \\
\frac{1}{4} & \quad \frac{90}{100} \\
\frac{1}{4} & \quad \frac{100}{100} \\
\frac{1}{4} & \quad \frac{90}{100} \times \frac{100}{100} \\
\text{Parentals} & \quad \frac{90}{100} \\
\text{Recombinants} & \quad \frac{90}{100} \\
\text{Recombinants} & \quad \frac{100}{100} \\
\text{Recombinants} & \quad \frac{100}{100} \\
\end{align*}
\]
RESULTS

The results of the experiment are summarized in Table 4. The number of individuals from each family exhibiting the specific genotype are noted in the table. Only parental genotypes were observed for all families sampled (with the exception of three abnormal cases in families M37 and M38). No recombinant genotypes were detected out of 665 scoreable individuals. This fact was used to estimate a confidence interval for the amount of recombination present in the population. Using an antisymmetric 95% confidence interval the amount of recombination was found to be between 0 and .004495 \( (L_{95^\%}: 0<P_r<.004495) \). Families M37 and M38 were produced with Eagle Lake trout as indicated in Figure 8. The observed occurrences of pgm1-s(90/90) in these families are abnormal genotypes.
Table 4—Summary of results

<table>
<thead>
<tr>
<th>Family</th>
<th>a 100/90</th>
<th>b 100/100</th>
<th>a 100/100</th>
<th>b 100/90</th>
<th>a 90/90</th>
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<td>M39</td>
<td>119</td>
<td>76</td>
<td>0</td>
<td>0</td>
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<tr>
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<td>77</td>
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<tr>
<td>M41</td>
<td>51</td>
<td>41</td>
<td>0</td>
<td>0</td>
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</tr>
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<td>M46</td>
<td>91</td>
<td>59</td>
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</tr>
</tbody>
</table>
DISCUSSION AND CONCLUSION

The results show no observed recombinants out of a sample size of 665 individuals. This suggests a very low rate of recombination between the structural elements and the regulatory elements of the gene in question (Lg: $0<P_r<0.004495$). This low rate of recombination suggests that the structural and the regulatory elements of the pgm1 gene are very tightly linked and may even be adjacent to each other on the chromosome. The fact that they are tightly linked also supports previous evidence that the regulatory element is cis-acting. Allendorf (1982) previously speculated that the observed intermediate expression of heterozygotes would be consistent with cis-regulation resulting in two independently regulated chromosomes. Chandlee and Scandalios (1984) have looked at various regulatory elements affecting enzyme loci in eukaryotes. Pgm1-t is the only cis-acting regulatory element that has been reported in a vertebrate other than a mouse.

The insertion of genes into an organism to modify the phenotype or
to correct a genetic defect has been carried out in mice and fruit flies (Palmiter et al. 1982; Spradling and Rubin 1982). Zhu et al. (1985) using egg microinjection have successfully inserted a novel gene into the genome of a goldfish. Hammer et al. (1984) have successfully corrected a growth hormone deficiency in mice by inserting a rat growth hormone gene into the genome of diseased mice. However, lack of appropriate control of these genes resulted in abnormal phenotypes-typically gigantism. This demonstrates the importance of determining how to regulate transferred genes correctly. If the pgm1-t regulatory element can be controlled, cloned, and successfully inserted into the genome of hatchery trout it could have profound effects upon the aquaculture of this agriculturally important species.

Glycolysis should be of greater metabolic importance at higher temperatures when there is less oxygen available and anaerobic metabolism becomes more important (Hochachka, cited in Allendorf 1983a). Therefore differences in developmental rates between fish with and without liver PGM expression should increase with temperature. Fish with liver PGM expression should thus be more resistant to higher water temperatures and better able to be reared at these higher temperatures,
than fish lacking PGM liver expression. Fish hatcheries using trout with liver PGM expression should be able to grow fish faster and at higher temperatures thereby reducing production costs. Fish with PGM liver expression may also be useful in stocking streams where water temperatures are marginal for trout survival.

Knowledge of the DNA sequence changes responsible for the expression or non-expression of PGM in liver would provide extremely valuable information about gene regulation in eukaryotes. Future research along these same lines is planned by Fred Allendorf over the next few years. This research includes both the cloning and the sequencing of the pgm1 regions (both pgm1-s and pgm1-t) from fish with and without liver PGM activity. This should allow for the identification of the sequence differences that are responsible for the differential liver-specific expression (Allendorf 1985). Continued research is also planned for the further elucidation of the metabolic effects of structural and regulatory allelic variation at the pgm1 locus.

The three observed abnormal genotypes, pgm1-s(90/90), of families M37 and M38 are impossible outcomes given the mating design used. There are two possible explanations for these genotypes. Allendorf
(1985) has discovered a third type of genetic variant affecting pgm1-s which he designated as a null allele or pgm1-s(n). Some individuals of a New Zealand strain of rainbow trout possessed no PGM activity in any tissue. These fish were apparently homozygous for the null allele. This null allele could be present in the Eagle Lake trout used for families M37 and M38. The parent thought to be homozygous for the structural alleles, pgm1-s(100/100), could actually have possessed a null gene at one of the alleles, pgm1-s(100/n). These two genotypes would then be electrophoretically indistinguishable and would both appear as pgm1-s(100/100). As segregation occurs an alternate structural allele could be paired with a null allele producing a pgm1-s(90/n) genotype that appears electrophoretically as a pgm1-s(90/90) genotype. In addition the interspecific cross of the Eagle Lake trout with the rainbow trout could produce developmental incompatibilities resulting in abnormal expression of the genes present.
LITERATURE CITED


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