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Bob

Dear Colleague:

Enclosed is a report entitled "Cutthroat Trout--a biochemical genetic assessment of their status and systematics." This report demonstrates the application of using biochemical genetic methods in studying the systematics of and hybridizations among western trouts (Salmo) and is a completion report for cutthroat trout studies sponsored by the University of Wyoming-National Park Service Research Center and the states of Nevada, Wyoming, and California.

This report is being submitted to the University of California, Division of Agricultural Sciences for publication as an Experiment Station Bulletin, which will facilitate its distribution and availability.

Sincerely,

Eric J. Loudenslager

/lg
Enclosure

Feel free to make any comments you want

CUTTHROAT TROUT

A Biochemical-Genetic Assessment of Their Status and Systematics

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and

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PREFACE

During the summer of 1976, a study of genetic variations in cutthroat trout (Salmo clarki) in Grand Teton and Yellowstone National Park was initiated using cytogenetic and biochemical genetic methods. The principal objective of the original study was to determine the origin of the fine spotted cutthroat trout in the Snake River, Wyoming. An ancillary objective was to evaluate, using chromosomal and electrophoretic markers, genetic characteristics of cutthroat trout subspecies and their hybrids with rainbow trout.

The first objective was accomplished from the sampling in 1976 and 1977. In order to fulfill the second objective, sampling of cutthroat and rainbow trout throughout their ranges was required. With the cooperation of several state and federal agencies, the necessary sampling and analyses have been completed. We have been successful in our attempts to use electrophoretic markers to identify cutthroat subspecies and their hybrids with rainbow trout. The results are currently being used by fish managers throughout western United States to plan and implement cutthroat trout management and restoration projects. This report presents a comprehensive summary of the results of biochemical genetic analyses of over 2,000 trout from 78 locations in Arizona, California, Idaho, Mexico, Montana, New Mexico, Nevada, and Wyoming. Included are sections on speciation and population genetics of cutthroat trout, hybridizations between cutthroat and rainbow trout, and the systematics of western Salmo. Our intention was to write a report in a format suitable for use by fisheries biologists, geneticists, and interested individuals from the general public.

We take pleasure in expressing our gratitude to those individuals who made the project possible; John Varely, Ron Jones and Bob Gresweld - U.S. Fish and Wildlife Service, Yellowstone National Park; Dale Lockard, Bob Sumner, and Pat

Coffin - Nevada Department of Wildlife; Mike Stone and Joe White - Wyoming Game and Fish Department; Steve Nicola and Eric Gerstung - California Department of Fish and Game; John Rinne and Bruce Anderson - U.S. Forest Service; Greg Mauser - Idaho Department of Fish and Game; and Dean Hendrickson - Arizona State University.

Bob Behnke, Dick Wallace, Fred Utter, Don Compton, Gary Thorgaard, and Craig Busack have shared their ideas on Salmo evolution, and each in their own way has influenced our presentations. Boyd Bentley helped run the analysis of most of the Nevada samples and tolerated EJL's invasion of his laboratory to run the remainder. Craig Busack and Bill Baker wrote the computer programs and assisted with the data analysis. The project was initiated while EJL was a graduate student of R.M. Kitchin, Department of Zoology, University of Wyoming, and completed while a post-doctoral geneticist, Department of Animal Science, University of California, Davis.

The study was supported financially by the Nevada Department of Wildlife through Dingell-Johnson Federal Aid to Fish Restoration funds, the University of Wyoming - National Park Service Research Center, the Wyoming Game and Fish Department, and the California Department of Fish and Game through Dingell-Johnson Federal Aid to Fish Restoration funds. The interest, cooperation, and support of these agencies and all the individuals who assisted us will be remembered for many years. We thank you all!

Introduction

Patterns of land use throughout the western United States are placing increasing demands on water resources. Impacts from logging, livestock grazing, irrigation needs, and dam construction have altered large segments of aquatic and riparian habitat. Recreational use from growing population centers has increased the exploitation of native fisheries and has frequently been accompanied by demands from sportsman for the introduction of exotic species. In combination, habitat loss and competition, and hybridization with exotic species are thought to be responsible for the catastrophic decline in the abundance of native trout populations (Deacon, et al., 1979). With the enactment of the Endangered Species Act of 1973, several state and federal agencies stepped up efforts to assess the status of the remaining native trout stocks. These assessments usually involve quantifying habitat and stream flow requirements, estimating the size of populations, and evaluating the taxonomic status and species purity of the populations.

