Enzyme heterozygosity and growth in rainbow trout: genetic and physiological explanations

MOIRA M. FERGUSON

Department of Zoology, University of Guelph, Guelph, Ontario, Canada N1G 2W1

The possibility of whether the association between enzyme heterozygosity and body size (fork length) is consistent among rainbow trout (*Oncorhynchus mykiss*) with different degrees of relationship and is affected by fish age was determined. Six-month-old full-sibs and progeny groups from five and 13 parents of each sex do not show the positive associations between multilocus heterozygosity and fork length which are detectable in larger pooled gamete matings ($25\text{♀} \times 25\text{♂}$). Moreover, the relationship between heterozygosity at single loci and fork length is inconsistent among families. These data suggest that chromosomal segments, marked by the enzyme loci, are responsible for the phenotypic effects (associative overdominance). Fish age affects both the strength and direction of the association between multilocus heterozygosity and fork length; the association is positive in 6-month-old rainbow trout but is weaker, negative, or differs between the sexes in fish at 1 year. Moreover, the strength of the relationship changes over time in repeated measurements on the same fish. A decline in growth of larger and more heterozygous fish, because of precocious sexual maturation, may partially explain the changing relationship between multilocus heterozygosity and fork length. Sexually mature males are significantly more heterozygous than immature males and show significantly reduced recent growth rates as measured by white muscle RNA concentration. However, females, who rarely mature at 1 year, show significantly higher recent growth rates. Thus, heterozygosity is differentially associated with the allocation of energy resources into somatic and reproductive tissue in male and female rainbow trout.

Keywords: associative overdominance, fish, growth-heterozygosity, RNA concentration, sexual maturation.

Introduction

The identity of the genetic loci responsible for associations between enzyme heterozygosity and characters related to fitness, and the genetic mechanism whereby they act (dominance or overdominance), is generally unknown (Mitton & Grant, 1984; Allendorf & Leary, 1986; Zouros, 1987; Zouros & Foltz, 1987). Both the enzyme loci (direct involvement explanation, Zouros & Foltz, 1987) and chromosomal segments marked by the enzyme loci (associative overdominance, Zouros & Foltz, 1987) have been proposed as responsible for the phenotypic correlations (Koehn *et al.*, 1988; Zouros *et al.* 1988, respectively). According to the associative overdominance hypothesis, the enzyme loci are in gametic disequilibrium with overdominant loci with effects on fitness or more homozygous individuals at

enzyme loci have increased the probability of being homozygous for deleterious recessive alleles at loci randomly spread throughout the genome due to either gametic-phase disequilibrium or inbreeding (Houle, 1989).

The associative overdominance and direct involvement hypothesis can be tested because they make contrasting predictions on the consistency of the heterozygosity/fitness association among the progeny of different crosses (Danzmann *et al.*, 1986; Mallet *et al.*, 1986; Leary *et al.*, 1987; Zouros *et al.*, 1988; Zouros & Mallet, 1989; Leberg *et al.*, 1990). Under the direct involvement of the enzyme loci, the relationship between specific genotypes and relative fitness should be consistent among progeny groups (among families, pooled gamete versus families) due to the consistent metabolic roles of the gene products. In contrast,

associative overdominance predicts that the heterozygosity/fitness association might differ among progeny groups depending upon breeding structure and linkage associations between enzyme loci and deleterious genes (Zouros & Foltz, 1987; Houle, 1989).

Multilocus heterozygosity is positively associated with body size among the progeny of pooled gamete matings in both the Arlee and Eagle Lake hatchery strains of rainbow trout (*Oncorhynchus mykiss*) (Danzmann *et al.* 1987, 1988). Surprisingly, a negative relationship was detected amongst older fish (about 1 year) derived from the self-sustaining natural population in the Ganaraska River, Ontario (Ferguson 1990). The first experiments reported here test the two general hypotheses for the detected associations between heterozygosity and body size by determining whether consistent associations are detectable among pooled gamete matings produced from fewer parents and full-sibs.

The direct involvement and associative overdominance hypotheses do not, however, address the differences in the direction of the multilocus heterozygosity/body size association observed among strains of rainbow trout sampled at different ages. One explanation is that the detection of the correlation is affected by precocial sexual maturation in males; females rarely mature at 1 year (Ferguson, 1990). This hypothesis was tested by examining the heterozygosity-size correlation in rainbow trout sampled at different ages and comparing the heterozygosity of precociously mature and immature males.

