INCREASING LEVELS OF GENETIC VARIATION IN ERWIN RAINBOW TROUT BY BACKCROSSING WITH ARLEE X ERWIN HYBRIDS

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Population Genetics Laboratory Report 89/5
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ABSTRACT

It is important to maintain genetic variation in broodstocks used for management purposes. The loss of genetic variation can adversely affect the performance of a broodstock in the wild and the hatchery environment. When the level of genetic variation in a broodstock is low, it can be enhanced by hybridization between stocks. The amount of variation introduced, however, depends on how much genetic divergence exists between the parental strains and the proportion of hybrid or backcross matings.

Compared to most other rainbow trout, Oncorhynchus mykiss, broodstocks, the Erwin strain maintained at the Ennis National Fish Hatchery has reduced genetic variation. Hybrids between the Erwin and Arlee strains have 24% more genetic variation, estimated by protein electrophoresis, than Erwin rainbow trout. A new broodstock, genetically more variable than the Erwin strain, can be created by backcrosses with the hybrids. In order for the increased genetic variation to be biologically meaningful, we suspect it will have to be at least 5%. This requires at least 25% of the matings to be backcrosses.

INTRODUCTION

State, federal, and Native American management agencies maintain numerous hatchery populations of salmonid fishes. These populations serve mainly as a readily available source of fish to augment natural reproduction or to establish natural reproducing populations in barren or rehabilitated waters. For threatened or endangered species, hatchery populations may be established primarily to serve as a captive source of some of the genetic diversity of the species. Subsequently, they may be used in restoration programs to establish other natural reproducing populations of the species.

From a genetics perspective, the main goal of the above hatchery programs should be to maintain genetic variation. The fishes are often introduced into diverse environments and may be expected to survive for appreciable amounts of time. This is especially true when the goal of an introduction is the establishment of a natural reproducing population. In these cases, genetic variation is essential in order for the populations to adapt to the new environment through the process of natural selection.

Loss of genetic variation can also adversely affect the performance of a broodstock in the hatchery environment. In fishes, inbreeding (mating of closely related individuals) is associated with reduced survival, reduced growth, and an increased percentage of morphologically deformed individuals (Kirpichnikov 1981; Kincaid 1983). In many organisms, including fishes, heterozygosity (a measure of genetic variability) has been found to be positively associated with survival, growth, disease resistance, and the ability of individuals to develop normally (Mitton and Grant 1984; Allendorf and Leary 1986; Zouros and Foltz 1987).

Protocols for maintaining genetic variation in hatchery broodstocks have been outlined in detail (Allendorf and Ryman 1987). The efficacy at which a hatchery program is maintaining genetic variation, however, is best evaluated through a monitoring program. A comprehensive monitoring program includes a temporal sequence of biochemical genetic and morphological attributes of a population (e.g. Leary et al. 1985a; Allendorf and Ryman 1987).

The United States Fish and Wildlife Service (USFWS) maintains a number of rainbow trout, Oncorhynchus mykiss, broodstocks at the Ennis National Fish Hatchery, Ennis, Montana. Since 1982 in cooperation with the USFWS we have been monitoring the genetic characteristics of these broodstocks. Compared to most other broodstocks, the Erwin strain has reduced genetic variation (Leary et al. 1983a). The USFWS now considers it desirable to increase the amount of genetic variation in this broodstock. A new, genetically more variable broodstock than the Erwin strain can be created by hybridization and backcrossing; that is crossing Erwin rainbow trout with hybrids between Erwin and another rainbow trout strain. The amount of genetic variation gained, relative to Erwin rainbow trout, however, depends on how much genetic divergence exists between the parental strains and the proportion of backcross matings.

Although more genetic variation can be gained through hybridization than backcrossing, the USFWS favors the latter procedure in this case because it desires to maintain a broodstock that spawns during the summer. At the Ennis National Fish Hatchery, the Erwin strain is the only one with this characteristic. Hybrids between late spawning Erwin and early spawning individuals of a fall strain are possible. Because spawning time has a genetic component in rainbow trout (Leary et al. 1989), however, it is

suspected these hybrids will spawn later than is usual for Erwin rainbow trout. It is hoped that backcrosses will spawn at a time more characteristic of Erwin rainbow trout.