This bulletin summarizes our examination of the population structure, speciation and hybridization of cutthroat trout using biochemical-genetic markers to characterize populations and assesses the current status of Salmo systematics. The topics are arranged to demonstrate the applicability of the approach to the development of management policies for rare species and its use as a tool in defining systematic relationships among western Salmo.

The report contains six chapters. The first is a review of western trout taxonomy and an introduction to electrophoretic methodology. The second outlines a detailed study of the population structure and differentiation found in cutthroat trout from major drainages and basins. The third describes the current systematics of inland cutthroat trout and discusses some problems. The

fourth is an examination of putative hybridization between cutthroat and rainbow trout. The fifth chapter presents a detailed analysis of genetic differentiation within and between subpopulations and subspecies of cutthroat trout and the final chapter discusses systematic relationships among western Salmo, pointing out areas of continued controversy.

I. THE PROBLEM AND THE APPROACH

1. Salmo Taxonomy

Many western trout populations are isolated in disjunct drainages and basins and exhibit extreme variation in morphology and life history. Diversity among populations within drainages and basins may be as great as among populations from separate drainages. Because taxa were described from limited sampling within drainages, the historical classifications of Salmo promoted a typological species concept. Consequently, efforts to classify Salmo using a binomial system of nomenclature which reflects the genetic, ecological, and evolutionary relations of populations has been controversial.

Several recent attempts have been made to clarify the evolutionary relationships among Salmo species. Miller (1950) proposed that all forms of western Salmo either belong to, or were derived from, two phyletic lines: the cutthroat trout, Salmo clarki, and the rainbow trout, S. gairdneri. Subsequent investigations (Behnke, 1972; Schreck and Behnke, 1971; Schreck et al. 1972; Miller, 1972) speculated that several taxa including Arizona trout, S. apache, gila trout, S. gilae, California golden trout, S. aguabontia, and red-banded trout, Salmo sp. represent distinct phyletic lines. However, most of these taxa evolved in allopatry and are thought to hybridize (Behnke, 1972; Miller, 1950, 1972) with other Salmo. The absence of diagnostic morphological characters as well as the lack of reproductive isolation has raised questions regarding the validity of many of the recognized taxa and their evolutionary relationships.

2. Hybridization

In the past, the rainbow trout has been established for recreational purposes outside of its native range. When rainbows were stocked into environments inhabited by cutthroat, gila, Arizona, or golden trout,

hybridization was invariably assumed to have occurred. The assumption of widespread hybridization coupled with the typological species concept has further complicated the identification and evaluation of the purity of populations which may represent stocks of native trout. However, despite being considered a principal cause of the decline in the abundance of native trout, the existence rainbow-native trout hybrids (S. clarki, S. apache, S. gilae, S. aguabonita) have been poorly documented. Adequate studies of hybridization require a thorough examination of the geographic variation in the species under study, and should include samples of known F_1 hybrids if wild caught putative hybrids are to be identified reliably (Neff and Smith, 1979). Although cutthroat-rainbow and rainbow-golden hydrids have been produced in hatcheries, thorough documentation of relative viability, growth, and development is only presently under investigation. Estimates of the fertility of F_1 hybrids, and evidence of F_2 and backcross matings are unavailable.

3. Electrophoretic Methodology

Fishery managers and fisheries scientists alike, have been examining the potential of new methods for solving taxonomic problems in Salmo, because traditional procedures have been insufficient. Techniques were sought which would provide a means of evaluating hybridization and speciation on a genetic basis, thus eliminating confounding environmental influences. Gel electrophoresis has become one of the more popular new methods. Identifiable, inherited markers can be used to study the influence of stocked congeners upon native populations as well as to study the population structure of geographically isolated groups of populations.