Materials and methods

Fish

The progeny of adult rainbow trout collected from the Ganaraska River, Port Hope, Ontario, or a derived hatchery stock, were used in all experiments. The hatchery stock was initiated in the early 1980s and has been maintained with large effective population sizes (> 500) at the Ontario Ministry of Natural Resources Normandale Fish Culture Station (Ferguson *et al.*, 1991). The Ganaraska River population is a relatively large self-sustaining stock and over 10,000 fish have been counted during the spawning run in recent years. The natural and hatchery sources can be considered a single strain, at least within the context of the present study, because no significant differences in allele frequencies have been detected between them (Ferguson & Ihssen, 1991; Ferguson *et al.*, 1991).

Six, pooled gamete crosses were made by addition of equal volumes of sperm from several males (premixed), with equal volumes of eggs from the same number of females (also premixed prior to combination with

sperm). Two progeny groups were produced from hatchery fish (A14 — 11 March 1987; B35 — 16 March 1988) and four progeny groups with wild fish (A200 — 15 April 1987; C14, C15, C16 — 20 April 1989). All progeny groups were produced by combining gametes of 25 females and 25 males except for C15 and C16 where 13 and five fish of each sex were used, respectively. Twelve full-sib families were produced on 15 April 1987 from the same rainbow trout used to produce A200.

All progeny groups were reared at the University of Guelph in raw well water, ranging from 6 to 12°C depending upon the season. The fish were fed to excess with commercial trout food and their photoperiod was adjusted monthly to follow ambient conditions. The ages of all fish represent days since fertilization.

Electrophoresis

Genotypes at eight polymorphic loci were determined in all fish with horizontal starch gel electrophoresis (Allendorf *et al.*, 1977) or cellulose acetate gel electrophoresis (Hebert & Beaton, 1989). The protein products resolved on high (> 8) (*bGlua*, *Pgm2*, *Sod*, *G3pl*, and *G11*) and low pH. (7–8) (*Idh2*, *Idh3,4*, and *Mdh3,4*) buffers were scored on cellulose acetate and starch, respectively. Multilocus heterozygosity was expressed as the observed number of heterozygous loci per fish. Two pairs of loci (*Idh3,4*; *Mdh3,4*) are duplicated and share alleles (isoloci) and therefore it is difficult to assign a specific allele to a given locus. Therefore, each duplicated pair was considered a single tetrasomic locus in all analyses where fish with a single electromorph were designated homozygotes while fish with two or more electromorphs were considered heterozygotes.

Body size and growth

Fork length, measured from the tip of the snout to the fork of the tail (nearest millimetre), was used as an estimate of body size. RNA concentration ($\mu\text{g/g}$ wet tissue) and an RNA:DNA ratio were used as estimates of recent growth as the amount of RNA in white muscle is positively associated with direct measures (Bulow, 1987; Ferguson & Danzmann, 1990). Previous studies with Ganaraska rainbow trout have shown that RNA concentration is a more sensitive indicator of recent growth (measured by changes in body size) than RNA:DNA ratio (Ferguson & Danzmann, 1990). The concentrations of RNA and DNA were measured in white muscle according to the fluorometric method of Karsten & Wollenberger (1972) as modified by Ferguson & Drahushchak (1989).

Statistics

The Pearson product-moment correlation coefficient (r) was used as a measure of association between the number of heterozygous loci per fish and growth (fork length, RNA, RNA:DNA ratio). The contribution of specific loci to the heterozygosity-growth relationship within a specific progeny group was determined with multiple linear regression where each locus was considered an independent variable and fork length the dependent variable (see Koehn *et al.*, 1988 for an example). Heterozygotes at a particular locus are assigned a value of 1 and homozygotes a value of 0. Thus, the contribution and significance of a specific locus can be determined by the significance and direction of the partial regression coefficient. Parametric ANOVAS were used to determine if mean numbers of heterozygous loci per fish were significantly different

among progeny groups. Finally, non-parametric t -tests (Wilcoxon two sample) were used to determine if mature and immature males differed in growth and heterozygosity because these data do not meet parametric assumptions.

Results

Progeny groups

The association between the number of heterozygous loci per fish and fork length is not consistent among 6-month-old progeny groups with different degrees of relationship (Table 1). Multilocus heterozygosity is significantly and positively associated with fork length in the pooled gamete mating C14. In contrast, progeny produced from pooled matings with fewer parents of each sex (C15, C16) show no significant association.