In this report we show that, based on protein electrophoresis, hybrids between the Arlee and Erwin strains have 24% more genetic variation than Erwin rainbow trout. Backcrossing the hybrids and Erwin strains, therefore, will create a broodstock genetically more variable than the latter. In order for this increased genetic variation to be biologicall meaningful, however, we expect it will require at least 25% backcross matings. We also present evidence that indicates that genetic differences exist between the Arlee strain at the Ennis National Fish Hatchery and the one it was founded from and that the genetic characteristics of the Erwin strain have not been temporally stable.

History of the strains

The Montana Department of Fish, Wildlife, and Parks (MDFWP) founded the Arlee strain in the 1940's and maintains it at the Jocko River State Trout Hatchery, Arlee, Montana. The strain spawns from late October through mid-December. By selecting early spawning individuals, the MDFWP established another Arlee strain that spawns from mid-August to late October. These are referred to as the regular and early Arlee strains, respectively.

The USFWS established its Arlee strain by acquiring eggs from the MDFWP. They received approximately 300,000 eyed eggs 18 December 1978 and about 200,000 more eyed eggs 21 December 1979. Assuming about three weeks until the eyed stage, these eggs came from fish spawned in late November. The USFWS, therefore, acquired regular Arlee rainbow trout. At the Ennis National Fish Hatchery, the fish are spawned in October and November.

The Erwin strain was founded (date unknown) by the USFWS at the Wytheville National Fish Hatchery, Wytheville, Virginia. Subsequently (date unknown), it was transferred to the Erwin National Fish Hatchery, Erwin, Tennessee. In 1978, it was transferred to the Ennis National Fish Hatchery where it is spawned June through September.

The Arlee x Erwin strain was created at the Ennis National Fish Hatchery 21 October 1987. Personnel mated 31 early maturing, two year-old Arlee females with 18 late maturing, two year-old Erwin males.

METHODS

Since 1979, we have annually obtained biochemical genetic information from regular Arlee rainbow trout maintained at the Jocko River State Trout Hatchery. All these samples came from adults during the spawning season. In this report, we use information only from the 1979 (60 three year-old females) and 1987 (34 four year-old females, 35 three year-old males) samples. We used these samples because the Ennis National Fish Hatchery acquired eggs during the 1979 spawning season and we also obtained a sample (N=50) of the 1987 year-class of Arlee rainbow trout maintained at the Ennis National Fish Hatchery . These fish were one year-old juveniles being raised for future broodstock. We can test for genetic differences between the broodstocks, therefore, by comparing progeny at the Ennis National Fish Hatchery to adults spawned at the Jocko River State trout hatchery in a year in which the former was founded and ones used to create the 1987 year-class.

We obtained two samples of juvenile Erwin rainbow trout maintained at the Ennis National Fish Hatchery. Fish (N=60) from the 1982 year-class were obtained from the Creston National Fish Hatchery, Creston, Montana. These

fish were being raised until a size suitable for stocking. Fish (N=50) from the 1988 year-class were obtained from the Ennis National Fish Hatchery. These six month old fish were being raised for future broodstock. We also obtained from the Ennis National Fish Hatchery 50 one year-old juveniles produced from the matings performed to create the Arlee x Erwin strain.

