I(a)

Mr. Craig Busack
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Dear Craig:

Thanks for a copy of your ms on Paiute trout. Normally, this paper would come to me for review by the Canadian Journal, but only last week I was requested to review a submitted paper on S. salar x S. trutta hybridization (based on electrophoresis), so I may not receive your paper as Johanna Reinhart, the editor, tries to spread out the reviewing process.

I might suggest that you could elevate the paper to a more confident level of sophistication by avoiding ~~the~~ statements that electrophoresis is superior to morphological data. ~~This~~ This type of comment is so characteristic of electrophoretic papers that it has become a cliche and makes the author appear defensive. ~~like a mono~~ Simply, one ~~technique~~ samples the metabolic genome, the other the regulatory. Both are useful and complementary. Merely point out that you detected hybridization in Cottonwood Creek specimens electrophoretically that you couldn't detect with meristic characters. Or, as you did to some extent, elaborate on the usefullness of both methods (especially in groups of relatively recent separation). For example,

2

I(b)

Yellowstone cutthroat trout, fine-spotted Snake River cutthroat trout, Bonneville cutthroat, and Colorado River cutthroat can't be consistantly separated by electrophoresis, yet, there are genetic based sharp ecological differences of great significana for fish management and ~~spott~~ striking differences in coloration and spotting useful for classification.

Although I believe you are correct that the Cottonwood Creek trout have some rainbow trout genes, I have some reservations. ~~The~~ Only the MDH-2 pattern ~~suo~~ strongly indicates rainbow influence. I note in Loudenslager and Gall's recent paper, S. c. henshawi from Poison Flat Creek has a higher proportion of the MDH-2 100 allele than Cottonwood Creek fish. I have never examined specimens from Poison Flat Creek and can't comment on their relative purity.

As I recall, Eldon Vestal brought Paiute trout to Cottonwood Creek in 1946 but most of the specimens came from Coyote and/or Corral Creek -- a stock(s) that you have no data on. The founder's principle could have played a role ~~by~~ greatly increasing the frequency of a rare allele. The fact that the

II (a)

Cottonwood Creek sample averages 24 gillrakers and all have basibranchial teeth (the character that is typically most sensitive to rainbow trout influence) leads me to believe that if there are rainbow trout alleles in the population, the effect is extremely slight.

I only hope that someone in Cal F. & G. doesn't use your data to claim that the Cottonwood Creek population must be eradicated because they are hybrids. This would create dissension and set be a set back for native trout restoration projects.

Except for someone with intense interest in the subject, the great elaboration on demonstrating that the Silver King Creek fish consist of Paiute trout and hybrids and that hybridization is progressing toward a climax hybrid swarm (until the creek was poisoned again), could be condensed.

If the reverse was true - that the hybrids and pure Paiutes are maintaining reproductive isolation -- then the great depth of detail would be called necessary. However, since there is not one stream in the Lahontan basin where native cutthroat and rainbow trout (or hybrids) coexist, and the 1949 plant of rainbows into Silver King Creek resulted in a hybrid swarm

by 1963, it is entirely predictable that hybridization, once underway, would eventually create a hybrid swarm, and there is no need to prove the obvious to such a degree.

I would point out that it was the Heenan Lake, Lahontan cutthroat that was stocked into White Cliff Lake and got into Silver King Creek to ~~beyond~~ further add to the hybrid swarm ~~of~~ 1963. Thus, for a fair discussion of Lahontan cutthroat influence, you should have data on the allelic frequency of the Heenan Lake stock.

If I do receive your paper for review, ~~you~~ I've probably given you all of the comments I would make. The content of your work is impressive and I would certainly recommend it for publication.

Enclosed is a copy of a rough draft of a paper on Great Basin trouts with some discussion of the Paiute trout. This paper was prepared for a symposium on desert fishes. The papers of the symposium will be published as a book by John Wiley. My paper will be considerably expanded and modified before publication.

Let's hope you do get back into the trout systematic and evolutionary research. Your paper indicates a potential for great things to come.

Sincerely,

August 19, 1980

Dear Dr. Behnke:

Here at long last is the Paiute cutthroat paper. We're submitting it today to CJFAS.

I think you'll find the conclusions much more to your liking than the conclusions of my thesis; I have reanalyzed everything trying to keep common sense biology as my goal, rather than over-detailed statistical analysis.

Your comments on my thesis were a primary motivation for the reworking of my ideas — thank you once again and I'd appreciate your comments on the MS.

I'm out of the ~~fish~~ trout business for the time being, doing my doctoral research on quantitative genetics of Gambusia affinis. I may go back to this type of work in a year or so as a post-doc with James Clayton in Winnipeg, but we'll have to see what turns up.

I hope you find the MS interesting.

Sincerely,

Craig Busack

INTROGRESSIVE HYBRIDIZATION IN POPULATIONS OF PAIUTE CUTTHROAT TROUT (*Salmo clarki seleniris*)

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Running head: Introgressive Hybridization in Paiute Cutthroat

Busack, C. A. and G. A. E. Gall. 1980. Introgressive hybridization in populations of Paiute cutthroat trout (*Salmo clarki seleniris*). Can. J. Fish. Aquat. Sci. 00:000-000.

Two populations of Paiute cutthroat trout (*Salmo clarki seleniris*) were compared meristically and electrophoretically with Lahontan cutthroat (*S. c. henshawi*) and rainbow trout (*S. gairdneri*) to elucidate population structure and verify the occurrence of introgressive hybridization. In Silver King Creek, both meristic and electrophoretic evidence indicated two populations were present, one appearing to be pure Paiute cutthroat, the other Paiute cutthroat introgressed with rainbow trout. Lahontan cutthroat introgression was a possibility in Silver King Creek, but could not be evaluated because of the strong merisitc and electrophoretic similarity of Paiute and Lahontan cutthroat. The other Paiute population, Cottonwood Creek, meristically appeared to be pure Paiute cutthroat, but electrophoretic data indicated it was

introgressed with rainbow trout. Introgression appeared to be incomplete in Silver King Creek, but complete in Cottonwood Creek. The meristic similarity of Cottonwood Creek trout to pure Paiute cutthroat was probably a result of strong selection by management agencies for a Paiute cutthroat phenotype. Electrophoresis was shown to be much more powerful than meristics in analyzing introgression. Electrophoretic data proved introgression had taken place, allowed estimation of sampling errors in Silver King Creek, and demonstrated that the two Silver King Creek populations were probably interbreeding. Meristic analysis alone was ineffective in these respects; however, it should still continue to be used in studies of introgressive hybridization.

Key words: Salmo clarki, Salmo gairdneri, Paiute cutthroat, Lahontan cutthroat, meristics, electrophoresis, introgression, hybridization, linkage disequilibrium, principal components.

Introduction

At least five closely related trout species of genus Salmo inhabit the western half of North America, from the eastern slope of the Rocky Mountains west to the Pacific coast. Natural sympatry of these species is rare, occurring only between rainbow (S. gairdneri) and cutthroat trout (S. clarki) in coastal drainages (Behnke 1972). Where artificial introductions have created zones of secondary contact between allopatric trout species, hybridization has often resulted or has been assumed to have occurred (Dangel et al. 1973), demonstrating incomplete prezygotic isolating mechanisms in these species. No critical evaluation of postzygotic isolating mechanisms in these fishes is yet available, but a great deal of circumstantial evidence suggests that trout hybrids are fertile; introgressive hybridization, the assimilation of genes from one species into the genome of another through hybridization and repeated backcrossing (Anderson, 1949), is a frequently cited contributing cause of the decline of many western trouts as distinctive forms. In particular, introgression of genes from the extensively introduced rainbow trout is thought to be a cause of the decline of the Little Kern golden trout (S. aguabonita whitei) (Schreck and Behnke 1971, Gold and Gall 1975a,b), Arizona trout (S. apache) and Gila trout (S. gilae) (Miller 1950, 1972; Behnke and Zarn 1976), redband trout (Salmo sp.) (Wilmot 1974), and several interior drainage cutthroat trout subspecies (Miller 1950; Behnke 1965, Behnke and Zarn 1976).

This paper presents the results of a study of introgressive hybribization in two populations of Paiute cutthroat trout (S.

clarki seleniris). The Paiute cutthroat is a Lahontan basin form native to a limited portion of the East Carson River drainage of California. It differs in appearance from the Lahontan cutthroat (S. c. henshawi), the trout native to the rest of the drainage, only in spotting (Behnke 1965). The body of the Lahontan cutthroat is heavily spotted, while that of the Paiute is virtually spotless; specimens collected for the fish's formal description by Snyder (1933) display usually no spots but at most nine body spots (Ryan and Nicola 1976). The distinctive spotting of the Paiute cutthroat has been the basis of an intensive management plan to control rainbow and possibly Lahontan cutthroat introgression in Paiute trout populations. The following summary of Paiute cutthroat management history was taken from Ryan and Nicola (1976) unless otherwise noted.

