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Heterozygosity and fitness in rainbow trout : marker loci or chromosomal segments?

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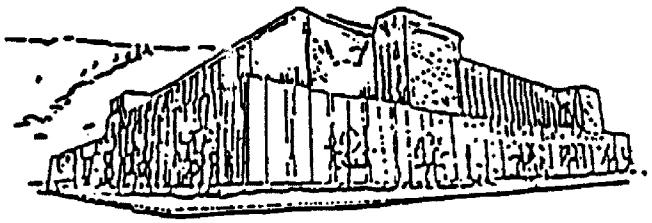

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HETEROZYGOSITY AND FITNESS IN RAINBOW TROUT: MARKER LOCI OR CHROMOSOMAL SEGMENTS?

by

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Heterozygosity and Fitness in Rainbow Trout: Marker Loci or Chromosomal Segments?

Committee Chair: Dr. Fred W. Allendorf

Previous studies, in rainbow trout (*Oncorhynchus mykiss*) and other species, have shown that increased heterozygosity at allozyme loci is correlated with traits associated with fitness. In this study, I test if the allozyme loci themselves are responsible for this observed relationship. The alternate explanation is that this relationship is due to increased heterozygosity at loci in linkage disequilibrium with the enzyme loci examined. If the effect of this heterozygosity-fitness association is due to linked chromosomal segments, then I would expect to find the same relationship between heterozygosity and condition factor at other nuclear loci that do not encode enzymes. I found that individuals that were more heterozygous at ten allozyme loci had significantly greater condition factor in two cohorts of rainbow trout (1996 $P<0.001$; 1997 $P<0.001$). In contrast, there was no evidence at ten microsatellite loci that increased heterozygosity was associated with greater condition factor in individual rainbow trout. Therefore, the observed relationship between heterozygosity at enzyme loci and condition factor appears to be due to the enzyme loci themselves, rather than increased heterozygosity at nearby linked loci.

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INTRODUCTION

A positive correlation has been observed between increased enzyme multilocus heterozygosity and increased fitness components such as growth, fecundity, survival, developmental stability, and oxygen consumption in populations of many species (Allendorf and Leary 1987; Mitton and Grant 1984; Britten 1996). Three hypotheses have been proposed to explain these positive associations between heterozygosity and components of fitness (*e.g.* David 1998; Savolainen and Hedrick 1995; Leary et al. 1992). First, this association may be a consequence of differences in inbreeding among individuals. Second, the associations may be due to heterozygous advantage at the loci examined. Lastly, the loci examined may be in gametic phase (linkage) disequilibrium with loci that affect the traits being studied, resulting in associative overdominance.

There may be associations of allozyme genotypes and components of fitness due to the presence of nonrandom mating or different levels of inbreeding in the population (Ledig et al. 1983). The assumption is that it is the presence of homozygosity at particular loci that results in a reduction in fitness parameters and not heterozygous advantage. Highly homozygous individuals are considered the progeny of closely related parents, while highly heterozygous individuals are the products of more distantly related parents. Individuals with increased homozygosity tend to have a higher likelihood of being homozygous for deleterious recessive alleles at loci spread at random throughout the genome. Therefore, if inbred individuals that have both low heterozygosity and low fitness are pooled in a sample with outbred individuals that have relatively higher values

of both heterozygosity and fitness, then a positive association of individual allozyme heterozygosity and fitness may occur, assuming there is no linkage between the allozyme loci and selected loci (Ledig et al. 1983).

Leary et al. (1987) showed with full-sib families of the Arlee strain of rainbow trout that the inbreeding explanation does not account for the observed associations in these fish. They found the same significant negative correlation between heterozygosity and developmental stability among individuals in full-sib families as they did in individuals randomly sampled from the population. They concluded, therefore, that differences in inbreeding are not responsible for the association between allozyme heterozygosity and developmental stability. This leaves heterozygosity at the loci examined or associative overdominance as possible reasons for the observed association between multilocus enzyme heterozygosity and components of fitness in this population.

Heterozygotes at allozyme loci may have an intrinsically higher fitness than homozygotes. One possible mechanism for this is that heterozygotes at allozyme loci may have increased biochemical efficiency compared to homozygotes because heterozygotes may produce enzymes with different catalytic properties, whereas homozygotes can produce only one form of an enzyme. The biochemical properties of enzymes in metabolic pathways can affect the efficiency of energy flow through an individual (*e.g.* Burton and Feldman 1983). It follows that organisms that utilize this energy more efficiently will have higher fitness compared to their metabolically less efficient counterparts. For example, work done with the phosphoglucose isomerase (PGI)

locus in *Colias* butterflies (Watt 1977; Watt et al. 1983; Watt et al. 1985; Watt 1992) has shown that individuals heterozygous for certain alleles had increased performance in flight time and capacity resulting in increased male mating success, increased survival, and increased female fecundity relative to homozygous individuals.

Linkage disequilibrium between the allozyme loci and other loci may result in apparent heterozygous advantage at the allozyme loci, even if the allozyme loci are having no effect on fitness. Genes are linked on chromosomes, so natural selection acting upon one gene will likely influence the allele and genotype frequencies at other tightly linked polymorphic genes (Lewontin 1964) if the alleles between the genes are nonrandomly associated. If linkage disequilibrium is the cause of the observed relationship between heterozygosity and fitness, then it is this disequilibrium between the marker enzyme loci and any other loci having an effect on the measured fitness factors that would produce the given results. This difference in fitness may be due to either the higher fitness of the heterozygotes, or the reduced fitness of deleterious alleles expressed in the homozygotes. There is, however, some evidence against this associative overdominance hypothesis (Pogson and Zouros 1994; Karl and Avise 1992; see discussion).

The advent of the polymerase chain reaction (PCR) has enabled researchers to study heterozygosity at many additional markers in the genome, specifically microsatellites. This measure allows me to test the hypotheses with a different class of markers, and compare these to results from the enzyme loci. If

the microsatellite loci and the enzyme loci show the same relationship between heterozygosity and fitness, then it can be assumed that the enzyme loci are in linkage disequilibrium with other loci that are driving the relationship. If these two classes of markers show a different association between heterozygosity and fitness, then it is the enzyme loci themselves that are responsible for this observed relationship.