Describing genetic diversity and quantifying allelic frequencies in populations are the fundamental observations on which genetic studies of

hybridization and speciation depend. Any technique that is to enumerate genetic aspects of populations must fulfill the following criteria (Lewontin, 1974):

1. Phenotypic differences caused by the substitution of one gene (allele) for another at a single genetic locus must be detectable as an unambiguous difference between individuals;
2. Allelic substitutions at one locus must be distinguishable in their effects from allelic substitutions at other loci;
3. All or a very large portion of allelic substitutions at a locus must be detectable and distinguishable from each other, irrespective of the intensity or range of their physiological effects;
4. The loci that are amenable to examination must be a random sample of genes with respect to the amount of genetic variation that exists in the population; and
5. Many individuals and many loci must be amenable to simultaneous analysis.

Gel electrophoresis of proteins, often referred to as biochemical-genetic analysis, is the technique presently available that best fulfills all five criteria. Proteins are composed of a linear arrangement of the twenty amino acids, the sequence of which is determined by the sequence of nitrogenous base pairs in the DNA molecule. As such, proteins are the phenotypic products of single structural genes. Deletions, frame shifts, and substitutions of base pairs within the DNA molecule will sometimes alter the amino acid sequence of the protein molecule without changing its function. These alterations can result in the production of proteins with different net ionic charges and conformations that are inherited in a simple Mendelian manner. Using an electric current, electrophoresis separates the proteins, obtained as aqueous extracts of crude tissue homogenates, in a supporting medium such as starch or polyacrylamide gel.

Because these water soluble proteins carry a specific net ionic charge in a

buffered solution, they will migrate at a repeatable rate within a specific electric field. Enzyme molecules that have an altered amino acid sequence migrate at a different rate in an electric field but possess the same enzymatic properties. If gels are stained histochemically for each enzyme, one at a time, inherited variation at a discrete number of genetic loci can be visualized as zones of enzyme activity in different regions of the gel. Codominant expression of the enzyme variants (i.e., visible staining of each isozyme form) facilitates designating individual genotypes from the banding pattern on the gel. Because the gels can accommodate many samples, several individuals can be analyzed side by side and the frequency of alleles determined directly from the population data.

The use of electrophoretic data in the study of genetic variation within species assumes that the banding variation on the gel is a reflection of genetic variation. The strongest evidence in support of Mendelian inheritance comes from the analysis of progeny from parents of known electrophoretic differences. Evidence from such inheritance studies has confirmed much of the observed enzyme variation in rainbow trout (Allendorf and Utter, 1973, 1976; Utter et al., 1974; Allendorf et al., 1975, 1976; Gall and Bentley, 1981). Enzyme variation in other western Salmo that is presumably homologous to the enzyme variants in S. gairdneri, are assumed to have the same genetic basis. In the absence of inheritance data, the banding patterns must be interpretable using a genetic hypothesis.

Allendorf and Utter (1976) suggested a uniform system of nomenclature for salmonids which we use throughout. Genetic loci are designated by an abbreviation which corresponds to the enzyme which it encodes (e.g., asparate amino transferase is abbreviated AAT). When more than one locus encodes several

Figure 1. Allozyme phenotypes of Pepatidase 1(PEP-3) in Salmo. PEP-1 is a dimeric molecule encoded by a single locus. Heterozygous individuals exhibit a 3 banded phenotype where as homozygous individuals exhibit a single band. In this sample of 30 individuals, four alleles are present. Individuals 1 and 2 are heterozygous for the 60/100 alleles; individuals 5-10 are homozygous for the 150 allele; individuals 11, 12, 17, 18, and 19 are heterozygous for the 100/160 alleles, individuals 13, 14, 15, 16, 23, 24, and 25 are homozygous for the 100 allele; individuals 20, 21, 22, 26, 27, 28, 29, and 30 are homozygous for the 160 allele.