The original range of the Paiute cutthroat was probably a 6 to 10 km stretch of Silver King Creek immediately below Llewellyn Falls. In 1912 the fish was transplanted to the barren Silver King Creek drainage above Llewellyn Falls, where it was collected by Snyder (1933). The Paiute cutthroat subsequently became extinct in its original range, presumably due to hybridization with rainbow and Lahontan cutthroat trout.

Rainbow trout were introduced into the Silver King Creek drainage above the falls in 1949 and Lahontan cutthroat were introduced in 1955 and 1956. Heavily spotted trout, presumably the result of hybridization, began appearing in the 1950's. These increased in frequency until only two headwater tributaries of

Table 1. Means ± standard errors and ranges observed in nine meristic characters, percentage of specimens in cutthroat samples possessing basibranchial teeth, and mean coefficients of variation. Means not sharing the same superscript letter are significantly different ($p \leq .05$); means without superscripts are significantly different from all others.

	Paiute cutthroats				Lahontan cutthroats			Rainbow trout	
Character	SKU	SKS	NFC	FMC	INL	MAC	MCC	RTH	RTW
Lateral series scales	174.2±1.4^{a} (155-190)	164.6±2.9^{b} (133-201)	168.6±1.7^{ab} (147-180)	166.2±1.6^{b} (140-184)	157.7±1.4 (137-180)	169.3±2.6^{ab} (145-200)	174.0±2.0^{b} (153-189)	142.8±0.9 (134-151)	127.0±0.8 (118-137)
Vertebrae	61.5±0.1^{a} (60-63)	62.2±0.2^{b} (61-65)	61.3±0.2^{a} (60-62)	61.7±0.1^{a} (60-63)	61.8±0.1^{ab} (61-63)	61.2±0.2^{a} (58-62)	62.2±0.1^{b} (60-63)	63.7±0.2 (63-66)	60.4±0.2 (58-62)
Proximal dorsal pterygiophores	12.7±0.1^{ac} (12-14)	13.4±0.1 (12-16)	12.6±0.1^{abc} (12-13)	12.7±0.1^{ac} (12-14)	12.2±0.1^{b} (11-13)	12.5±0.1^{ab} (12-14)	12.6±0.1^{abc} (12-14)	14.0±0.1 (13-15)	13.0±0.1^{c} (12-14)
Proximal anal pterygiophores	12.6±0.1^{a} (11-14)	12.6±0.1^{a} (12-14)	12.6±0.1^{a} (12-14)	12.6±0.1^{a} (12-13)	13.5±0.1 (13-14)	12.8±0.1^{a} (12-14)	12.5±0.1^{a} (12-13)	12.9±0.1^{a} (12-14)	11.4±0.1 (10-13)
Pectoral rays	14.5±0.1^{a} (13-16)	14.0±0.1^{bc} (13-15)	14.0±0.1^{bc} (13-15)	14.0±0.1^{bc} (13-15)	14.4±0.1^{ac} (12-15)	14.5±0.1^{a} (13-15)	13.8±0.1^{b} (13-15)	15.1±0.1 (14-16)	13.8±0.1^{b} (13-15)
Pelvic rays	9.2±0.1^{a} (9-10)	9.0±0.1^{a} (9-10)	9.0±0.0^{a} (9)	9.1±0.1^{a} (8-10)	9.0±0.0^{a} (9)	9.6±0.1 (9-10)	9.0±0.0^{a} (9-10)	10.2±0.1^{b} (10-11)	10.1±0.1^{b} (9-11)
Branchiostegal rays	11.4±0.1^{ac} (11-13)	11.5±0.1^{ac} (11-12)	10.9±0.1^{b} (10-12)	11.6±0.1^{a} (10-12)	10.2±0.1 (9-11)	10.7±0.2^{b} (9-12)	10.9±0.1^{b} (10-12)	11.7±0.1^{a} (11-13)	11.1±0.2^{bc} (10-13)
Gill rakers	24.5±0.2^{a} (23-27)	22.7±0.4^{b} (19-27)	24.0±0.3^{ad} (22-27)	24.2±0.2^{a} (22-27)	24.0±0.2^{ad} (21-27)	23.2±0.2^{bd} (21-25)	23.7±0.3^{ad} (21-26)	19.5±0.3^{c} (16-21)	20.2±0.2^{c} (19-21)
Pyloric caeca	59.3±1.5^{a} (40-77)	51.9±1.7^{bc} (35-67)	53.5±1.1^{bcd} (45-61)	57.5±1.0^{ad} (44-66)	43.7±1.0 (35-51)	50.9±2.1^{bc} (41-68)	50.7±1.5^{b} (40-75)	57.0±1.3^{acd} (44-70)	54.0±1.2^{bcd} (44-63)
Percentage with basibranchial teeth	97.4	46.7	100.0	100.0	100.0	90.0	96.6	---	---
Mean coefficient of variation (%)	5.6	6.6	3.9	4.8	4.8	6.2	5.5	5.3	5.0

Silver King Creek, Four Mile Canyon and Fly Valley creeks, both above barriers, were free of spotted fish. Waters containing the putative hybrids were chemically treated in 1964 to destroy all fish, and were restocked later that year with trout from the hybrid-free creeks.

A 1968 survey found that one third of the fish in the treated area were heavily spotted. From that time, until the inception of the present study in 1976, the management program in the drainage consisted of manually removing spotted fish. The most intensive activity occurred in 1973 and 1974, when the entire area was electrofished and all fish with any body spots were removed. In 1975 only fish with more than five body spots were removed, 28% of those collected.

The Paiute cutthroat was introduced into barren waters of the north fork of Cottonwood Creek, Mono County, California in 1946. Heavily spotted trout appeared there in 1964, apparently the result of hybridization with rainbow trout introduced from a portion of the stream below a barrier. In 1970, the stream was electrofished and then chemically treated; fish with fewer than five spots, 75% of those collected, were returned to the creek (Wong 1975). The most heavily spotted fish in a large (163 fish) 1973 sample from Cottonwood Creek had only six body spots.

The present study was designed primarily to elucidate the population structure of the trout in Silver King Creek. The population was being managed as if the spotted fish were of mixed ancestry and the unspotted fish were genetically pure Paiute

cutthroats, but other population structures were possible, ranging from a single introgressed population to a population consisting of rainbows, Lahontan cutthroats, Paiute cutthroats, and a mixture of hybrid types. Secondly, we wished to characterize the Cottonwood Creek population of Paiute cutthroats. The 1975 survey found no heavily spotted fish in Cottonwood Creek, implying that the 1970 management activities had returned the population to a "pure" state. It was unclear, however, if the population was genetically pure or merely phenotypically acceptable. The third and final purpose of the study was to compare the utility of meristic analysis with that of electrophoretic analysis of protein variation in characterizing introgressive hybridization in trout.

Materials and Methods

Seven samples of Paiute and Lahontan cutthroat trout were collected in California in mid-1976 by the California Department of Fish and Game (CDFG) and the U.S. Forest Service. No rainbow trout were sampled specifically for this study; instead, the electrophoretic data of Busack et al. (1979, 1980) and unpublished meristic data of Gold and Gall for two domestic rainbow trout strains were used. Designations, descriptions, and sizes (in parentheses) of the nine samples follow.

Paiute Cutthroats.-FMC. Fourmile Canyon Creek, Alpine County (40). This population was one of those used to restock Silver King Creek after the 1964 treatment. It should represent pure Paiute cutthroat.

NFC. North Fork of Cottonwood Creek, Mono County (19). Fish

were sampled where spotted fish had been observed in the past.

SKU. "Unspotted" specimens (having five or fewer body spots) from Silver King Creek above Llewellyn Falls, Alpine County (41).

SKS. "Spotted" specimens (having more than five body spots) from Silver King Creek above Llewellyn Falls (31).

Lahontan Cutthroats.-INL. Hatchery reared specimens from eggs taken at Independence Lake, Little Truckee River drainage, Nevada and Sierra counties (43).

MAC. Macklin Creek, Middle Yuba River drainage, Nevada County (30). This population is probably the result of an introduction from the Truckee River drainage (Behnke and Zarn 1976).

MCC. Murray Canyon Creek, East Carson River drainage, Alpine County (29). This population was thought to be an undisturbed remnant population of Lahontan cutthroat.

Rainbow Trout.-RTH. CDFG's Hot Creek hatchery strain (276 for electrophoresis and 25 for meristics). This is a McCloud River strain with some Mt. Whitney strain ancestry (Busack and Gall 1980).

RTW. CDFG's Mt. Whitney hatchery strain (245 for electrophoresis and 24 for meristics). This strain, which was introduced into Silver King Creek in 1949 (Ryan and Nicola 1976), is derived from a variety of native California rainbow trout strains, and has some Lahontan cutthroat and Kamloops rainbow trout (S. g. kamloops) ancestry (Busack and Gall 1980).