Microsatellite loci have a much higher rate of mutation then enzyme loci (Pamilo and Palsson 1998). They also typically have many more alleles per locus then enzyme loci. Both of these properties of this molecular marker can make the comparisons between them and enzyme loci difficult to interpret. We must, therefore, attempt to account for these discrepancies. Population geneticists have long assumed that alleles at many loci appear to mutate in a stepwise pattern (Ohta and Kimura 1973). The majority of the microsatellite loci analyzed also appear to mutate in this manner, with the newly mutated variants differing from the original allele by one or a few repeat units (Valdez et al. 1993). If this assumption is correct, it follows that the difference between microsatellite alleles as measured with base pair repeat units will provide information about the time since the alleles diverged from a common ancestor (Slatkin 1995; Goldstein et al. 1995).

It has been shown that there is a significant, positive correlation between an increase in this level of coancestry and the increase of some fitness components in natural populations of several vertebrates (Coulson et al. 1998; Coltman et al. 1998). This correlation uses d^2, which is the squared distance in

repeat units between two microsatellite alleles at a locus. Individuals with a small mean d^2 would be from parents of more recent ancestry than individuals with a large mean d^2.

Mean d^2 is a measure that takes into account homozygosity, but in microsatellites with several alleles, the contribution of homozygosity may be swamped by the contribution of allele length variation (Coulson et al. 1998). Mean d^2 can potentially act as a more sensitive technique to examine an association between heterozygosity and fitness. For example, the two studies that used this technique did not find an association between increased heterozygosity and increased fitness at microsatellite loci, but did find an association with d^2. Therefore, individuals that have large differences between allele sizes will have an increased d^2 and should be more heterozygous at surrounding marker loci as well.

Many studies have shown positive associations between multilocus protein heterozygosity and components of fitness in rainbow trout (*Oncorhynchus mykiss*). Leary et al. (1983, 1984a) demonstrated that an increase in an individual's heterozygosity at allozyme loci is positively associated with more stable development, as measured through fluctuating asymmetry of five meristic characters. Danzmann et al. (1988) expanded on this work and found other significant relationships between components of fitness such as body size, oxygen consumption (VO_2), condition factor, and egg size with an increase in observed multilocus heterozygosity. The question remains, however, are these associations due to heterozygosity at the loci examined, or associative overdominance.

In this study, I test if it is the individual enzymes examined or chromosomal segments marked by these enzyme loci that are mainly responsible for an association between multilocus heterozygosity and a fitness component, condition factor. I compared levels of multilocus heterozygosity at both enzyme and microsatellite loci to condition factor. If the relationship between heterozygosity and fitness is observed in both enzymes and noncoding molecular markers in rainbow trout, then it can be concluded that it is the chromosomal segments that are mainly responsible for the association observed between multilocus enzyme heterozygosity and fitness components. If microsatellites do not show this relationship and allozyme loci do, then it is more likely the products of the allozyme loci themselves that are responsible for the observed association.

MATERIALS AND METHODS

Rainbow trout

I used Arlee rainbow trout, maintained by Montana Fish, Wildlife and Parks at the Jocko River State Trout Hatchery, Arlee, MT. This strain of rainbow trout has been maintained in isolation at this hatchery for at least 19 generations (47 years), and has an average amount of genetic variation compared to other natural and hatchery populations of rainbow trout (Leary et al. 1983).

Condition factor was calculated as $(W/L^3)*1{,}000$ (Brown 1957), where "W" and "L" are the weight in milligrams and length in millimeters. Condition factor is an indicator of the overall health of the fish, independent of length. Indeed, when length is regressed on condition factor, there is no trend (1996:

slope=0.000; 1997: slope=0.000). Danzmann et al. (1988) used condition factor in conjunction with egg size and VO_2, and found that all these measures of fitness were significantly positively correlated with an increase in the number of heterozygous enzyme loci measured, indicating that condition factor is consistent with other measures of fitness in rainbow trout.

Two cohorts (1996 N=109; 1997 N=108) of rainbow trout were sampled for condition factor and individual heterozygosity at allozyme and microsatellite loci. I collected the 1996 year class about 22 weeks after hatching and the 1997 year class were collected about 26 weeks post-hatching. Average condition factors for the 1996 and 1997 year classes were 1.202 and 1.277, respectively. These fish were raised in a spring-fed water source ranging in temperature from 8-10°C. Fish were given a lethal dose of MS-222, bagged, put on ice immediately, transported to the laboratory (about 30 minutes), and fish weights and lengths were immediately determined.

Allozymes

Horizontal starch gel electrophoresis was used to screen the products of 10 loci previously known to be polymorphic that encode for 10 enzymes in muscle, liver, or eye tissue of each fish (Danzmann et al. 1988). The common allele in all cases was **100* and are coded as the "1" allele in Table 4. The other alleles in Table 4 are coded from slowest to fastest mobility when more than two alleles are present. The loci, observed alleles, and enzyme commission number (EC) are creatine kinase (*CK-A1*76*; EC 2.7.3.2), beta-glucosaminidase (*bGLUA*70, *80*;

EC 3.2.1.52), isocitrate dehydrogenase (*mIDHP-2*140,* and *sIDHP-1*40, *71, *114;* EC 1.1.1.42), lactate dehydrogenase (*LDH-B2*76* and *LDH-C*95*; EC 1.1.1.27), malate dehydrogenase (s*MDH-B1,2*83, *95*; EC 1.1.1.37), phosphoglucomutase (*PGM-2*90*; EC 5.4.2.2), phospoglycerate kinase (*PGK-2*90, *110*; EC 2.7.2.3) and superoxide dismutase (*sSOD-1*152*; EC 1.15.1.1).

All salmonid fishes, including rainbow trout, are derived from a tetraploid ancestor of some 50-100 million years ago (Ohno 1974; Allendorf and Thorgaard 1984). Because of this tetraploid ancestry some loci are still functionally duplicated, and are referred to as isoloci. It has been shown in Arlee rainbow trout that *sIDHP-1,2** can be treated as two independent loci, with the variation all being contained in the *sIDHP-1** locus (Danzmann et al. 1985). Since it is not possible with isoloci to assign variation to the individual locus of each pair, the one isolocus in this study, *sMDH-B1,2**, was treated as a single tetrasomic locus. Genotypes of *sMDH-B1,2** were scored using the method of Leary et al. (1983), so that individuals were considered to be heterozygous if they possessed two different alleles, regardless of dosage.