Electrophoresis

Horizontal starch gel electrophoresis was used to determine the genetic characteristics of the sampled populations at genes producing enzymes present in eye, liver, or muscle tissue. Electrophoresis followed the procedures described by Allendorf and Utter (1979). Buffers used to make the gels and stains used to reveal the position of particular enzymes in the gel after electrophoresis followed the protocols of Harris and Hopkinson (1976) and Allendorf et al. (1977). Nomenclature of loci (genes) and alleles (form of a gene) follows the procedures of Leary et al. (1987) with modifications to conform with recommendations recently proposed by an American Fisheries Society committee (Shaklee et al. 1989). With the exception of the 1979 Arlee and 1982 Erwin samples, the following enzymes with the loci that encode them in parentheses were analyzed in each fish: adenylate kinase (AK-1,2), alcohol dehydrogenase (ADH), aspartate aminotransferase (AAT-1,2,3,4), creatine kinase (CK-A1,2; CK-B; CK-C1,2), dipeptidase (PEPA-1,2), glucose phosphate isomerase (GPI-A; GPI-B1,2), glyceraldehyde-3-phosphate dehydrogenase (GAPDH-3,4), glycerol-3-phosphate dehydrogenase (G3PDH-1,2), iditol dehydrogenase (IDDH), isocitrate dehydrogenase (sIDH-1,2; mIDH-1,2), lactate dehydrogenase (LDH-A1,2; LDH-B1,2; LDH-C), malate dehydrogenase (sMDH-A1,2; sMDH-B1,2; sMDHp-1,2; mMDHp-2); N-acetyl-beta-glucosaminidase (bGLUA), phosphoglucomutase (PGM-1,2; PGM-1r), phosphogluconate dehydrogenase (PGDH), phosphoglycerate kinase (PGK-

2), superoxide dismutase (sSOD-1), tripeptide aminopeptidase (PEPB), and xanthine dehydrogenase (XDH). In the 1982 Erwin sample, the products of bGLUA, CK-C1,2, and PGK-2 were not analyzed. In the 1979 Arlee sample, the products of only ten polymorphic (genetically variable) loci were analyzed.

In rainbow trout, the common allele at some pairs of loci produces a protein with identical function and electorphoretic mobility. For example, AAT-3 and AAT-4 both produce an aspartate aminotransferase present in muscle. After electrophoresis, the protein produced from the common allele at each locus occupies the same position in the gel. Such pairs of loci are commonly termed isoloci and their existence can conclusively be detected only when one or both loci are polymorphic. In these situations, however, it is not possible to determine at which locus of the pair a variant allele exists. In order to estimate allele frequencies at the isoloci (AAT-3,4, sIDH-1,2, sMDH-A1,2, sMDH-B1,2, sMDHp-1,2), therefore, we considered each pair to be a single gene with four instead of two copies per individual.

We used contingency table chi-square analysis to determine if allele frequencies at polymorphic loci significantly differed between samples. If so, this would indicate the samples came from genetically different populations. Significant differences between year-classes would indicate the genetic characteristics of the strain may not have been temporally stable.

We used average observed heterozygosity as an estimate of the amount of genetic variation in the samples. At any particular locus, an individual that possesses two different alleles is said to be heterozygous and genetically variable. Individuals that possess two copies of the same allele at a locus are termed homozygous and are genetically invariant at the locus under consideration. Average observed heterozygosity is calculated by: 1)

determining the number of genes at which an individual was heterozygous, 2) averaging these values over all individuals in the sample and 3) dividing this value by the number of loci analyzed. Thus, an average observed heterozygosity of 0.10 indicates that on the average individuals in the sample were heterozygous at ten percent of the loci examined. We used the Wilcoxon two-sample test to determine if average observed heterozygosity significantly differed between samples. If so, this would indicate the samples came from populations with different levels of genetic variation. Fish were considered heterozygous at isoloci using the criterion of Leary et al. (1983b).

Meristics

In salmonid fishes, the counts of bilateral meristic characters may differ between the left and right side of an individual. This condition is generally termed asymmetry and represents a developmental accident because the counts should be the same on each side. Previous studies have shown that the number of asymmetric characters per individual in populations of salmonid fishes is negatively associated with the number of heterozygous protein loci (Leary et al. 1984). Furthermore, the loss of substantial genetic variation from a population is often associated with unusually high levels of asymmetry (Leary et al. 1985a,c). Thus, differences in levels of asymmetry between year-classes of a strain may indicate a loss of genetic variation. Furthermore, a high level of asymmetry in a strain may indicate a substantial loss of genetic variability.

We took the counts of five bilateral meristic characters on the left and right side of each fish in the samples except those from the Jocko River State Trout Hatchery: rays in the pectoral and pelvic fins, gill rakers on the upper and lower first branchial arches, and mandibular pores. The Wilcoxon

two-sample test was used to determine if the mean total count (left plus right) of the characters and average number of asymmetric characters per individual significantly differed between samples.