Sample sizes for individual meristic characters and protein systems varied little from the numbers of fish given above, with two exceptions: only 19 INL and 14 MAC fish were available for pyloric

caeca counts.

Meristic Analysis

Counts of lateral series scales, vertebrae, pectoral fin rays, pelvic fin rays, branchiostegal rays, gillrakers, and pyloric caeca were made according to Behnke (1965) and Schreck and Behnke (1971). Two nonstandard characters were also counted: proximal dorsal pterygiophores (PDP's) and proximal anal pterygiophores (PAP's). Pterygiophore counts are merely indirect counts of dorsal and anal fin rays, because a one-to-one correspondence exists between rays and pterygiophores. All S. clarki specimens were also examined for the presence of basibranchial teeth.

Sample means, standard errors, and coefficients of variation were calculated for each character in each population. Analysis of variance (ANOVA) was carried out for each character to obtain error mean squares for use in further analyses. Means were compared using Student-Newman-Keuls multiple range tests (Sokal and Rohlf 1969). A multivariate comparison of samples was made by the extraction of principal components from the nine character correlation matrix. The Euclidean distance between each pair of samples was calculated as the square root of the Mahalanobis distance (Sneath and Sokal 1973). These distances were used to construct an UPGMA dendrogram (Sneath and Sokal 1973).

Electrophoretic Analysis

Horizontal starch gel electrophoresis as described by Busack et al. (1979, 1980) was used to examine twenty loci encoding the following twelve proteins (locus designations given in parentheses):

alcohol dehydrogenase (ADH), alpha-glycerophosphate dehydrogenase (AGPDH-1,2) creatine kinase (CK-2), isocitrate dehydrogenase (IDH-3,4), malate dehydrogenase (MDH-1,2,3,4) malic enzyme (ME), para-albumin (PALB), phosphoglucoisomerase (PGI-1,2,3), phosphoglucomutase (PGM), sorbitol dehydrogenase (SDH-1,2), superoxide dismutase (SOD), and xanthine dehydrogenase (XDH). The SKU and SKS samples were later examined for variation at two peptidase loci using techniques to be described in a later paper (Loudenslager and Gall, unpublished).

Gel interpretation and nomenclature were also as described by Busack et al. (1979, 1980). As in those previous papers, genotypes could not be determined at the individual IDH loci (IDH-3 and IDH-4) nor at two MDH loci (MDH-3 and MDH-4). Thus, IDH-3,4 and MDH-3,4 were each treated as systems with two identically polymorphic loci in computing allele frequencies. Gall and Bentley (1980) have recently shown para-albumin to be encoded by two loci polymorphic for alleles whose products have identical electrophoretic mobility. However, in the present study, results were not sufficiently repeatable to confidently score allele dosages; hence, PALB was interpreted as being encoded by a single locus.

Heterozygosities were calculated according to Nei and Roychoudhury (1974). Maximum nonsignificant allele frequency ranges were found using the G-statistic (Sokal and Rohlf 1969). Standard genetic distances between samples were calculated by the method of Nei (1972), and then used to construct an UPGMA dendrogram.

To facilitate an examination of linkage in the SKU, SKS, and NFC

samples, loci were made diallelic by pooling the frequencies of minor alleles. Three methods were then used to measure linkage disequilibrium between pairs of loci: (1) A G-test of independence of genotypes. (2) Calculation of D', the linkage disequilibrium (D) expressed as a proportion of the maximum possible disequilibrium (D_{max}) (Lewontin 1964); D was estimated using the maximum likelihood iterative method of Hill (1974) with specific procedures suggested by Hedrick et al. (1978). (3) Calculation of the squared correlation coefficient (r^2) of allele frequencies (Hill and Robertson 1968).

Results and Discussion

Meristics

Table 1 summarizes the meristic data collected from the nine trout samples. For discussion, the cutthroat samples were divided into two groups: Group A represents populations in which hybridization was assumed to not have taken place (FMC, INL, MAC, and MCC), whereas Group B includes populations in which hybridization may have taken place (SKU, SKS, and NFC).

Table 1 near here

Considerable heterogeneity was observed in the Group A samples. INL was the most distinctive of the four samples, with significantly fewer lateral series scales, branchiostegal rays, and pyloric caeca than the other three samples. As expected, the FMC Paiute cutthroats were meristically very similar to the Lahontan cutthroat samples, differing only in number of brachiostegal rays and pyloric caeca. For both of these characters, the Paiute cutthroats displayed significantly higher means than the Lahontan cutthroats.

The two rainbow trout samples, RTH and RTW, differed significantly in six of the nine characters; in number of pectoral rays, the rainbows exhibited the highest and lowest means of all the samples. The extreme heterogeneity of the rainbow samples limited the number of characters useful in discriminating between rainbow trout and the Group A cutthroats. The cutthroats exhibited significantly higher lateral series scale and gill raker means, but lower pelvic ray means, than the rainbows, all in agreement with the results of Behnke (1965). Basibranchial teeth, treated here as a discrete character state rather than a meristic character, are widely accepted as occurring only in the cutthroat (Behnke 1965; Miller 1950, 1972), thus discriminate between rainbows and cutthroats.

Two criteria for hybridization were considered in examining the Group B cutthroat samples. The first, derived from Hubbs (1955), is that hybrid populations should be intermediate between the parental species in meristic counts. Second, introgressed populations are expected to display high variability relative to the parental forms (Anderson 1949), a consequence of the creation of novel genotypes through genetic recombination.

The unspotted fish from Silver King Creek (SKU) were meristically very similar to the pure Paiute cutthroats (FMC) differing significantly in only two characters, lateral series scales and pectoral rays. In each case, the SKU mean was well within the range of Lahontan cutthroat means. It is unlikely, however, that these differences between SKU and FMC indicate

Lahontan cutthroat introgression; for both characters discriminating between Paiute and Lahontan cutthroat, branchiostegal rays and pyloric caeca, the SKU and FMC samples were indistinguishable. The SKU sample also demonstrated a coefficient of variation typical of rainbow trout and Group A cutthroats (Table 1).

The sample of spotted Silver King Creek fish, SKS. differed significantly from the Group A cutthroats in number of PDP's and in percentage having basibranchial teeth. The Group A samples averaged fewer than 13 PDP's, while the SKS mean was 13.4, well within the rainbow trout range. Virtually every Group A specimen possessed basibranchial teeth, but only about half the SKS specimens had them. For these two characters, then, the SKS sample exhibited evidence of rainbow trout introgression. When compared with SKU, SKS differed significantly for five additional characters: lateral series scales, vertebrae, pectoral rays, gill rakers, and pyloric caeca. SKS appeared more rainbow-like than SKU in lateral series scales and gill rakers, although the SKS means fell within the range of Group A variation. In pyloric caeca, the SKS mean was intermediate between the Lahontan cutthroat and rainbow trout values. This could be taken as an indication of Lahontan cutthroat introgression, but the SKS mean is so close to that of NFC, where no Lahontan cutthroat introgression was possible, that it must be regarded as very weak evidence.

The second expected indicator of introgression was high variability relative to the parental forms. SKS specimens exhibited wider ranges of counts (Table 1) than all other samples in lateral

series scales, PDP's, gillrakers, and pyloric caeca. Especially conspicuous was the variability in lateral series scales and gillrakers, where the lowest SKS values were well within the rainbow trout range, and the highest SKS values equalled or exceeded the highest Group A counts. The mean CV of SKS for the characters which discriminated between rainbow and cutthroat was about 50% higher than that of all other samples. Considering all characters, mean CV of SKS was again highest, but not by nearly so large a margin.

In summary, SKS was meristically distinct from SKU and exhibited evidence of introgression with rainbow trout and possibly with Lahontan cutthroat trout. The evidence fits both the expectations outlined earlier, meristic intermediacy and high variability.

The final Group B sample, NFC, meristically was more similar to the pure Paiute cutthroat sample, FMC, than was SKU. NFC differed significantly from FMC only in branchiostegal rays, where the NFC mean was well within the Group A range. None of the NFC specimens was spotted, and all had basibranchial teeth. NFC also displayed the lowest mean CV of all samples, both over all characters and over the four characters discriminating between rainbow and cutthroat trout. Based on meristic analysis, NFC appeared to represent a pure Paiute cutthroat population.

Meristic intermediacy between the putative parental species is a necessary, but not a sufficient condition for identification of an introgressed population. Sampling populations consisting of: 1) rainbow and cutthroat trout living in sympatry but not interbreeding, or 2) rainbow and cutthroat trout interbreeding but

with production of sterile hybrids, would result in mean values intermediate between rainbow and cutthroat trout, and high coefficients of variation. To ascertain that introgression has taken place in SKS, backcrossing must be demonstrated. Direct demonstration of backcrossing is impossible with meristic analysis, but the alternatives (the two hypothetical populations discussed above) can be eliminated by examining the individual specimens in SKS. Both alternatives predict the existence of a breeding population of rainbow trout in Silver King Creek, which presumably would be represented in the SKS sample.