Microsatellites

Microsatellite analysis was done at 10 loci: *FGT3; OGO3; OMY77; ONEμ3; ONEμ8; ONEμ11; OTS1; OTS3; SSA14*; and *SSA197* (see Table 1 for annealing temperatures, sequences and their references). Amplification procedures followed those reported by the original authors, with minor modifications. The primer sets were selected on the basis of their scorability,

clearness of the product (*i.e.* the amount of stutter bands), and if the locus was previously shown to be non-duplicated. Amplification products were size fractioned in a 7% denaturing polyacrylimide gel and visualized with a Hitachi FMBIO-100® fluorescent imager.

Data analysis

I performed regression analysis of condition factor on the number of heterozygous loci for each individual separately for both allozymes and microsatellites using MINITAB for Windows, version 11.21. A two-sample T-test was performed to test for significance between the mean condition factor of the heterozygotes versus this mean of the homozygotes at each locus. A one-way ANOVA was performed to test for significant differences in the mean condition factors of the different genotypes.

Hardy –Weinberg proportions, linkage disequilibrium, and frequency of null alleles were analyzed using version 3.1a of GENEPOP (Raymond and Rousset 1995). F_{IS} values were calculated as described in Weir and Cockerham (1984). The sequential Bonferroni test, as described in Rice (1989), was applied to all probability values in tables 2 and 3.

RESULTS

Genotypic distributions

There were several loci that did not conform to Hardy –Weinberg proportions (Tables 2 and 3). *FGT3** and *OGO3** had a significant deficit of

heterozygotes in both year classes. At *OGO3**, two individuals in the 1997 year class had no detectable product after several PCR amplifications, which suggests that these individuals were homozygous for a null allele (**a*). I re-analyzed the genotype distribution at this locus using the maximum likelihood method of Dempster et al. (1977), but now included the null allele. I assumed that the fish lacking *OGO3** amplification were null homozygotes, and recalculated the allele frequencies, assuming the homozygous fish for other alleles either remained homozygotes, or became heterozygotes with the null allele. The estimated frequency of *OGO3*a* under these assumptions was 0.132. These new observed genotypic proportions do not differ significantly from Hardy-Weinberg expectations. The null allele frequency for the 1996 *OGO3*a* was estimated as 0.105 using the method as described in Brookfield (1996).

The method described in Brookfield (1996) was also used to calculate the null allele frequencies for both cohorts at *FGT3*a*, giving 0.063 and 0.108, respectively. When a chi-square test is applied using these new frequencies at each locus, they are no longer significantly different then what is expected under Hardy-Weinberg proportions. However, due to the circular nature of calculating the frequency of the null allele frequencies in these loci could not be adjusted.

Two additional microsatellite loci differed significantly from Hardy-Weinberg expectations (Table 3; *OTS3** in the 1996 cohort and *SSA14** in the 1997 cohort). The enzyme locus *bGLUA** also did not conform to Hardy-Weinberg expectations (Table 2). Because these loci did not consistently differ

from expected allele frequencies in both cohorts, the results were attributed to either a low frequency null allele or sampling error.

I tested for allele frequency differences in each locus across cohorts. Allele frequencies for the ten enzyme loci were consistent across both cohorts, except for *LDH-C** ($P<0.001$) and *sSOD** ($P<0.05$). The microsatellite allele frequencies were also consistent across both cohorts, except for *OMY77** ($P<0.001$). In four loci, there were rare alleles that were detected in only one of the two cohorts (Table 5).

Allozymes

A significant, positive correlation between the number of heterozygous enzyme loci per fish and condition factor was observed in both cohorts (Figure 2; $P<0.001$).

There were ten enzyme loci examined in each year class, giving a total of 20 individual locus comparisons. When all these loci are considered, heterozygotes had a significantly higher mean condition factor then homozygotes (Table 2; ANOVA $P < 0.0001$). There was no significant interaction of mean condition factor in heterozygotes versus homozygotes between the cohorts (Table 2; $P=0.139$), indicating that the positive heterozygosity-fitness correlation in enzymes is not due to a few loci with a large effect, but rather to all the observed loci having some small effect.

It has been suggested that, in enzymes, the common homozygote should have comparable fitness to the heterozygotes, and it is the alternate allele

homozygote that is driving this negative correlation between the level of homozygosity and some fitness parameter (Smouse 1986). By comparing the mean condition factor of the different genotypes at the enzyme loci for both cohorts, I found no such trend (Table 4). An alternate homozygote may have a much-reduced mean condition factor in one cohort, but it was never consistent between the two years of data. Similarly, loci where the common allele had a higher mean condition factor than the other genotypes, including the heterozygotes, did not show this same pattern in the alternate year. The general trend between year classes was that the heterozygotes had higher mean condition factor values then their homozygous counterparts in the population. So the observed relationship may be due to the higher fitness of the heterozygotes, or the reduced fitness of deleterious alleles expressed in the homozygotes.

Microsatellites

No significant positive regression between the number of heterozygous microsatellite loci per fish and condition factor was observed in either of the two cohorts (Figure 2). There were ten microsatellite loci examined in each cohort, for a total of 20 individual locus comparisons. When all these loci are considered, heterozygotes did not have a significantly higher mean condition factor then homozygotes (Table 3).

None of the 10 microsatellite loci had a significant effect in either of the two cohorts. The regression analysis of the association between an increase in the number of heterozygous microsatellite loci and an increase in condition factor did

not change significantly when any single locus was excluded from the analysis, indicating there were no locus of large affect.

There is no indication of a significant positive association between heterozygosity and condition factor in microsatellites even when I corrected for the large number of mutations and, therefore, large number of alleles that are associated with microsatellites. No significant regression was observed between mean d^2 at ten microsatellite loci and condition factor for both cohorts (Figure 3). Several loci have from a 40 to 90 base pair difference between alleles. I adjusted for this large allele size difference by taking an adjusted d^2, which is simply the squared allele size difference divided by the standard deviation of this difference (Coltman et al. 1998). The mean of this adjusted d^2 regressed to the condition factor for these individuals in both cohorts was still not significant (Figure 4).

The R^2 values in the 1996 and 1997 enzyme-condition factor regressions were 0.131 and 0.150, respectively. Many similar studies associating increased enzyme heterozygosity with an increase in fitness parameters usually have values ranging from 0.050 - 0.100 (Pogson and Zouros 1994). This shows that more than 12% of the variation in condition factor can be described by the relatively few loci that were measured in the genome. Conversely, the R^2 values in the 1996 and 1997 microsatellite-condition factor regressions were 0.000 and 0.002, respectively.