RESULTS

Arlee Rainbow Trout

Comparison of allele frequencies indicates that the genetic characteristics of Arlee rainbow trout maintained at the Jocko River State Trout Hatchery may have changed between 1979 and 1987. Significant allele frequency differences at two loci exist between the samples (Table 1). These differences could reflect true genetic differences or chance departures from homogeneity due to sampling error. In order to distinguish between these possibilities we compared the chi-square value at each locus to the modified level of significance proposed by Cooper (1968). This accounts for encountering chance departures from homogeneity when multiple tests are performed between samples by dividing the 0.05 level of significance by the number of tests (in this case eight). The chi-square value at sIDH-1,2 has a probability level below the modified significance of 0.006. Thus, we conclude slight genetic differences exist between Arlee rainbow trout spawned in 1979 and 1987 at the Jocko River State Trout Hatchery.

Genetic differences also exist between the 1987 year-class of Arlee rainbow trout from the Ennis National Fish Hatchery and the samples from the Jocko River State Trout Hatchery. There are significant allele frequency differences between the 1979 Jocko River and Ennis samples at three loci and all these differences are significant at the modified level of 0.006 (Table 2). At six loci, the allele frequencies are statistically heterogenous

between the 1987 Jocko River and Ennis samples and at sMDH-B1,2 the difference is significant at the modified level of 0.003 (Table 2). Despite the allele frequency differences between these latter two samples, they have comparable levels of average observed heterozygosity ($P > 0.10$; Table 2). We could not calculate average observed heterozygosity for the 1979 Jocko River sample because genotypes of individuals were not available.

Erwin Rainbow Trout

The available data indicate genetic differences exist between our samples of the 1982 and 1988 year-classes of Erwin rainbow trout. Significant allele frequency differences exist between the samples at three loci and at LDH-B1 and sSOD-1 the differences are significant at the modified level of 0.006 (Table 3). Despite these genetic differences, however, both samples have comparable levels of average observed heterozygosity ($P > 0.50$; Table 3) and asymmetry ($P > 0.50$; Table 5).

Arlee x Erwin Compared to Erwin Rainbow Trout

The primary purpose of creating the Arlee x Erwin strain was to produce fish that would ripen at about the same time as the Erwin strain. This would allow a high proportion of backcrosses to be made with the Erwin strain, and the potential to create a genetically more variable strain with Erwin spawning characteristics. The amount of genetic variation gained, however, will depend on the proportion of backcross matings and the amount of genetic divergence between the strains. Because of the latter factor, we compared the genetic characteristics of the 1988 Erwin year-class and Arlee x Erwin fish.

The Erwin and Arlee x Erwin strains are genetically quite different. Significant allele frequency differences exist between them at eight loci. At bGLUA, G3PDH-1, sIDH-1,2, and sMDH-B1,2 the differences are significant at the

modified level of 0.004 (Table 4). Furthermore, the Arlee x Erwin strain has 24% more genetic variability, as measured by average observed heterozygosity, than the Erwin strain ($P < 0.05$; Table 4). Despite this difference in heterozygosity, however, both strains have similar levels of asymmetry ($P > 0.50$; Table 6).

Comparison of Meristic Counts

In general, fish in our sample of the 1982 Erwin year-class have higher meristic counts than those in the 1988 year-class. Out of five meristic characters, the mean counts of three significantly differ between the samples and in all these cases the 1982 fish have a higher mean (Table 5). In contrast to these results, fish from the 1987 Arlee, 1988 Erwin, and 1987 Arlee x Erwin year-classes all have similar meristic counts. None of the 15 pairwise comparisons between these samples were statistically significant (Table 6).

DISCUSSION

Genetic Differences between Year-Classes and Arlee Rainbow Trout Strains

Because our 1979 and 1987 Jocko River Arlee rainbow trout samples constitute adults that were ripe during only a portion of the spawning season, this confounds interpretation of the genetic differences between them. Previous data indicate that genetic differences exist among the progeny of fish in this strain spawned on different days of the season (Leary et al. 1989). In 1979, we sampled females that were ripe during the early part of the spawning season and in 1987 we sampled individuals ripe during the middle portion of the season. The genetic differences between these samples, therefore, probably reflect, at least partially, differences among individuals

spawning at different times. We cannot exclude the possibility, however, that these differences also may partially reflect genetic changes in the Arlee broodstock over generations.