To examine the individual specimens from SKS, principal components were extracted from the nine character correlation matrix based on all specimens in the study which had counts for all nine characters (219 fish). To faciltate a simple two dimensional analysis, only the first two components (PC), which accounted for 49% of the variances, were used in the multivariate sample descriptions (Table 2). In PC I, lateral series scales, PDP's, pelvic rays, and gillrakers were weighted most heavily, separating the cutthroats from the rainbows. PC II weighted vertebrae, PAP's, and pectoral rays most heavily; the major effect of this component was to separate the two rainbow trout samples from each other.

The diagram of the bivariate principal component sample spaces (Fig. 1) supports the observations made from the univariate meristic analysis. The sample space of FMC, the pure Paiute cutthroat sample, coincided well with that of the Lahontan cutthroats. SKU was more variable than FMC, but the two sample spaces coincide well.

Table 2. Coefficients of principal components I and II. PC I accounted for 29% of the total variance, PC II accounted for 20%.

Character	PC I	PC II
Lateral series scales	0.74	0.44
Vertebrae	-0.28	0.64
PDP's	-0.61	0.36
PAP's	0.24	0.70
Pectoral rays	-0.31	0.60
Pelvic rays	-0.81	-0.08
Branchiostegal rays	-0.34	0.30
Gill rakers	0.81	0.21
Pyloric caeca	-0.12	0.27

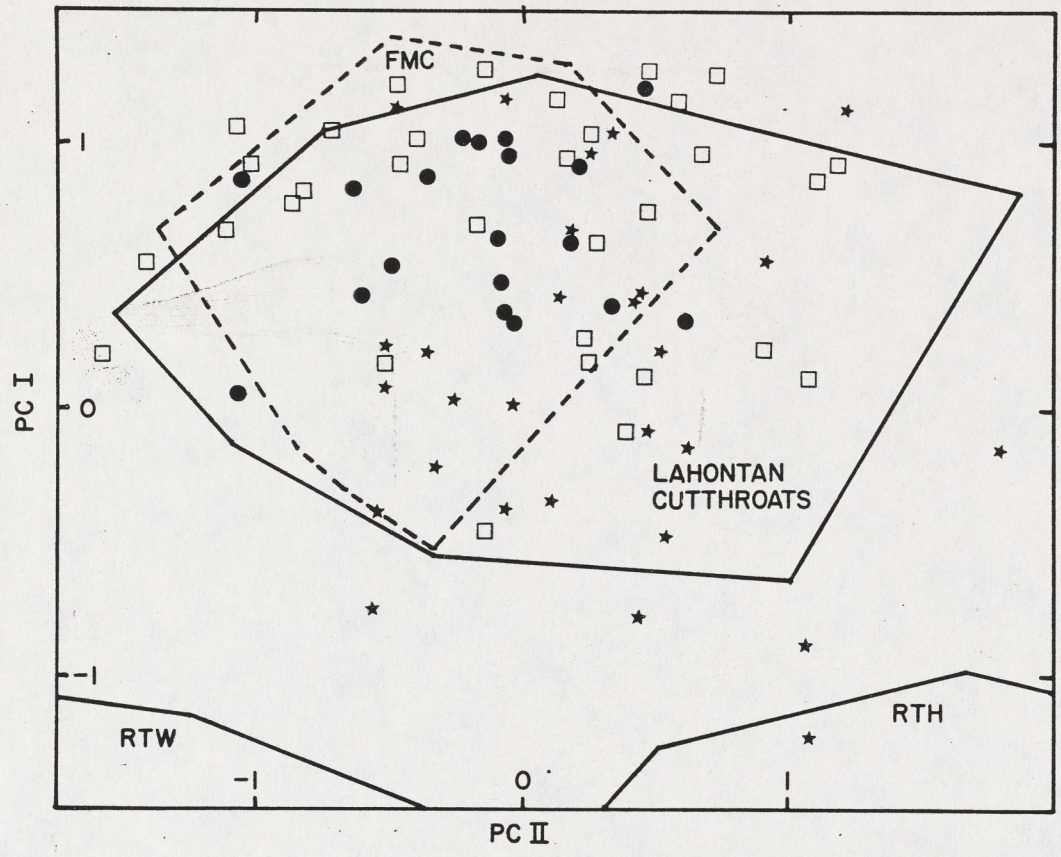

FIG. 1. Principal component scores for Paiute cutthroat, Lahontan cutthroat, and rainbow trout. Individual SKU (open squares), SKS (stars), and NFC specimens (solid circles) are shown; for the other groups, the smallest possible convex polygon bounding the sample space is shown.

NFC had low variability but coincided well with SKU and FMC. The introgressed sample SKS was more similar meristically to RTH than to RTW, although RTW was a sample of the strain of rainbow trout which was planted in Silver King Creek. Perhaps RTW was not meristically similar to the Mt. Whitney rainbow of 1949 because of genetic drift, or because of the introduction of Kamloops rainbow trout into the Mt. Whitney strain in the mid-1950's (Busack and Gall 1980). The SKS sample space was larger than that of any other sample. Of the 27 SKS specimens examined for all nine characters, only one fell within the rainbow trout space, and only six between the Paiute cutthroat space (FMC, NFC, and SKU combined) and the rainbow trout space. No clumping of SKS specimens which could be construed as representing a group of rainbow trout was evident. The conclusion is that the rainbow trout characteristics of SKS are the result of introgressive hybridization; if rainbow trout exist in Silver King Creek, they are rare.

Meristic data indicate the SKU and SKS samples probably represent two populations of trout, one of Paiute cutthroat and the other of Paiute cutthroat introgressed with rainbow trout and possibly Lahontan cutthroat. The alternative hypothesis, that a single introgressed population exists with phenotypic extremes similar to those of pure Paiute cutthroat, is untenable for two reasons. First, it is unlikely such a high correlation between characters would exist so that unspotted fish would also have basibranchial teeth, high lateral series scale counts, nine pelvic rays, etc. Second, even if this correlation did exist, there is too

high a proportion of unspotted fish in the creek (at time 50%), for them to represent the extreme of a normal distribution.

Electrophoresis

The nine samples were identically monomorphic at five loci: AGPDH-2, MDH-1, PGI-2, SDH-2, and XDH. The cutthroats from both groups A and B were also identically monomorphic at CK-2 and SOD, loci at which the rainbow trout samples were polymorphic (Busack et al. 1979). At three other loci (ADH, PGI-1, and PGI-3) low level polymorphisms were found but not considered useful in detecting hybridization because the rare allele at each locus was observed in only one sample. Allele frequencies at the remaining ten loci, and heterozygosities calculated over all 20 loci, are presented in Table 3. Maximum nonsignificant allele frequency ranges for selected loci are presented in Figure 2.

Three of the four Group A cutthroat samples, FMC, MAC, and MCC, were virtually identical electrophoretically, all being monomorphic or nearly so for the same alleles at all ten loci. The fourth sample, INL, was as distinctive electrophoretically as it was meristically. Besides being substantially more polymorphic than the other samples at IDH, ME, and SDH-1, INL exhibited four alleles not found in any other sample: AGPDH-1(60), IDH-3,4(115), and the variant alleles at PGI-1 and 3. The high genetic identity of Paiute and Lahontan cutthroat will make detection of Lahontan cutthroat introgression in Silver King Creek impossible.

The Group A cutthroats were most distinct from rainbow trout at three loci: MDH-2, ME, and SDH-1. In the case of MDH-2 and SDH-1

Table 3. Allele frequencies at 10 polymorphic structural gene loci, and mean heterozygosities for 20 loci, in Paiute cutthroat, Lahontan cutthroat, and rainbow trout.