DISCUSSION

A significant, positive regression was observed between an increase in multilocus enzyme heterozygosity and condition factor in rainbow trout. An absence of an effect was observed for a similar regression in the microsatellite loci. This suggests that this association is due to the enzyme loci, rather than chromosomal segments marked by these loci, in rainbow trout.

Allozymes

Is the observed relationship that, with an increase in multilocus enzyme heterozygosity there is a positive correlation with an increase in fitness, due to heterozygote superiority, or is it just that alternate allele homozygotes are inferior in fitness? It appears to be that the heterozygotes are superior. The heterozygous fish have a higher condition factor than either the common allele or rare alternate allele homozygote (Table 4). Also, the alternate allele homozygote does not have a significantly different mean condition factor than the common allele homozygote when all the loci are considered (ANOVA $P = 0.307$).

It also appears that this heterozygosity-condition factor association is due to many loci of small effect, rather than a few loci with large effect. The loci which showed a significantly higher mean condition factor for heterozygotes versus homozygotes were not repeated in either of the two cohorts examined.

These results are in concordance with previous work done in rainbow trout. Leary et al. (1993) used null alleles at lactate dehydrogenase (LDH) loci to

determine if individual loci could affect fitness parameters. Null alleles result in no detectable enzymatic activity. Individuals heterozygous or homozygous for null alleles, therefore, have reduced amounts of enzyme produced, which decreases efficiency compared to active allele homozygotes. If the null allele, however, is in linkage disequilibrium with other loci having a heterotic effect on a particular fitness factor, then the null allele heterozygote could have higher or similar fitness compared to "normal" allele heterozygotes. They found that individuals heterozygous for the null allele had reduced LDH concentration and reduced developmental stability, indicating that single loci can have detectable effects on developmental stability. If there is a chromosomal effect, it is apparently being masked by the LDH loci. Leary et al. (1984b) also traced major morphological differences in rainbow trout to a regulatory locus, *PGM-1r**. This locus is responsible for the production of the product of a phosphoglucomutase locus (*PGM-1**) in the liver of rainbow trout. Most rainbow trout do not express *PGM-1** in the liver.

Both the above instances demonstrate that the amount of enzyme present in an individual rainbow trout can have an effect. However, these examples deal with a highly specific class of variation (presence or absence of activity) and may not apply to variation that is normally observed at allozyme loci to the entire genome of an individual (Leary et al. 1993). Thus, the two explanations (associative overdominance versus individual genes) for the relationship between multilocus protein heterozygosity and fitness are still plausible.

This relationship between multilocus enzyme heterozygosity and fitness components has been observed in at least eight different year classes of this population, showing the robustness of the heterozygosity-fitness correlation in enzymes in Arlee rainbow trout (Leary et al. 1983, 1984a, 1985, 1992; Danzmann et al. 1988).

Microsatellites

There was no suggestion of an effect in microsatellite loci that, with an increase in heterozygosity, there is an increase in condition factor. It is possible that this association exists, but due to the intrinsic properties of microsatellite loci we were unable to detect them. Microsatellites have a very high rate of mutation when compared to non-repetitive regions of the genome (Jeffreys et al. 1988). This high mutation rate could mask the observed relationship that, with an increase in the number of heterozygous loci measured, you get in increase in fitness parameters. If this is true, then the associative overdominance hypothesis could still be correct. However, the analysis of d^2 showed this was not true in rainbow trout, and so the original conclusion that the observed association is due to the enzyme loci themselves is still valid.

Two of the measured microsatellite loci appeared to have null alleles present. Null alleles have previously been documented in other microsatellite loci (Spruell, et al. 1999). Null alleles at microsatellite loci could be artifacts because the primers do not exactly match. The occurrence of null alleles at microsatellite loci should not affect the phenotype of the individuals because microsatellites are

in non-coding regions of the genome (Watt 1994). Also, if individuals at microsatellite loci were scored as homozygous, but were actually heterozygous with a null allele, it should not change the results of this study. Indeed, when *FGT3* and *OGO3,* two loci that likely had null alleles, were removed from the regression analysis between the number of heterozygous loci and mean condition factor, there was still no association (1996: P=0.882; 1997: P=0.763).

Alternative explanations

Perhaps allozyme loci are more likely to be in linkage disequilibrium with other loci than microsatellite loci. If this were true, then the conclusion in this study could be invalid, and the enzyme loci measured are actually marking a segment of chromosome that contains other loci that are responsible for the heterozygosity-fitness correlation.

Genes are not distributed at random throughout the genome. In humans, it has been shown that the genome is comprised of two main regions, gene-rich and gene-poor (Williams et al. 1994). Assuming this genome structure can be applied to other vertebrates, it is possible that the measured enzyme loci used in this study are found in these gene-rich regions of the genome, and the microsatellite loci are found in the gene-poor regions of the genome. If this is true, then the associative overdominance hypothesis could still be what is driving the observed correlation between heterozygosity and condition factor in rainbow trout. In pink salmon (*Oncorhynchus gorbuscha*), however, no such patterns in the mapping of allozymes or microsatellites has been observed (Lindner et al. In

prep.; Matsuoka 1998). Also, Schulte et al. (1997) have mapped a microsatellite locus in the enzyme locus *LDH-B2**.

Significance

Other studies have also found results of enzyme analysis that were not consistent with other molecular markers. Pogson and Zouros (1994), for example, compared growth rate and heterozygosity at seven enzyme loci and eight nuclear restriction fragment length polymorphisms (RFLP's) detected by anonymous cDNA probes in the scallop *Placopecten magellanicus*. They found a significant positive association between an increase in growth rate with an increase in heterozygosity at the enzyme loci, but they did not, however, observe this correlation in the same individuals at the DNA markers. They concluded that these differences between enzyme and DNA markers provide evidence against the associative overdominance hypothesis.

These results suggest the need for caution when drawing conclusions from a single class of genetic markers. These genetic markers are used to estimate genetic drift and gene flow that are two of the forces that determine the patterns of genetic structure in natural populations. These forces should affect all loci uniformly if the allelic variation is selectively neutral (McDonald 1994). If allozyme heterozygosity is maintained in a population through overdominance, then the effects of genetic drift would be masked, and population parameters such as N_e would be inflated. If there is selection at allozyme loci, and only one class of genetic markers is used, the patterns of gene flow among populations could be obscured (Karl and Avise 1992, Pogson et al. 1995). However, many studies

show concordance when several different classes of markers are utilized (Allendorf and Seeb submitted). The results presented here do not, however, invalidate the analysis of biochemical genetic data using neutral models because it has been shown that most molecular polymorphisms behave in an essentially neutral fashion (Avise 1994; Lynch 1996).