Arlee rainbow trout maintained at the Ennis National Fish Hatchery were founded from individuals spawning in the middle to later portion of the season at the Jocko River State Trout Hatchery. Because this represents only a small part of the spawning season we expected genetic differences between the Jocko River and Ennis broodstocks. Whether the observed differences are totally attributable to establishing the Ennis broodstock from a restricted part of the spawning season or also partially reflect changes among generations in one or both strains cannot be determined.

In 1983 and 1984, all fish in the Erwin strain that would potentially produce future broodstock were screened for the presence of the LDH-B1* null allele. All fish with this allele were not allowed to produce future broodstock. This selection has been successful. We did not detect this allele in the sample from the 1988 year-class. Because of sampling error, we cannot be certain the allele no longer exists in the broodstock. It is unlikely ($P < 0.05$), however, that if it is present it exists at a frequency greater than 0.04.

The above selection may partially account for genetic differences at loci other than LDH-B1 between the 1982 and 1988 Erwin year-class samples. During the selection process, future broodstock was retained from only two spawning dates each year. Since future broodstock came from only a small part of the spawning season in these years, allele frequencies very likely changed at loci other than that at which the selection was targeted.

The amount of genetic change in the Erwin strain attributable to selection, however, may not be as great as the data suggest. The 1982 year-class sample came from production fish being raised at the Creston National Fish Hatchery. These fish were probably produced from only a fraction of the spawning season and may not accurately reflect the genetic characteristics of the Erwin strain.

Meristic Comparisons

The counts of rainbow trout meristic characters are affected by genetic (Leary et al. 1985b) and environmental variation (Garside 1966; MacCrimmon and Kwain 1969; MacGregor and MacCrimmon 1977). Our data indicate that Arlee and Erwin rainbow trout have similar mean meristic counts when raised at the Ennis National Fish Hatchery. Ferguson and Danzmann (1987) also found that these fish had similar mean meristic counts when raised in the same environment. Because of the substantial genetic differences between the strains, we would have predicted the fish would have different meristic counts. We find these results, therefore, somewhat surprising.

There is good evidence indicating that developmental differences exist between Arlee and Erwin rainbow trout. As measured by hatching time, Arlee develop faster than Erwin rainbow trout (Ferguson et al. 1985a). Hybrids between these strains also developed at rates different than the parental strains and tended to have higher mean meristic counts (Ferguson et al. 1985b, Ferguson and Danzmann 1987). Apparently the developmental differences between Arlee and Erwin rainbow trout are not reflected in the counts of the meristic characters in individuals from the pure strains but are reflected by higher counts in hybrids.

In contrast to the above results, we found that Arlee x Erwin rainbow trout had meristic counts similar to the parental strains. We are not certain why these two comparisons between hybrids and parental strains produced different results. The discrepancy may partially reflect the fact that the two data sets are not strictly comparable. Ferguson and Danzmann (1987) produced hybrids from the parents they used to create individuals of the parental strains. In our data, individuals used to produce hybrids were not used to create parental strain fish. Thus, unlike the data of Ferguson and Danzmann (1987) our results are potentially confounded by the hybrids and parental individuals not having a common parentage.

Unlike the Arlee, Erwin and Arlee x Erwin strains, we detected substantial meristic differences between the two Erwin year-classes (Table 5). These differences could partially reflect the genetic differences between the year-classes. We suspect, however, they are mainly the consequence of raising the fish in two different environments. The water temperature at Ennis National Fish Hatchery is a constant 12°C while at Creston National Fish Hatchery it fluctuates between 3 and 12°C. Such differences in temperature are known to be capable of altering the counts of rainbow trout meristic characters (Garside 1966; MacGregor and MacCrimmon 1977; Leary et al. unpublished data).

Asymmetry Comparisons

Like meristic counts, levels of asymmetry in fishes are affected by genetic (Leary et al. 1984, 1985a,c) and environmental variation (Valentine and Soule' 1973; Ames et al. 1979; Jagoe and Hanes 1985; Leary et al. unpublished data). Despite the genetic differences between the two Erwin year-class samples they had comparable levels of asymmetry. We feel this

mainly reflects the fact that these differences did not appreciably alter levels of genetic variation in the year-classes and thus disrupt normal development. It is more likely that the different environments in which these fish were raised would result in different rather than similar levels of asymmetry.