		Paiute cutthroats				Lahontan cutthroats			Rainbow trout	
Locus	Allele	SKU	SKS	NFC	FMC	INL	MCC	MAC	RTH	RTW
AGPDH-1	140	0	0	.026	0	0	0	0	.002	.009
	100	1.000	1.000	.974	1.000	.953	1.000	1.000	.998	.991
	60	0	0	0	0	.047	0	0	0	0
IDH-3,4	170	0	0	0	0	.214	0	.150	.001	.382
	140	1.000	1.000	.987	1.000	.488	1.000	.850	.699	.340
	115	0	0	0	0	.268	0	0	0	0
	100	0	0	.013	0	.030	0	0	.173	.075
	60	0	0	0	0	0	0	0	.127	.203
MDH-2	130	.939	.433	.526	.988	.900	.914	.967	0	.014
	100	.061	.567	.474	.012	.100	.086	.033	1.000	.986
MDH-3,4	125	0	.097	0	0	0	0	0	0	.006
	100	.994	.822	1.000	1.000	1.000	1.000	1.000	.999	.750
	85	.006	.081	0	0	0	0	0	.001	.244
ME	125	.951	.661	.842	1.000	.893	1.000	1.000	0	0
	100	.049	.339	.158	0	.107	0	0	.697	.645
	85	0	0	0	0	0	0	0	.303	.355
PALB	105	.962	.683	.816	1.000	.930	1.000	1.000	.462	.552
	100	.038	.317	.184	0	.070	0	0	.538	.448
PGM	100	1.000	1.000	.895	1.000	1.000	1.000	.967	.684	1.000
	85	0	0	.105	0	0	0	.033	.316	0
SDH-1	250	.988	.839	.964	1.000	.779	1.000	1.000	0	0
	40	.012	.161	.036	0	.221	0	0	1.000	1.000
Heterozygosity		.017	.116	.071	.001	.142	.010	.032	.125	.181

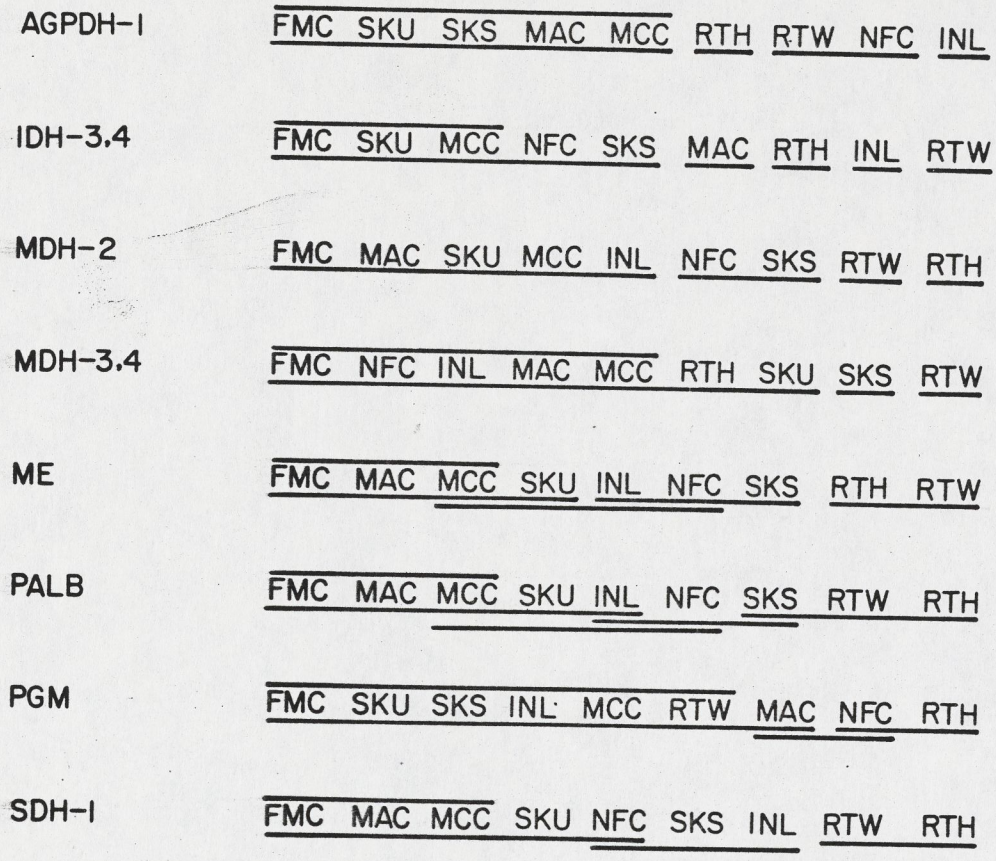

FIG. 2. Relationships among Paiute cutthroat, Lahontan cutthroat, and rainbow trout in allele frequencies. Underlined samples did not differ significantly in frequency ($p>.05$); overlined samples were identically monomorphic.

the two species were nearly monomorphic for alternate alleles. At ME the cutthroats exhibited ME(125) in high frequency, but the rainbows exhibited the ME(100) at a frequency of about .67 and ME(85) at a frequency of about .33. PALB also helped distinguish between the two species; the cutthroats were nearly monomorphic for PALB(105), while the rainbows exhibited the PALB(100) and PALB(105) in approximately equal frequencies. In general, the rainbow trout exhibited more alleles, resulting in higher heterozygosities than the cutthroats. Low within-population heterozygosity of several cutthroat subspecies has been demonstrated by Loudenslager and Gall (1980).

A tacit assumption of the study to this point has been the genetic purity of the Lahontan cutthroat samples. Loudenslager and Gall (1980), in their extensive sampling of the Lahontan basin, found many of the same rare cutthroat alleles found in this study. In some cases, however, the occurrence of these alleles correlated well with planting records of rainbow trout (Loudenslager, pers. comm.). It must be acknowledged, then, that some of the low level polymorphism seen in the Group A samples may be an artifact of rainbow trout introgression. This possibility is illustrated by MAC, which is weakly polymorphic at three protein systems. The INL sample, on ther other hand, displayed some polymorphisms which can be explained by rainbow trout introgression (MDH-2, ME, PALB, SDH-1), but also other polymorphisms which cannot (AGPDH-1, IDH, PGI-1, PGI-3). The conclusions yet to be drawn regarding the purity of the Group B samples will not be affected if the Group A samples

do exhibit low level introgression.

In the meristic assessment of the Group B samples, two possible criteria for detection of introgression were used, intermediacy and high variability relative to the parental species. The same criteria are appropriate for electrophoretic analysis. The presence of both rainbow and cutthroat alleles would indicate intermediacy and would normally result in higher heterozygosity, a measure of genetic variability. However, in the present study, the large discrepancy in heterozygosity between rainbow and cutthroat trout made it unlikely that introgressed populations would be more variable than rainbows. Consequently, high variability in a cutthroat population would be taken as evidence of introgression if rainbow trout alleles also occurred in the population.

SKU was electrophoretically indistinguishable from FMC. It did, however, exhibit low level polymorphisms at five systems (MDH-2, MDH-3,4, ME, PALB, SDH-1). One allele found in SKU, MDH-3,4(85), was found in the rainbow trout samples but not in the Group A samples, or in any of the Lahontan cutthroats sampled by Loudenslager and Gall (1980). Thus, SKU exhibited electrophoretic variation consistent with a hypothesis of low level rainbow trout introgression.

SKS differed significantly from the Group A samples at MDH-2, ME, and PALB. At MDH-2 the common rainbow and cutthroat alleles occurred in approximately equal frequencies in SKS. PALB(100), also common in rainbow trout, occurred in much higher frequency than in the Group A samples. ME(125) was found in lower, and ME(100) in

higher frequency than in the Group A fish. Another difference between SKS and the Group A samples, although not a statistically significant one, was the presence in SKS of two MDH-3,4 rainbow trout alleles which were absent from the Group A samples. SKS also differed from SKU in the frequency of SDH-1(40), the common rainbow allele. Additional differences between SKU and SKS were provided by Loudenslager (unpub.), who compared the two samples at two diallelic peptidase loci which readily distinguish rainbow from cutthroat trout. At both PEP-1 and PEP-2, SKS exhibited the rainbow trout alleles in significantly higher frequencies (.350 and .267) than did SKU (.038 and .012). In summary, SKS was electrophoretically distinct from SKU, and displayed high genetic variation consistent with the idea of rainbow trout introgression.

NFC, which appeared meristically to be pure Paiute cutthroat, exhibited electrophoretic variation consistent with the hypothesis of rainbow trout introgression. The most compelling evidence of this was found at MDH-2, where like SKS, NFC displayed the common rainbow and cutthroat alleles in intermediate frequencies. NFC was also the only cutthroat sample to display AGPDH-1(40), an allele found in low frequency in the rainbow trout samples. Finally, at ME, PALB, and PGM, NFC had common rainbow trout alleles in higher frequencies than in FMC and SKU, though not in significantly higher frequencies than all the Group A samples.

A multivariate approach to the electrophoretic data was undertaken to verify that introgressive hybridization had occurred. Each SKU and SKS specimen was scored for the number of rainbow trout

alleles present at PEP-1, PEP-2, MDH-2, MDH-3, ME and SDH-1. All low level polymorphism was assumed to be derived from rainbow trout. It was also assumed that all MDH-3,4 variation occurred at MDH-3, probably a good assumption since MDH-4 variation has been found in only a very few rainbow trout (Busack et al. 1979) and no cutthroat trout. By this scoring scheme, pure cutthroats would have scores of 0; pure rainbow trout would have scored of 12. A similar scoring scheme was followed for NFC, but using only five loci (AGPDH-1, MDH-2, ME, PGM, and SDH-1), so scores ranged from 0 to 10. Resulting frequency distributions of allele scores demonstrate that no rainbow trout were found in any of the three samples (Table 4). In SKU, 35 of 40 specimens were pure cutthroat, compared to 4 of 29 in SKS, and 4 of 19 in NFC.