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Table 1. Ten microsatellite loci used in this study. F>: Forward; R>: Reverse.

Primer	Primer sequence	Annealing temperature	Reference
FGT3	F>5' CAAGAAATTTGTGGAGCGG	53°C	Sakamoto et al. 1994
	R>5' GAAGCCCTGTTTGACTTTTAGC		
OGO3	F>5' CATGTAAGGAATGCAGTTTAGTGTC	55°C	Olsen et al. 1998
	R>5' ACGTTAGGAGTGAGGCGGT		
OMY77	F>5' CGTTCTCTACTGAGTCAT	60°C	Morris et al. 1996
	R>5' GGGTCTTTAAGGCTTCACTGCA		
ONEμ3	F>5' TCTCCTTGGTCTCTCTGTCCCTT	53°C	Scribner et al. 1996
	R>5' CTATCAGCCAATCGCATCAGGAC		
ONEμ8	F>5' AACATTCTGGGATGACAGGGGTA	55°C	Scribner et al. 1996
	R>5' CTGTTCTGCTCCAGTGAAGTGGA		
ONEμ11	F>5' GTTTGGATGACTCAGATGGGACT	56°C	Scribner et al. 1996
	R>5' TCTATCTTTCCTGTCAACTTCCA		
OTS1	F>5' GGAAAGAGCAGATGTTGTT	53°C	Banks et al. 1999
	R>5' TGAAGCAGCAGATAAAGCA		
OTS3	F>5' CACACTCTTTCAGGAG	52°C	Banks et al. 1999
	R>5' AGAATCACAATGGAAG		
SSA14	F>5' CCTTTTGACAGATTTAGGATTTC	52°C	McConnell et al. 1995
	R>5' CAAACCAAACATACCTAAAGCC		
SSA197	F>5' GGGTTGAGTAGGGAGGCTTG	55°C	O'Reilly et al. 1996
	R>5' TGGCAGGGATTTGACATAAC		

Table 2. Allozyme analysis in two cohorts of rainbow trout at ten loci. Expected heterozygosity (H_s), inbreeding coefficient (F_{IS}), and number of alleles (A) are given. Δ= sign of difference in condition factor (CF) between homozygotes and heterozygotes. *=P <0.05, **=P <0.01, ***= P<0.001.

Locus	Cohort	A	H_s	F_{IS}	Homozygotes		Heterozygotes		Δ
					No.	CF	No.	CF	
CK-A1	1996	2	0.090	0.163	99	1.201	8	1.217	+
	1997	2	0.137	-0.080	92	1.276	16	1.307	+*
bGLUA	1996	3	0.628	0.122*	48	1.199	59	1.205	+
	1997	3	0.634	0.051	43	1.276	65	1.283	+
mIDHP-2	1996	2	0.353	0.233	78	1.198	29	1.215	+
	1997	2	0.393	0.105	70	1.266	38	1.306	+*
sIDHP-1	1996	4	0.507	-0.180	42	1.179	65	1.218	+**
	1997	4	0.639	-0.144	27	1.250	81	1.290	+
LDH-B2	1996	2	0.071	-0.052	99	1.203	8	1.187	-
	1997	2	0.054	-0.029	102	1.279	6	1.297	+
LDH-C	1996	2	0.274	0.216	84	1.204	23	1.193	-
	1997	2	0.113	-0.064	95	1.277	13	1.306	+
sMDH-B1,2	1996	3	-	-	63	1.189	44	1.221	+*
	1997	2	-	-	58	1.279	50	1.282	+
PGK-2	1996	3	0.468	0.100	62	1.185	45	1.226	+**
	1997	3	0.489	0.016	56	1.275	52	1.286	+
PGM-2	1996	2	0.122	0.079	95	1.199	12	1.229	+
	1997	2	0.080	-0.031	99	1.274	9	1.350	+*
sSOD-1	1996	2	0.377	-0.090	63	1.192	44	1.217	+
	1997	2	0.284	-0.079	75	1.263	33	1.320	+***

Table 3. Microsatellites analysis in two cohorts of rainbow trout at ten loci. Expected heterozygosity (H_s), inbreeding coefficient (F_{IS}), and number of alleles (A) are given. Δ= difference in condition factor (CF) between homozygotes and heterozygotes. *= P<0.05, **= P<0.01, ***= P<0.001.

					Homozygotes		Heterozygotes		
Locus	**Cohort**	**A**	**H_s**	**F_{IS}**	**No.**	**CF**	**No.**	**CF**	**Δ**
FGT3	1996	11	0.840	0.137***	30	1.198	79	1.205	+
	1997	10	0.850	0.234***	38	1.288	70	1.276	-
OGO3	1996	5	0.650	0.266***	57	1.206	52	1.200	-
	1997	5	0.708	0.240***	49	1.266	57	1.292	+
OMY77	1996	9	0.708	0.081	38	1.193	71	1.208	+
	1997	9	0.652	0.071	42	1.291	66	1.273	-
ONEU3	1996	5	0.501	-0.026	53	1.204	56	1.202	-
	1997	5	0.577	0.125	54	1.290	54	1.270	-
ONEU8	1996	4	0.595	0.044	47	1.213	62	1.195	-
	1997	4	0.617	-0.011	40	1.266	68	1.289	+
ONEU11	1996	2	0.448	-0.188	51	1.208	58	1.198	-
	1997	3	0.463	-0.069	55	1.289	53	1.271	-
OTS1	1996	7	0.754	-0.021	25	1.202	84	1.203	+
	1997	8	0.797	0.010	23	1.261	85	1.285	+
OTS3	1996	5	0.653	0.003*	38	1.187	71	1.212	+
	1997	5	0.666	0.077	42	1.273	66	1.285	+
SSA14	1996	8	0.808	-0.022	19	1.183	90	1.207	+
	1997	9	0.804	0.179***	36	1.277	72	1.282	+
SSA197	1996	2	0.500	0.064	58	1.213	51	1.192	-
	1997	2	0.492	0.012	55	1.273	53	1.288	+

Table 4. Allele frequencies and mean condition factors (CF) for each genotype at ten enzyme loci in two cohorts of rainbow trout. See text for allele mobilities.