Table 1 Multiple regression between heterozygosity at eight enzyme loci (independent variables) and fork length (cm) (dependent variable) in 6-month-old Ganaraska rainbow trout with different degrees of relationship. The association (r , Pearson product-moment correlation coefficient) between multilocus heterozygosity and fork length is also given

		Locus								
Progeny group	<i>bGlua</i>	<i>G3p</i>	<i>Gl1</i>	<i>Idh2</i>	<i>Idh3,4</i>	<i>Mdh3,4</i>	<i>Pgm2</i>	<i>Sod</i>	<i>r</i>	
Pools (25♀ × 25♂)										
A200	NS	NS	NS	NS	NS	NS	NS	NS	0.196	
C14	NS	+	NS	NS	NS	NS	+	NS	0.343*	
Pools (C15:13♀ × 13♂; C16:5♀ × 5♂)										
C15	NS	NS	M	NS	–	NS	NS	NS	0.066	
C16	NS	M	NS	–	NS	NS	NS	NS	0.181	
Families (1♀ × 1♂)										
A37	M	M	M	NS	+	–	M	+	0.350*	
A39	NS	M	M	NS	NS	+	M	M	0.052	
A41	NS	M	M	NS	M	NS	NS	NS	–0.018	
A42	M	NS	M	M	M	M	NS	NS	–0.053	
A43	NS	M	M	+	M	NS	M	+	0.408**	
A46	NS	M	M	NS	NS	NS	NS	NS	–0.156	
A48	NS	M	NS	M	NS	M	+	M	0.230	
A49	NS	M	M	NS	–	M	NS	NS	–0.150	
A50	NS	M	NS	M	–	M	M	NS	–0.018	
A52	NS	NS	M	–	M	NS	M	NS	–0.352*	
A53	M	M	M	M	M	M	NS	NS	0.235	
A54	NS	M	M	M	NS	NS	NS	M	0.103	

Sample sizes: A200 = 43, C14 = 44, C15 = 46, C16 = 39, A37 = 39, A39-A54 = 40.

– partial $t = P < 0.05$, Homo > Het.

– partial $t = P < 0.05$, Het > Homo.

NS partial $t = P > 0.05$.

M = locus monomorphic in progeny group.

* $P < 0.05$, ** $P < 0.01$.

Moreover, progeny from 12 full-sib families show no consistent relationship between multilocus heterozygosity and fork length. Significant positive correlations are detectable in two families and a negative relationship in a third. Overall, the correlation coefficients are negative in six families and positive in six families.

The relationship between heterozygosity at specific loci and fork length is inconsistent among families and pooled progeny groups when each locus is treated as an independent variable in multiple regression (Table 1). For three loci (*Idh2*, *Idh3,4*, and *Mdh3,4*), the partial regression coefficients are significantly positive in one family and significantly negative in at least one other. Thus, heterozygotes at a specific locus are larger than homozygotes in some families and the reverse is true in others.

Fish age

The strength and the direction of the relationship between multilocus heterozygosity and fork length in the progeny of pooled gamete matings of rainbow trout changes over time (Fig. 1). A significant positive correlation was detected in C14 sampled at 193 days. Older fish show a weaker or negative relationship between multilocus heterozygosity and fork length. Fish from A200 (sexes combined) show a significant positive association at 291 days. This relationship is not significant in the same fish sampled at 376, 473, 465, and 604 days. Similarly, no relationship was detected in

A14 at 283 days and B35 at 395 days. Significant negative associations were detected from repeated measurement of A14 (same fish) at 378, 421, and 441 days.

Repeated measurements of A200 and A14 indicate that the association between multilocus heterozygosity and fork length differs between the sexes over time (Fig. 2). Females from A200 show a significant positive association at 291 days. However, the relationship is not significant at 376, 473, 565, and 604 days. Males do not show any significant association on any sampling day. In A14, females show no significant association at 378, 421, and 441 days while males show significantly negative associations on all 3 days. On all sampling dates with both progeny groups (eight comparisons), males show more negative correlation coefficients between multilocus heterozygosity and fork length than females (correlation coefficients displaced downwards in Fig. 2).

RNA concentration is a more sensitive indicator of the subtle and complex changes in growth at 1 year than fork length. As previously mentioned, no signifi-

Fig. 1 Pearson product-moment correlation coefficients (r) between the number of heterozygous loci per fish and fork length in the progeny of four pooled gamete matings (25 ♀ and 25 ♂) of Ganaraska rainbow trout and sampled between 187 and 604 days from fertilization (sexes combined). Linked points represent repeated measurements on the same fish. Sample sizes (age): (○) A14 (283) = 40, A14 (378–441) = 75, (●) B35 (395) = 137, (△) C14 (193) = 44, (▲) A200 (187) = 43, A200 (291–604) = 165.