Table 4 near here

Because electrophoretic variants at different loci assort independently when loci are unlinked, as all of these probably are (May et al. 1979), each fish's pattern of variation over all loci reveals something of its ancestry. An F_1 hybrid would be expected to have one rainbow trout allele at each locus. A fish exhibiting no rainbow trout alleles at one locus, but two at another, is most parsimoniously explained as an F_2 or backcross individual. Specimens demonstrating this type of variation are evidence of the fertility of hybrids, and thus, proof of introgression. None of the specimens in SKU, SKS, or NFC could be identified as F_1 hybrids, even assuming the low level cutthroat polymorphisms to be natural. All fish in the three samples appeared either to be pure cutthroat or the product of rainbow trout introgression.

Table 4. Frequency distribution of rainbow trout allele scores in SKU, SKS, and NFC. Maximum possible scores are 12 in SKU and SKS, 10 in NFC.

	Allele scores									
Sample	0	1	2	3	4	5	6	7	8	9
SKU	35	1	2	1	1	---	---	---	---	---
SKS	3	2	6	3	4	5	1	---	4	1
NFC	4	6	6	2	---	---	1	---	---	---

For SKU to be merely the unspotted extreme of a single randomly mating population, alleles at five or six loci would have to be nearly perfectly correlated with spotting. It is much more likely that the electrophoretic evidence, like the meristic, domonstrates the existence of two populations in Silver King Creek, an essentially pure Paiute cutthroat, and a population introgressed with rainbow trout.

The fish in the SKU sample which exhibit introgression are possibly the product of sampling error. Assuming a normal distribution of body spotting in the introgressed population, some fish would have very few spots, and could have been included in the SKU sample. Since the two populations differ in allele frequency at several loci, mixing them in the SKU sample should cause linkage disequilibrium (Cavalli-Sforza and Bodmer 1971). Conversely, if pure Paiute cutthroat have up to nine body spots, the five-or-fewer spotting criterion may have assigned a few fish from the pure population to SKS. This should also cause linkage disequilibrium.

The same six loci (PEP-1, PEP-2, MDH-2, MDH-3, ME, and SDH-1) were used in all possible pairwise comparisons for linkage disequilibrium analysis in both SKU and SKS. Three indicators of disequilibrium were calculated (Table 5): a G-statistic testing independence of genotypes, the proportional linkage disequilibrium (D'), and the squared correlation coefficient of allele frequencies (r^2). The squared correlation coefficient may be used to compare the disequilibrium found with that expected by sampling alone. The relationship between the sample size, N, and the expectation of

Table 5. Estimates of D' and r^2 (in parentheses) in the SKU and SKS samples for 15 two-locus comparisons. Expected values of r^2, based on sample size and 50% recombination, are .012 in SKU and .016 in SKS. Asterisks indicate significance for G tests of independent distribution of genotypes.

	PEP-2		MDH-2		MDH-3		ME		SDH-1	
	SKU	SKS	SKU	SKS	SKU	SKS	SKU	SKS	SKU	SKS
PEP-1	1.000*	.186	1.000**	.311***	.994***	.256	1.000	.488*	1.000	.118
	(.329)	(.023)	(.494)	(.082)	(.988)	(.041)	(.001)	(.172)	(.000)	(.007)
PEP-2			.480	.400	1.000*	.439	.292	.372	1.000	.154
			(.152)	(.137)	(.329)	(.179)	(.063)	(.128)	(.000)	(.008)
MDH-2					1.000*	.541**	.474*	.227	1.000	.192
					(.494)	(.255)	(.110)	(.044)	(.000)	(.014)
MDH-3							1.000	.328	1.000	.380*
							(.001)	(.087)	(.000)	(.047)
ME									1.000*	.431
									(.247)	(.018)

* $p \leq .05$
** $p \leq .01$
*** $p \leq .005$

r^2 is

$$E(r^2) = 1/(4Nc+1) \quad \text{(Hill and Robertson, 1968)},$$

where c is the recombination rate.

Seven of the fifteen SKU comparisons showed significant disequilibrium, based on G tests, with D' ranging from .47 to 1.00. Theoretically, these disequilibria could be caused by selection, but linkage disequilibria between allozyme loci are seldom observed, even in Drosophila (Charlesworth et al. 1979), which have as few as three linkage groups. Thus, these large values of disequilibrium in SKU are probably the product of population mixing. Inspection of SKU genotypes revealed that a large amount of variation, including all MDH-3 and PEP-1 variation, was found in a single fish. Deleting this fish from the sample would reduce the number of significant disequilibria to two. These could be removed by deleting two more fish. Assuming, then, that all SKU disequilibrium was caused by mixing, the five-or-fewer spotting criterion allowed three fish from the introgressed population into the SKU sample of 41 fish.

Among the SKS trout, four of fifteen comparisons were significant, with D' ranging from .31 to .49. Examination of the preserved specimens which exhibited no rainbow trout alleles revealed they had four, ten, and 20 body spots, deleting the first two from the SKS sample still resulted four significant disequilibria. The persistence of the disequilibria may indicate interbreeding between the pure population and the introgressed population (Nei and Li, 1973).

NFC was examined for linkage disequilibrium at all pairwise comparisons of the AGPDH-1, MDH-2, ME, PGM, and SDH-1 loci. No significant disequilibria were found.

Comparison of Meristic and Electrophoretic Results

Euclidean and genetic distances (Table 6) for each pair of samples were highly correlated ($.90 \leq$ Spearman's rank correlation coefficient $[r_s] \leq .97$, with approximately 95% confidence). This was due, in part, to the inclusion of rainbow trout, which were quite distinctive both electrophoretically and meristically from the cutthroats. Among cutthroat samples alone the correlation was still significant ($.25 \leq r_s \leq .82$, with approximately 95% confidence).

Phenetic relationships based on the distance calculations are presented in Figures 3 and 4 as UPGMA dendrograms. Meristically, there was no clear pattern of relationship among the cutthroat populations except FMC and SKU were similar to each other and INL was distinct from the other cutthroat. In contrast, genetic distance estimates suggested that the SKU and FMC Paiutes were genetically virtually identical to the MCC and MAC Lahontan cutthroats. Secondly, SKS and NFC were more closely related to each other than to the other cutthroats. It was the presence of rainbow trout alleles in SKS and NFC that resulted in this clustering of the introgressed populations.

There was no correlation between meristic measures (CV's) and genetic measures (heterozygosities) of intrasample variability. A large difference in heterozygosity was seen between rainbow and cutthroat trout (excluding INL); within the cutthroats samples of

Table 6. Euclidean distances, based on nine meristic characters (above diagonal), and genetic distances, based on 20 loci (below diagonal) for all populations.

	FMC	SKU	SKS	NFC	INL	MAC	MCC	RTH	RTW
FMC	---	1.05	1.88	1.82	4.31	2.45	2.12	6.46	6.56
SKU	.000	---	2.21	1.61	4.05	2.77	2.05	6.48	6.83
SKS	.032	.026	---	2.41	4.18	3.30	2.11	5.55	6.46
NFC	.015	.012	.008	---	2.95	2.45	1.76	6.91	6.84
INL	.030	.029	.054	.041	---	3.10	3.67	7.06	7.56
MAC	.002	.003	.034	.017	.021	---	2.83	5.67	6.05
MCC	.000	.000	.027	.011	.030	.003	---	6.87	7.33
RTH	.273	.258	.165	.195	.252	.272	.260	---	6.00
RTW	.234	.220	.123	.165	.175	.218	.222	.083	---