	CK-A1		*bGLUA*		*mIDHP-2*		*sIDHP-1*		*LDH-B2*	
Allele	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997
1	0.953	0.926	0.495	0.481	0.771	0.731	0.659	0.518	0.963	0.972
2	0.047	0.074	0.266	0.259	0.229	0.269	0.229	0.261	0.037	0.028
3	-	-	0.238	0.259	-	-	0.051	0.119	-	-
4	-	-	-	-	-	-	0.061	0.101	-	-
	CF		CF		CF		CF		CF	
Genotype	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997
11	1.201	1.276	1.199	1.292	1.200	1.274	1.179	1.250	1.204	1.279
12	1.217	1.307	1.226	1.304	1.215	1.306	1.215	1.300	1.187	1.297
22	1.183	-	1.218	1.241	1.183	1.222	1.303	1.254	-	-
13	-	-	1.193	1.269	-	-	1.209	1.290	-	-
23	-	-	1.207	1.282	-	-	1.068	1.257	-	-
33	-	-	1.142	1.276	-	-	-	1.434	-	-
14	-	-	-	-	-	-	1.276	1.316	-	-
24	-	-	-	-	-	-	1.203	1.234	-	-
34	-	-	-	-	-	-	-	1.303	-	-
44	-	-	-	-	-	-	-	1.287	-	-

Table 4 continued. Allele frequencies and mean condition factors (CF) for each genotype at ten enzyme loci in two cohorts of rainbow trout. See text for allele mobilities.

	LDH-C		*sMDH-B1,2*		*PGK-2*		*PGM-2*		*sSOD-1*	
Allele	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997
1	0.836	0.940	0.867	0.766	0.696	0.676	0.935	0.958	0.748	0.829
2	0.164	0.060	0.114	0.141	0.121	0.134	0.065	0.042	0.252	0.171
3	-	-	0.019	-	0.182	0.190	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-

	CF		CF		CF		CF		CF	
Genotype	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997
11	1.205	1.277	1.191	1.279	1.184	1.287	1.199	1.274	1.200	1.261
12	1.197	1.306	1.246	1.301	1.261	1.301	1.226	1.350	1.217	1.320
22	1.192	-	1.271	-	1.178	1.107	1.232	-	1.096	1.343
13	-	-	1.213	-	1.199	1.289	-	-	-	-
23	-	-	1.074	-	1.223	1.233	-	-	-	-
33	-	-	-	-	1.188	1.232	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-	-	-
34	-	-	-	-	-	-	-	-	-	-
44	-	-	-	-	-	-	-	-	-	-

Table 5. Allele frequencies of ten microsatellite loci in two cohorts of rainbow trout. The size of each allele, in base pairs, are included in the key.

	FGT3		*OGO3*		*OMY77*		*ONEU3*		*ONEU8*	
Allele	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997
1	0.266	0.229	0.005	0.014	0.014	0.009	0.665	0.573	0.064	0.046
2	0.046	0.096	0.133	0.128	0.477	0.541	0.037	0.060	0.385	0.353
3	0.018	0.023	0.509	0.368	0.142	0.151	0.055	0.064	0.500	0.495
4	0.115	0.193	0.252	0.220	0.183	0.161	0.229	0.294	0.050	0.106
5	0.106	0.073	0.101	0.138	0.009	0.041	0.014	0.009	-	-
6	0.202	0.170	-	0.132	0.005	0.005	-	-	-	-
7	0.073	0.101	-	-	0.041	0.055	-	-	-	-
8	0.041	0.014	-	-	0.050	0.005	-	-	-	-
9	0.005	0.018	-	-	0.078	0.032	-	-	-	-
10	0.119	0.083	-	-	-	-	-	-	-	-
11	0.009	-	-	-	-	-	-	-	-	-

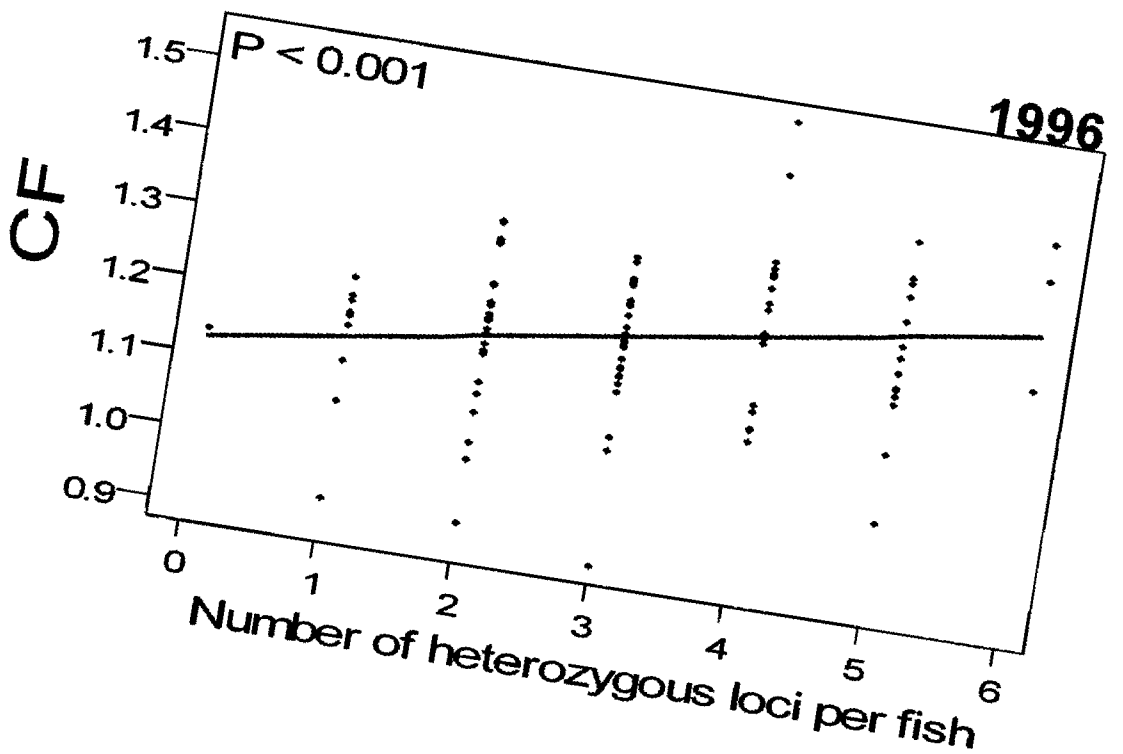
KEY:					
	1=194	1=191	1=97	1=197	1=152
	2=206	2=195	2=99	2=199	2=154
	3=212	3=197	3=103	3=201	3=156
	4=216	4=199	4=105	4=217	4=158
	5=218	5=203	5=109	5=219	
	6=220	6=null	6=111		
	7=222		7=115		
	8=224		8=129		
	9=230		9=143		
	10=236				
	11=246				

Table 5 continued. Allele frequencies of ten microsatellite loci in two cohorts of rainbow trout. The size of each allele, in base pairs, are included in the key.