Although the Arlee x Erwin fish are genetically more variable than Erwin rainbow trout, levels of asymmetry do not vary significantly between them or Arlee rainbow trout. Ferguson (1986) also found Arlee and Erwin rainbow trout had similar levels of asymmetry but she observed reduced asymmetry in hybrids between the strains. Again we cannot completely reconcile this discrepancy, but suspect it is at least partially due to our hybrids and parental individuals not sharing common parents while those of Ferguson's did. These results, regardless of their cause, stress what we feel is an important point. Asymmetry as a means of monitoring levels of genetic variation is likely to be much more informative when comparisons are made within rather than between strains.

Backcrossing Arlee x Erwin to Erwin Rainbow Trout

The amount of genetic variation gained by crossing hybrids and Erwin rainbow trout is highly dependent on the proportion of backcross matings performed. In order to demonstrate this point, we have calculated the expected levels of genetic variation in Erwin rainbow trout for various proportions of backcross matings (Table 7). The parameter calculated is average expected heterozygosity. This is the expected percentage of heterozygous individuals per locus under a random mating model, which at a locus is one minus the sum of the squared allele frequencies. These values are summed over all loci and divided by the total number of loci examined to

yield average expected heterozygosity. A value of 0.05, therefore, means that on the average individuals in the population will be heterozygous at five percent of their loci.

In the above calculation, we assumed the allele frequencies in the individuals used in the backcross matings will be the same as those in the parental strains. Thus, in backcross progeny the allele frequencies will be the average of the parental frequencies. If X is the proportion of backcross matings, P_E the frequency of an allele in Erwin rainbow trout, and P_H the frequency of this allele in the backcross progeny, then the frequency of the allele in the backcross population will be $XP_H + (1 - X)P_E$.

In order for an increase in heterozygosity to be biologically meaningful, we suspect it will have to be on the order of five percent or more. This is because a ten percent reduction in heterozygosity through inbreeding is usually required to have a detectable deleterious affect on phenotypic characters (Falconer 1981). From Table 7, it is apparent that in order to attain about a five percent increase in heterozygosity more than 25% of the matings should be backcrosses. This should not be interpreted to mean that if nature is so unkind as to make it impractical to perform at least 25% backcross matings that none should be performed. More matings can be conducted in the future.

For a given percentage of backcross matings the actual increase in heterozygosity may be somewhat different than our predicted values. The allele frequencies in the backcross parents may not be the same as in the parental populations especially if there is little overlap in spawning time. Thus, in order to determine the actual increase in heterozygosity and its potential effects an electrophoretic and asymmetry analysis should be

conducted on individuals being retained for future broodstock after the backcross matings have been performed.

ACKNOWLEDGEMENTS

Financial support and samples were provided by the United States Fish and Wildlife Service and the Montana Department of Fish, Wildlife and Parks.

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TABLE 1

Allele frequencies at polymorphic loci in samples of Arlee rainbow trout spawned in 1979 and 1987 at the Jocko River State Trout Hatchery. Chi-square equals contingency table chi-square test for homogeneity of allele frequencies between samples. D.F. equals degrees of freedom. At sMDH-B1,2, the 74 and 83 alleles were combined to avoid small expected numbers. * = $P < 0.05$, *** = $P < 0.001$.

Locus	Alleles	Sample and allele frequencies 1979	1987	Chi-square	D.F.
CK-A1	100	0.967	0.935	1.304	1
	76	0.033	0.065		
LDH-B2	100	0.933	0.964	1.304	1
	76	0.067	0.036		
mIDH-2	100	0.723	0.833	4.417*	1
	140	0.277	0.167		
mMDHp-2	100	0.981	1.000	2.346	1
	55	0.019	-		
PGM-2	100	0.966	0.964	0.004	1
	90	0.034	0.036		
sIDH-1,2	100	0.611	0.688	25.462***	3
	114	-	0.058		
	71	0.033	0.065		
	40	0.356	0.188		
sMDH-B1,2	100	0.867	0.877	0.118	1
	83	0.133	0.109		
	74	-	0.015		
sSOD-1	100	0.808	0.768	0.628	1
	152	0.192	0.232		