Fig. 2 Pearson product-moment correlation coefficients (r) between the number of heterozygous loci per fish and fork length in 70 female (○) and 95 male (●) rainbow trout from (a) A200 measured between 291 and 604 days from fertilization and in 35 female and 40 male progeny from (b) A14 measured at 378, 421, and 441 days.

association between multilocus heterozygosity and recent growth, as measured by RNA concentration in white muscle, was detected in females but not in males (Fig. 3).

The importance of precocial sexual maturation to the heterozygosity-growth correlation was examined in a more direct experiment with B35 sampled at 395 days. The predictions were tested that precociously mature males are more heterozygous and show lower recent growth than immature males. Spermiated males have significantly higher numbers of heterozygous loci per fish and show lower recent growth rates (muscle RNA and RNA:DNA ratio) than immature males (Table 2). Spermiated and immature males do not differ significantly in fork length.

Discussion

Progeny groups

The association between multilocus heterozygosity and fork length differs among progeny groups with dif-

Fig. 3 The relationship between the number of heterozygous loci per fish and recent growth as measured by RNA concentration in white muscle of (a) female and (b) male progeny of a pooled gamete mating (B35) at 395 days. The vertical bars represent the standard error of the mean. The numbers below the bars indicate the number of fish sampled in each heterozygosity class. Females: $r = 0.326$, d.f. = 59, $P < 0.01$; Males: $r = 0.073$, d.f. = 74, $P > 0.05$.

ferent degrees of relationship. Pooled gamete crosses sampled at 6 months show positive associations whereas full-sib families, and pooled gamete crosses with lower numbers of parents show no consistent associations. This inconsistency among progeny groups is not compatible with the direct involvement of the enzyme loci but suggests that chromosomal segments marked by the enzyme loci are responsible for the phenotypic correlations (associative overdominance).

A variety of genetic conditions could account for the phenotypic correlations under associative overdominance (Houle, 1989). The enzyme loci could be closely linked to the marked chromosomal segments such that occasional cross-overs between the enzyme locus and the unknown segment would lead to a change in the direction between genotype and fitness value. The differences among families in the direction of the association at single loci is consistent with this explanation. Alternatively, electrophoretic homozygosity could be correlated with homozygosity for recessive deleterious genes because of either inbreeding or gametic-phase disequilibrium. The lack of association in families and detectable associations in pooled gamete crosses is compatible with this second hypothesis. To distinguish between these possibilities is one of the greatest challenges to students of this problem, however, recent advances in molecular biology make it possible to evaluate the fitness effects of nucleotide variation within chromosomal segments tightly linked to the enzyme loci.

These conclusions assume that interactions among enzyme and marked chromosomal segments in different genetic backgrounds are responsible for the inconsistency among cross types. However, reduced polymorphism in progeny groups produced with low numbers of parents could militate against detection of

Table 2 Mean (SEM) concentrations of nucleic acids in white muscle ($\mu\text{g/g}$ wet weight of tissue), fork length (cm) and number of heterozygous loci per fish in mature and immature male rainbow trout at 395 days (from fertilization) from a pooled gamete mating (B35)

Measure	Mature	Immature	Significance
Heterozygosity	2.88 (0.52)	2.15 (0.14)	*
Fork length	14.5 (0.5)	13.7 (0.4)	
DNA	78 (7)	101 (3)	**
RNA	301 (44)	485 (13)	***
RNA:DNA	3.88 (0.50)	5.13 (0.28)	*
n	8	68	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (1 tail) Wilcoxon two-sample test.

cant association between multilocus heterozygosity and fork length was detected in B35. However, a positive correlations in such groups. This proposed explanation cannot, however, account for the observed inconsistencies in the direction of the correlations both at the multilocus and single locus levels. Furthermore, the lack of significant correlation in C15 and C16 is not the result of lower numbers of heterozygous loci relative to the larger pooled mating (C14) because no significant differences in the mean number of heterozygous loci per fish were detected between them (One-way ANOVA; $F_{2,126} = 1.411$; $P = 0.248$).