	ONEU11		*OTS1*		*OTS3*		*SSA14*		*SSA197*	
Allele	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997
1	0.661	0.647	0.422	0.340	0.028	0.014	0.280	0.326	0.518	0.564
2	0.339	0.344	0.124	0.193	0.138	0.110	0.161	0.188	0.482	0.436
3	-	0.009	0.165	0.142	0.486	0.468	0.055	0.046	-	-
4	-	-	0.128	0.115	0.298	0.303	0.096	0.133	-	-
5	-	-	0.064	0.101	0.050	0.106	0.037	0.028	-	-
6	-	-	0.028	0.009	-	-	0.106	0.106	-	-
7	-	-	0.069	0.087	-	-	0.018	0.023	-	-
8	-	-	-	0.014	-	-	0.247	0.147	-	-
9	-	-	-	-	-	-		0.005	-	-
10	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-

KEY:					
	1=142	1=155	1=77	1=135	1=110
	2=144	2=157	2=79	2=137	2=114
	3=146	3=161	3=81	3=145	
		4=163	4=83	4=147	
		5=167	5=85	5=149	
		6=169		6=151	
		7=245		7=153	
		8=165		8=157	
				9=133	

Figure 1. Regression of condition factor (CF) on the number of heterozygous enzyme loci per fish in two cohorts of rainbow trout.



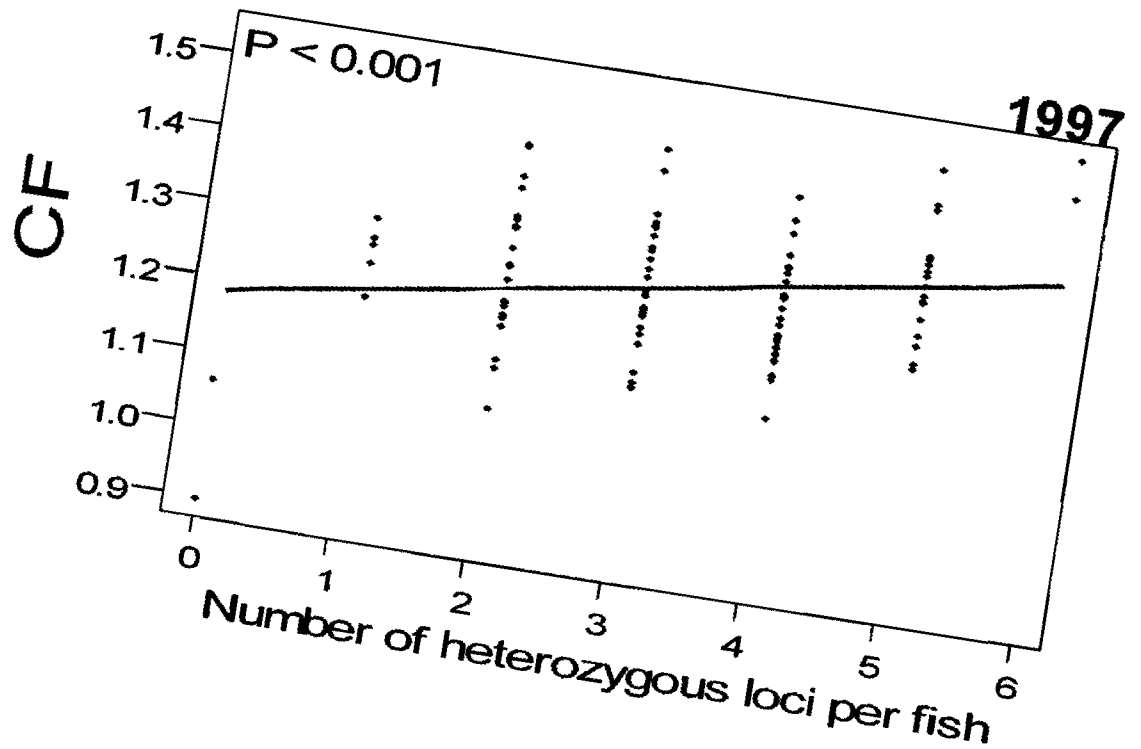


Figure 2. Regression of condition factor (CF) on the number of heterozygous microsatellite loci per fish in two cohorts of rainbow trout.