TABLE 2

Allele frequencies at the polymorphic loci in samples of Arlee rainbow trout from the Jocko River State Trout Hatchery in 1979 (Jocko 1979) and 1987 (Jocko 1987) and the Ennis National Fish Hatchery in 1987 (Ennis 1987). Chi-square is contingency table chi-square test for homogeneity of allele frequencies between adjacent samples. Degrees of freedom for each comparison is one less than the number of alleles except at sIDH-1,2 between the Jocko 1979 and Ennis 1987 samples. In this comparison the 114 and 71 alleles were combined into a single category to avoid small expected numbers yielding one degree of freedom. NA = not analyzed, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Locus	Alleles	Jocko 1979	Chi-square	Ennis 1987	Chi-square	Jocko 1987
bGLUA	100	NA	-	0.310	6.859**	0.478
	72			0.690		0.522
CK-A1	100	0.967	1.392	0.990	4.388*	0.935
	76	0.033		0.010		0.065
CK-C1	100	NA	-	0.890	1.038	0.928
	38			0.110		0.072
G3PDH-1	100	NA	-	0.990	0.124	0.986
	140			0.010		0.014
IDDH	100	NA	-	0.980	0.094	0.986
	40			0.020		0.014
LDH-B2	100	0.933	1.544	0.970	0.085	0.964
	76	0.067		0.030		0.036
LDH-C	100	NA	-	0.930	0.996	0.891
	95			0.070		0.109
mIDH-2	100	0.723	10.497**	0.900	2.195	0.833
	140	0.277		0.100		0.167
mMDHp-2	100	0.981	0.923	0.950	7.053**	1.000
	55	0.019		0.050		-
PGK-2	100	NA	-	0.660	0.085	0.667
	110			0.180		0.167
	90			0.160		0.167
PGM-1r	a	NA	-	1.000	6.341*	0.940
	b			-		0.060

TABLE 2 - CONTINUED

Locus	Alleles	Jocko 1979	Chi-square	Ennis 1987	Chi-square	Jocko 1987
PGM-2	100	0.966	0.047	0.960	0.020	0.964
	152	0.034		0.040		0.036
sIDH-1,2	100	0.611	22.473***	0.760	8.427*	0.688
	114	-		0.070		0.058
	71	0.034		0.015		0.065
	40	0.356		0.155		0.188
sMDH-B1,2	100	0.867	30.953***	0.885	19.886***	0.877
	83	0.133		0.035		0.109
	74	-		0.080		0.015
sSOD-1	100	0.808	0.738	0.760	0.024	0.768
	152	0.192		0.240		0.232
Average observed heterozygosity		NA		0.075		0.082

TABLE 3

Allele frequencies at the polymorphic loci in two year-classes of Erwin rainbow trout maintained at the Ennis National Fish Hatchery. Chi-square is contingency table chi-square test for homogeneity of allele frequencies between samples. All comparisons have one degree of freedom. Average observed heterozygosity is computed for the 1988 year-class without information from bGLUA, CK-C1,2, and PGK-2. NA = not analyzed, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

		Sample and allele frequencies		
Locus	Alleles	1982	1988	Chi-square
bGLUA	100	NA	0.860	-
	72		0.140	
CK-C1	100	NA	0.694	-
	150		0.306	
G3PDH-1	100	0.892	0.850	0.875
	140	0.108	0.150	
LDH-B1	100	0.867	1.000	14.479***
	null	0.133	-	
LDH-C	100	1.000	0.960	4.979*
	95	-	0.040	
mIDH-2	100	0.717	0.635	1.594
	140	0.283	0.365	
PEPA-1	100	0.967	1.000	3.334
	115	0.033	-	
PGK-2	100	NA	0.510	-
	110		0.370	
	90		0.120	
PGM-2	100	0.958	0.980	0.856
	90	0.042	0.020	
sIDH-1,2	100	0.954	0.945	0.192
	40	0.046	0.055	
sSOD-1	100	0.750	0.560	8.884**
	152	0.250	0.440	
Average observed heterozygosity		0.045	0.042	