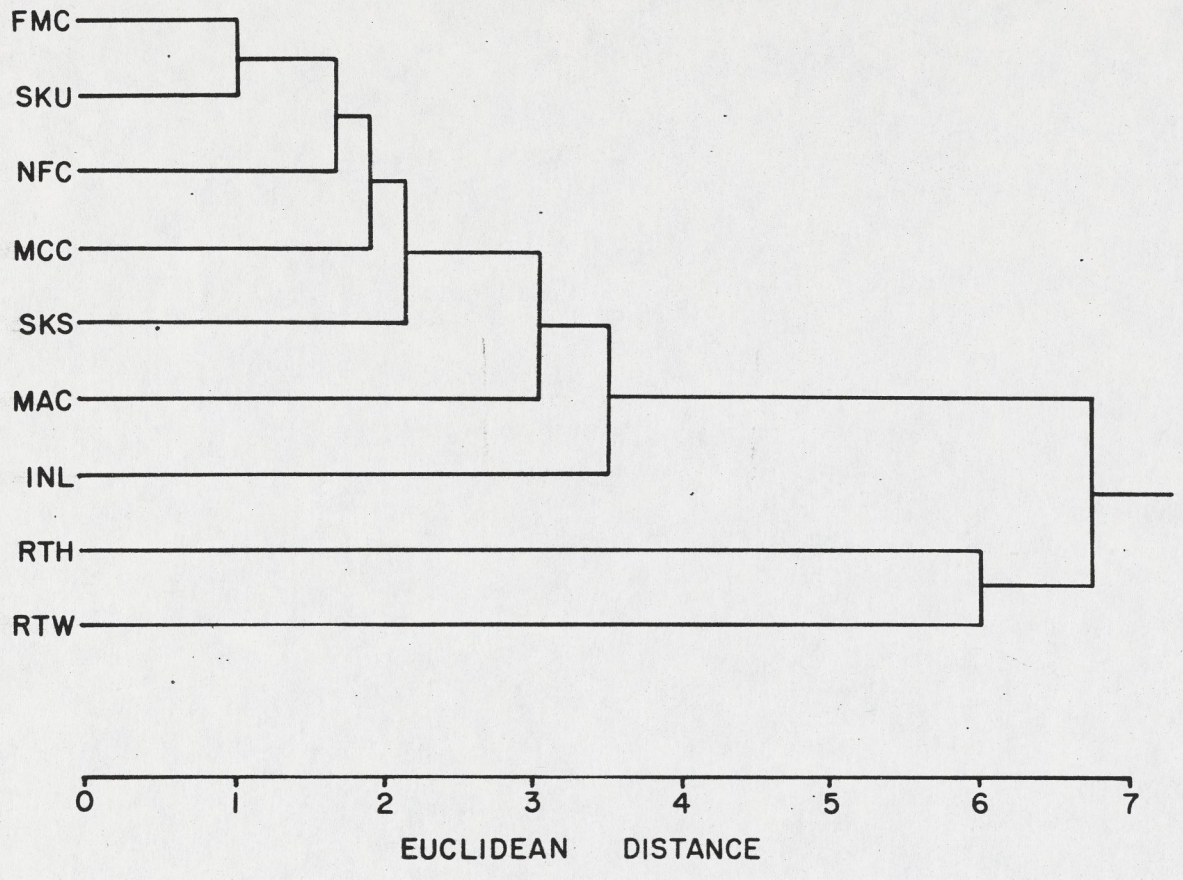

FIG. 3. Euclidean distance dendrogram based on means of nine meristic characters. The cophenetic correlation coefficient is .98.

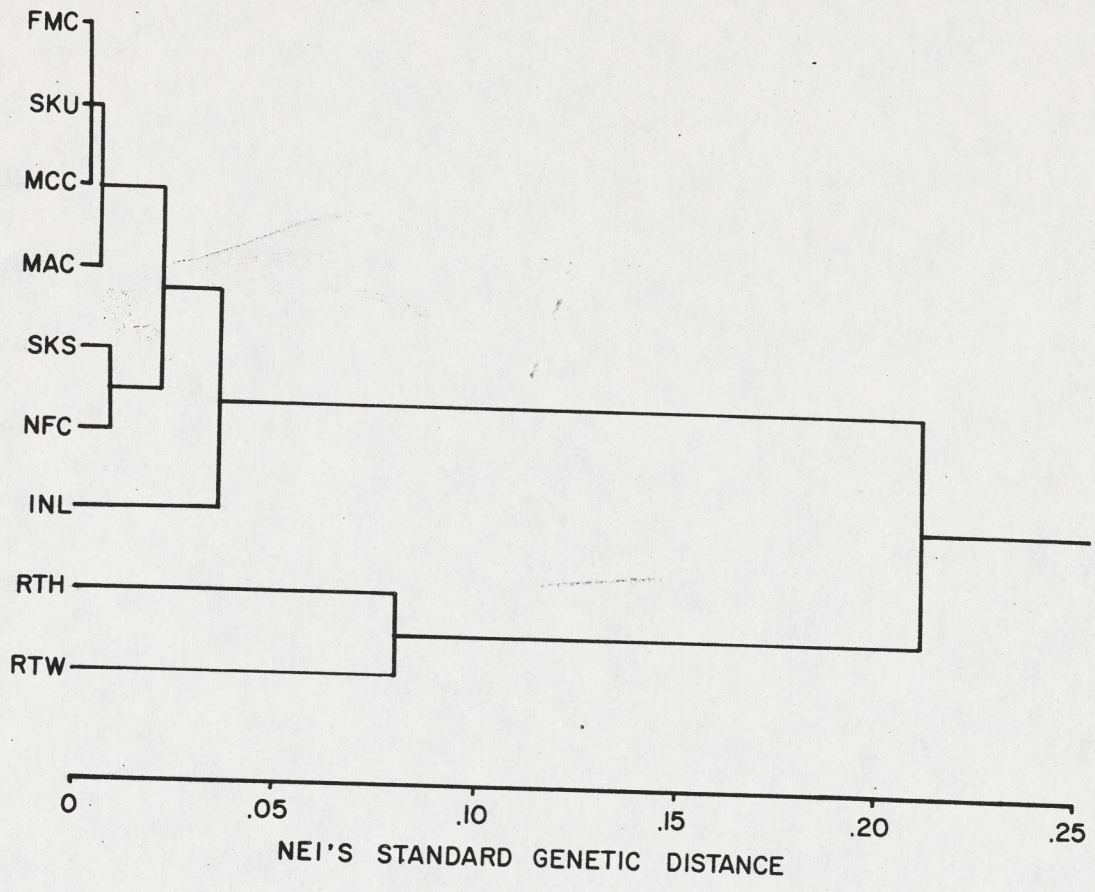

FIG. 4. Genetic distance dendrogram based on allele frequencies at 20 structural gene loci. The cophenetic correlation coefficient is .96.

introgressed populations were more heterozygous than nonintrogressed populations. Meristically no such trend existed. One of the introgressed samples, NFC had the lowest mean CV of all samples while the other, SKS, had the highest.

A bivariate principal component scattergram of SKS and NFC specimens is presented in Figure 5. Rainbow trout allele scores have been added as numerals to each appropriate data point. SKS specimens with extremely negative PC I and high positive PC II coordinates also tended to have high rainbow trout allele scores. Thus, within this sample, meristic outliers tended to be electrophoretic outliers. Also, the specimens meristically nearest to the rainbow trout sample spaces had high allele scores. In NFC no correlation between principal component coordinates and allele scores was evident.

Conclusions

Silver King Creek contains two trout populations: one appears both meristically and electrophoretically to be pure Paiute cutthroat, the other appears by both analyses to be the product of rainbow trout introgression. In contrast, Cottonwood Creek contains fish which meristically appear to be Paiute cutthroats, but electrophoretically show clear evidence of rainbow trout introgression. The high meristic uniformity (Table 1, Fig. 1) unimodal distribution of rainbow trout alleles (Table 4) and lack of correlation between principal component and allele scores (Fig. 5) in the NFC sample all indicate only one population is present.

The difference in population structure in the two creeks may be

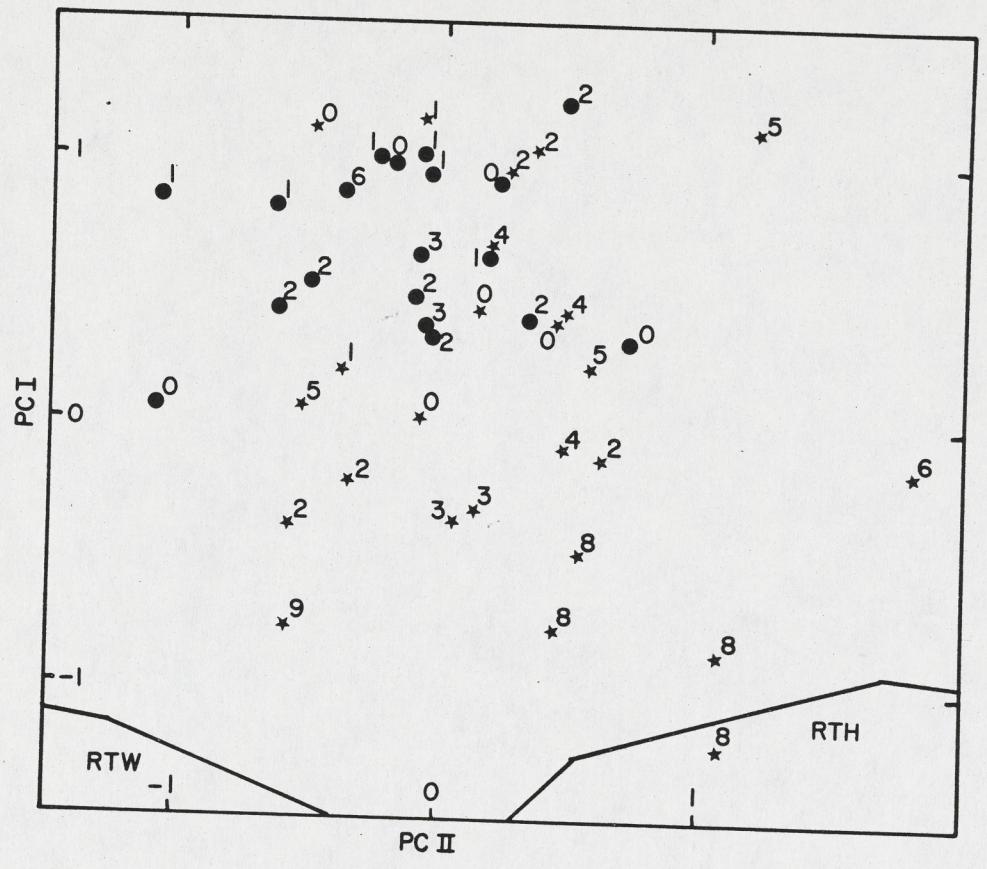

FIG. 5. Principal component scores and rainbow trout allele scores of SKS (stars) and NFC specimens (solid circles).