Despite the acknowledged importance of protein polymorphism in evolutionary processes, the role of the enzyme loci in selective processes remains controversial (e.g. Zouros *et al.*, 1988; Koehn *et al.*, 1988; Houle, 1989; Gaffney *et al.*, 1990; Koehn, 1990; Zouros, 1990). The dichotomy is in part due to the different approaches that laboratories have used to address the problem. For instance, studies where the direct involvement and associative overdominance hypotheses have been tested by examining the inconsistency in the heterozygosity–fitness correlation among progeny groups have provided support for associative overdominance (Beaumont *et al.*, 1985; Foltz & Chatry, 1986; Danzmann *et al.*, 1986; Mallet *et al.*, 1986; Danzmann *et al.*, 1988; Zouros *et al.*, 1988; Leberg *et al.* 1990; present study). As an exception, Leary *et al.* (1987) argued that the strength and direction of the correlation between multilocus heterozygosity and the number of asymmetric meristic characters is as strong among full-sibs as it is among individuals randomly sampled from populations of salmonid fishes (Leary *et al.*, 1984). In contrast, physiological studies (Koehn & Shumway, 1982; Garton *et al.*, 1984; Mitton *et al.*, 1986; Hawkins *et al.*, 1986, 1989; Koehn *et al.*, 1988) have provided mechanistic biochemical connections between enzyme phenotypes and fitness. These studies generally argue that the phenotypic effects are determined by heterozygosity at the enzyme loci rather than marked chromosomal segments because the metabolic basis for the correlations derive from differences in net energy status. Thus, given the dichotomy of approaches and conclusions (Koehn, 1990; Zouros, 1990), it is conceivable that the identity of the loci responsible for the fitness correlations will only be established with experiments that encompass both approaches.

Fish age

Fish age affects both the strength and direction of the correlation between multilocus enzyme heterozygosity and fork length in pooled gamete crosses of rainbow

trout. Positive associations were detected in fish at 6 months but the association is either weak, non-detectable, or the sexes differ in older fish. These data complement previous studies with this species; Danzmann *et al.* (1987; 1988) observed positive associations in fish less than 6 months old while Ferguson (1990) observed negative associations in older fish.

These data are consistent with the hypothesis that sexual maturation of more heterozygous individuals affects the association. Correspondingly, more heterozygous individuals would have faster growth rates before the spawning season, resulting in precocial maturation and slower growth at the onset. Thus, the detection of negative correlations in males and the significant differences in heterozygosity between mature and immature males suggests that sexual maturation has important influences on the heterozygosity–growth correlation. More heterozygous males presumably divert finite energy reserves into gamete production earlier, resulting in the detectable declines in recent growth.

A corollary of the maturation hypothesis is that the sexes should differ in the relative effect because of differential maturation at 1 year. Because females rarely mature so young, they should not show equivalent declines in growth as males resulting in the maintenance of the heterozygosity–growth correlation. The detection of positive associations between heterozygosity and recent growth (RNA concentration) in females from B35 supports this idea. However, despite a lack of sexual maturation, females still showed a decline in the heterozygosity–fork length correlation. This suggests that other factors affect the allocation of resources into somatic and reproductive tissue in homozygotes and heterozygotes.

Declines in the heterozygosity–fork length correlation with age and sex-specific differences were detected in both progeny groups where repeated measurements were taken over time. However, the progeny groups differed in the magnitude of the decline and qualitative differences in the relationship were detectable. There are many explanations for this difference, including factors that affect growth and maturation rates (and chance). For example, Ferguson (1990) observed that a significant negative association between multilocus heterozygosity and fork length was observed only among those fish fed excess rations and not those designed to promote low growth. This apparent variation in the magnitude of the heterozygosity–fork length correlation in relation to environmental factors might suggest that such results are too phenotypically labile to be of much biological significance. However, studies with another species of salmonid fish, indicate that sex-specific associations between heterozygosity and body

size are not an artifact of laboratory rearing. Mature male brook trout, *Salvelinus fontinalis*, from Mykiss Lake, Algonquin Provincial Park, Ontario show significant negative associations between multilocus heterozygosity and fork length whereas heterozygosity was positively associated with fork length in females (Liskauskas & Ferguson, 1991). Differences in growth rates prior to sexual maturation and differential allocation of energy towards gametic and somatic tissue have been proposed as mechanisms for the different relationships between body size and heterozygosity in male and female brook trout.

Studies with other species, especially marine invertebrates, have shown that the heterozygosity-growth correlation changes with age (Rodhouse *et al.*, 1986; Zouros & Foltz, 1987). No direct relationship between gonad development and heterozygosity, however, has been detected (Zouros *et al.*, 1988). Thus, the significantly higher heterozygosity in mature relative to immature male rainbow trout provides some of the most direct evidence that heterozygosity is associated with gonadal development in animals.

The dependence of the heterozygosity-growth correlation on fish age has important implications for studies where the objective is to determine the genetic mechanisms of such correlations. For instance, the lack of detectable and consistent correlations within full-sib families sampled at reproductive age could be taken as evidence for associative overdominance even though the results could be explained by maturation effects. A careful choice of sampling time and concomitant examinations of pooled gamete progeny would partially alleviate this problem.

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