TABLE 4

Allele frequencies at the polymorphic loci in the Arlee x Erwin and 1988 year-class of Erwin rainbow trout maintained at the Ennis National Fish Hatchery. Chi-square is contingency table chi-square test for homogeneity of allele frequencies between samples. D.F. = degrees of freedom. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Locus	Alleles	Sample and allele frequencies		Chi-square	D.F.
		Erwin	Arlee x Erwin		
bGLUA	100	0.860	0.460	35.650***	1
	72	0.140	0.540		
CK-A1	100	1.000	0.960	4.082*	1
	76	-	0.040		
CK-C1	100	0.694	0.800	3.006	1
	150	0.306	0.180		
	38	-	0.020		
G3PDH-1	100	0.850	1.000	16.216***	1
	140	0.150	-		
IDDH	100	1.000	0.930	7.254**	1
	40	-	0.070		
LDH-B2	100	1.000	0.980	2.020	1
	76	-	0.020		
LDH-C	100	0.960	1.000	4.082*	1
	95	0.040	-		
mIDH-2	100	0.635	0.790	5.779*	1
	140	0.365	0.210		
mMDHp-2	100	1.000	0.970	3.046	1
	55	-	0.030		
PGK-2	100	0.510	0.540	5.797	2
	110	0.370	0.240		
	90	0.120	0.220		
PGM-2	100	0.980	1.000	2.020	1
	90	0.020	-		
sIDH-1,2	100	0.945	0.825	17.071***	1
	114	-	0.045		
	71	-	0.005		
	40	0.055	0.125		

TABLE 4 - Continued

Locus	Alleles	Sample and allele frequencies		Chi-square	D.F.
		Erwin	Arlee x Erwin		
sMDH-B1,2	100	1.000	0.915	17.755***	2
	83	-	0.060		
	74	-	0.025		
sSOD-1	100	0.560	0.600	0.328	1
	152	0.440	0.400		
Average observed heterozygosity		0.068	0.084		

TABLE 5

Mean total counts of five bilateral meristic characters and average number of asymmetric characters per individual in the 1982 and 1988 year-classes of Erwin rainbow trout maintained at the Ennis National Fish Hatchery. Probability is based on Wilcoxon two-sample test for homogeneity of means. ** = $P < 0.01$, *** = $P < 0.001$, N.S. = not signficant.

	Year-class and mean		
Character	1982	1988	Probability
Lower gill rakers	22.92	21.66	***
Mandibular pores	14.68	14.68	N.S.
Pectoral rays	28.45	28.88	N.S.
Pelvic rays	20.20	19.90	**
Upper gill rakers	18.35	16.58	***
Asymmetry	1.60	1.48	N.S.

TABLE 6

Mean total counts of five bilateral meristic characters and average number of asymmetric characters per individual in three strains of rainbow trout maintained at the Ennis National Fish Hatchery. Based on the Wilcoxon two-sample test all pairwise comparisons are not signficant (P > 0.05)

	Year-class, strain, and means		
Character	1987 Arlee	1987 Arlee x Erwin	1988 Erwin
Lower gill rakers	21.70	21.62	21.66
Mandibular pores	15.50	15.20	14.68
Pectoral rays	28.74	28.58	28.88
Pelvic rays	19.70	19.66	19.90
Upper gill rakers	16.82	17.00	16.58
Asymmetry	1.27	1.54	1.48

TABLE 7

Average expected heterozygosity in the 1988 year-class of Erwin rainbow trout, 1987 year-class of Arlee x Erwin rainbow trout, and in hypothetical populations created by backcrossing Arlee x Erwin to Erwin rainbow trout. Percent increase is percentage increase in average heterozygoisty in the hypothetical populations relative to the 1988 Erwin year-class.

Population	Heterozygosity	Percent increase
Erwin	0.0625	-
Percent backcross matings		
5	0.0630	0.75
10	0.0634	1.44
25	0.0647	3.47
50	0.0666	6.49
75	0.0680	8.80
100	0.0692	10.66
Arlee x Erwin	0.0698	11.74