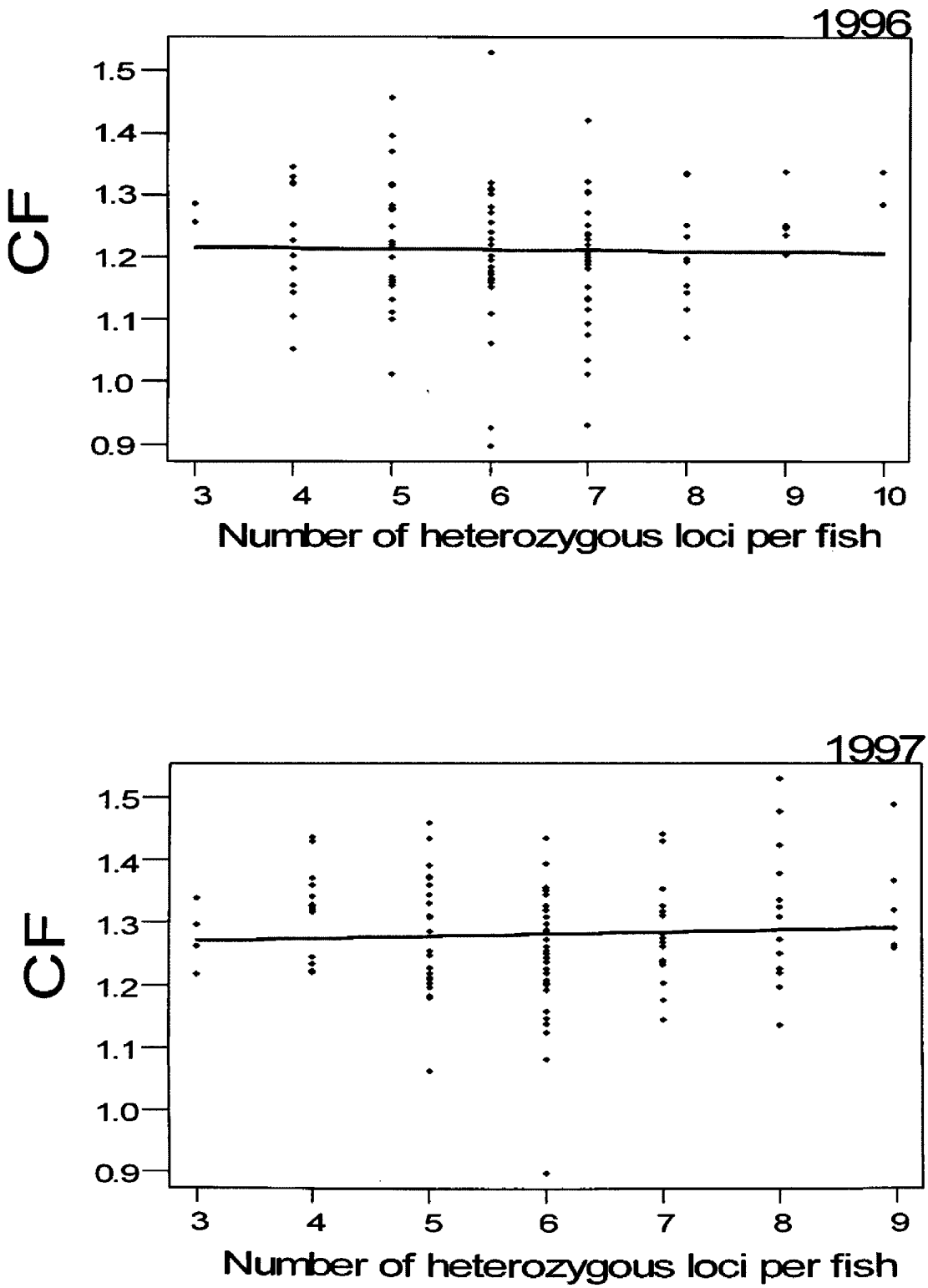


Figure 3. Regression of condition factor (CF) on d^2 at microsatellite loci in two cohorts of rainbow trout.

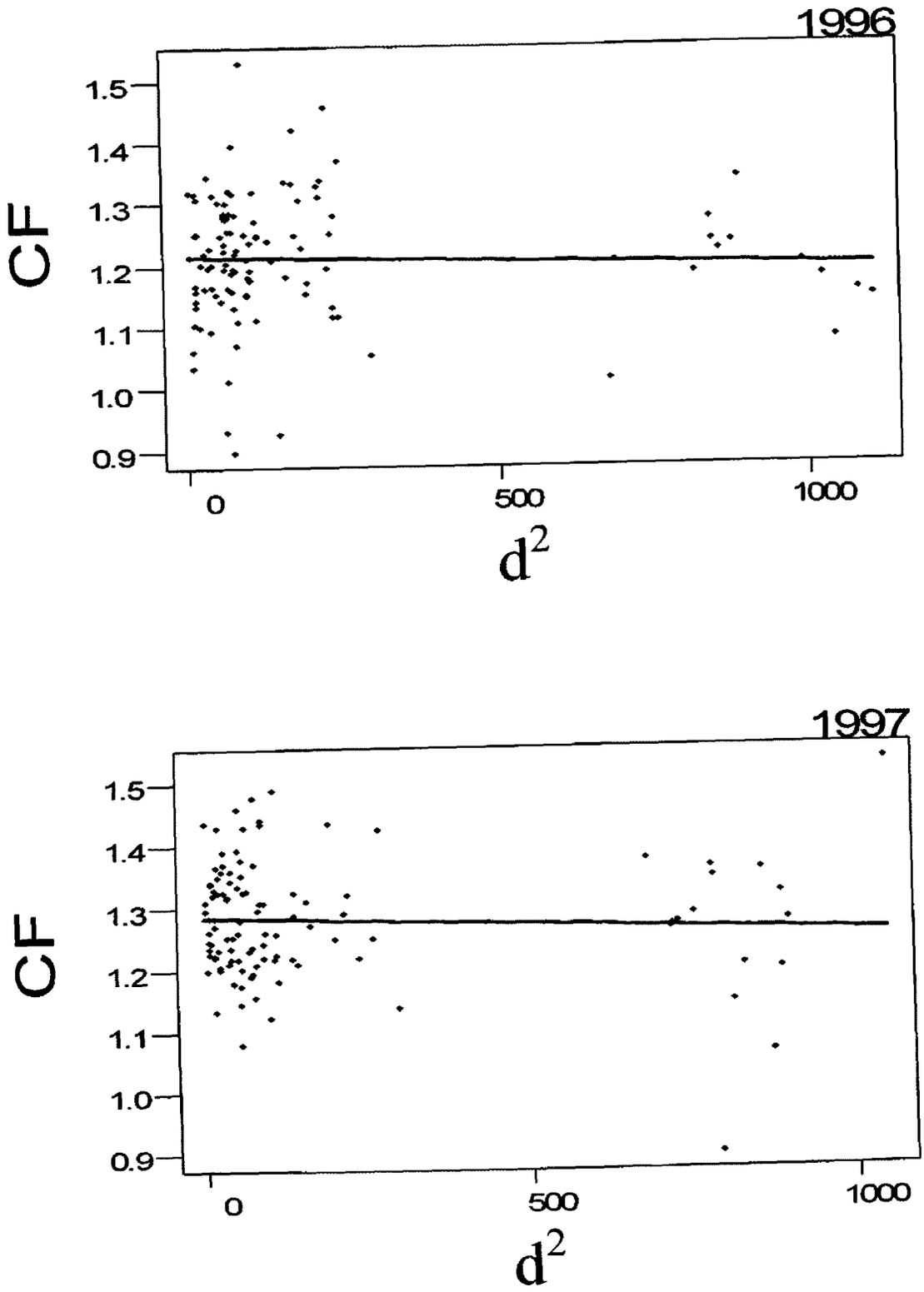


Figure 4. Regression of condition factor (CF) on d^2, adjusted to account for large allele size differences (see text for detailed explanation) in microsatellite loci in two cohorts of rainbow trout.