explained by introducing a simple introgression model. Consider a stream inhabited by species A into which species B is introduced. Let x_o denote the frequency of species A in the breeding population at the first breeding season in which both species particpate. Assuming constant population size, discrete generations, random mating, and equal fitness of all fish, the frequency of purebred species I fish t generation after mixing (x_t) will be $(x_o^2)^t$. Under this model, the frequency of species A drops to a negligible level very rapidly unless x_o is close to 1 (Fig. 6). Introgression may be considered complete when purebred species A fish no longer exist.

Fig 6 here

A reasonable estimate of the population size of Silver King Creek is 1,000 fish; Wong (1975) estimated the Cottonwood population at 500 fish. This disparity in population sizes makes the theoretical impact per rainbow trout transplanted much greater in Cottonwood Creek than in Silver King Creek. Not enough is known to estimate x_o for either population, but it is known that in Silver King Creek the rainbow trout were the survivors of a single introduction of 5,000 hatchery fingerlings (Ryan and Nicola 1976). Trout fingerlings typically have a low survival rate, so x_o in Silver King Creek may have been quite large. In contrast, the rainbow trout in Cottonwood Creek came from an established population downstream from the Paiutes. These transplanted fish would be expected to have a high survival rate, and several transplantations may have occurred. These factors could easily have made the effective x_o much lower in Cottonwood Creek than in

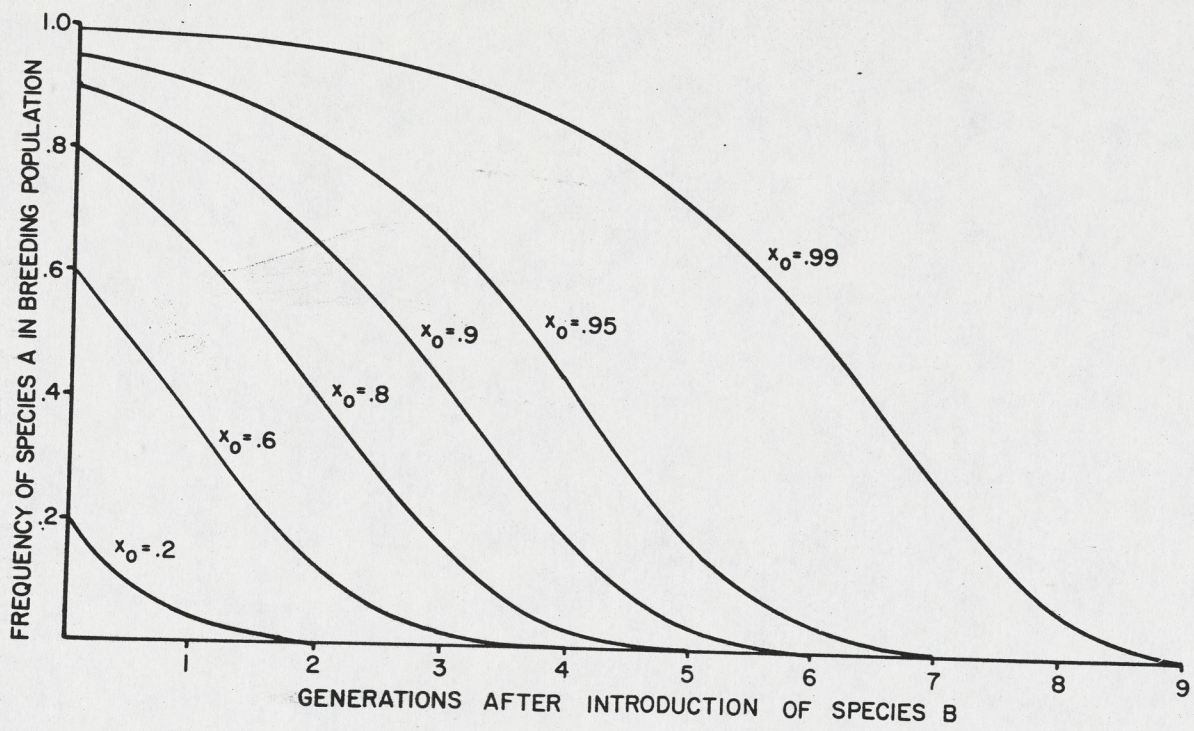

FIG. 6. Continuous-time representation of decline in frequency of purebred species A after species B is introduced, assuming constant population size, discrete generations, random mating, and equal fitness of all individuals. Original frequency of species A is denoted by x_0.

Silver King Creek. In light of the model, the difference in population structure between the two creeks probably does not reflect a difference in the outcome of introgression, but rather, different stages in the process; introgression is not yet complete in Silver King Creek, but is complete in Cottonwood Creek.

Management agencies have doubtless retarded the introgression process in Silver King Creek by removing large numbers of fish from the introgressed population, but there is evidence that introgression may have also been retarded by assortative mating. When the SKU and SKS samples were collected, spotted fish were much more common in fast water than in slow water. Probably 75% of the SKS fish were collected in fast water. Interbreeding between the pure and introgressed populations in Silver King Creek may thus be largely limited to the margins of fast water areas. This habitat preference may simply be a case of fish with rainbow trout ancestry preferring rainbow trout-like habitat (Mc Afee 1966), or it be a case of introgressed fish better exploiting a new habitat than nonintrogressed fish. Anderson (1949) speculated that introgression commonly leads to the occupation of new habitats, and Lewontin and Birch (1966) demonstrated experimentally that it is possible.

The Cottonwood Creek population provided the major discrepancy of the study; NFC fish electrophoretically showed clear evidence of rainbow trout introgression, yet meristically were indistinguishable from pure Paiute cutthorat. This situation most likely did not arise naturally. Spotted fish were common in Cottonwood Creek before the 1970 chemical treatment, and rainbow trout still live

downstream from the Paiute cutthroat population, below a barrier. Thus, it is improbable that the situation of meristics disguising introgression is caused by natural selection. It was probably caused instead by management activities. The 1970 removal of spotted fish may have effectively selected against all rainbow trout meristic traits as well, since polygenic traits tend to remain highly correlated for many generations in introgressed populations. This would also explain the low CV's observed in NFC. Rainbow trout allozyme alleles, which segregate independently, could not have been removed nearly as efficiently.

A study using meristic analysis alone would have arrived at the erroneous conclusion that NFC represented a pure Paiute cutthorat population. Perhaps cases like this are rare, however, much more often meristics and electrophoresis will agree, as is the case in Silver King Creek. Even so, electrophoresis can be a great deal more powerful. Electrophoresis demonstrated that introgressive hybridization had taken place in Silver King Creek; the meristic results merely were consistent with the hypothesis of introgressive hybridization. Linkage disequilibrium analysis of the electrophoretic data made sampling errors estimable in the SKU and SKS samples; this was not possible with the meristic data.

Morphological analyses such as meristics cannot be abandoned however, for three reasons. First, electrophoresis sometimes will not resolve the problem. In this study it was impossible to evaluate the possibility of Lahontan cutthroat introgression in Silver King Creek because S. c. henshawi and S. c. seleniris are

electrophoretically identical. Two other cutthroat subspecies, utah and pleuriticus, are also known to be electrophoretically identical (Loudenslager and Gall 1980). Second, in restoration operations, management agencies need to ascertain that the form they are attempting to salvage maintains a phenotypic fidelity to its original description. This can only be done on the basis of morphology. Third, electrophoresis is not a field technique. It can be used to elucidate population structure and evaluate identification methods, but what ultimately must be developed is a simple field-applicable technique to discriminate between introgressed and non-introgressed fish. Electrophoresis will play an integral part in the development of this technique, but the technique itself will almost certainly be based on morphology.

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Paiute Trout

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