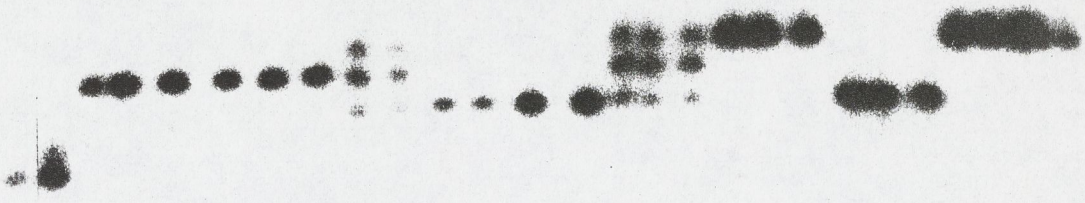
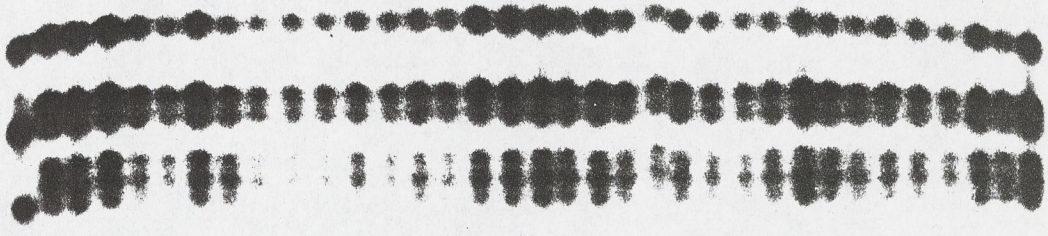


Figure 2. Isozyme phenotypes of malate dehydrogenase (MDH) in heart extracts of S. clarki henshawi and S. gairdneri. MDH is a dimeric molecule encoded by 4 loci. In S. gairdneri MDH 1 and 2 migrate to the same position and MDH 3 and 4 migrate to the same positions within the gel matrix, giving rise to a three banded phenotype. In S. clark henshawi MDH 3 and 4 comigrate, but MDH 1 and 2 do not, giving rise to a six banded phenotype.



forms of the same enzyme, the locus producing the enzyme with the least anodal migration is designated one, the next migrating product as two, and so on. Alleles are designated by the migration rates of their protein product relative to the most common form observed in rainbow trout.

The active molecule of many enzymes is made up of more than one polypeptide chain, usually two (dimeric) or four (tetrameric). Alleles encode for polypeptide chains, so when different alleles are present, such as in a heterozygote, the active enzymes present can be made up of either or both forms of the polypeptide chains. In such cases multiple banding is observed on the gel after staining (Figure 1). When multiple bands on the stained gel represent the expression of different alleles at a single locus they are called allozymes. Since the PEP-3 molecule is dimeric, heterozygous individuals express a 3 banded pattern, whereas homozygous individuals exhibit only a single band.

Malate dehydrogenase (MDH) is also a dimeric molecule (Figure 2), however, it is encoded by 4 loci. In S. gairdneri (sample 1), the products of two loci, MDH-1,2, encode for proteins that migrate to the same position in the gel. Similarly, the other two loci, MDH-3,4, encode for products that migrate to the same position in the gel slightly more anodal to the protein from MDH-1,2. Since the protein is dimeric, a 3 banded pattern is visible; the centrally migrating band represents the heterodimer. In contrast, the MDH-1 and MDH-2 loci of S. clarki henshawi (samples 2-35) encode for proteins that migrate to different positions in the gel. This results in three slow migrating bands, representing homodimers for loci 1 and 2, and the corresponding heterodimer. The proteins from the MDH-3,4 loci of S. c. henshawi comigrate, so they appear the same as the single homodimeric band observed for S. gairdneri. The two intermediate bands represent the heterodimeric molecules of the MDH-1 and MDH-2

polypeptides combined with the single form of MDH-3,4. These enzyme types are referred to as isozymes because the differences are due to different loci. Allelic variation at one of the loci would result in additional bands; these latter bands are referred to an allozymes, but may be indistinguishable from some bands referred to as isozymes.

II. GENETIC RELATIONSHIPS AMONG CUTTHROAT TROUT SUBSPECIES

1. Background

The cutthroat trout, Salmo clarki, is a polytypic species which exhibits the most extensive continental distribution of the western North American trouts (Behnke, 1972). Behnke (1965) recognized a coastal subspecies, S. c. clarki, consisting of anadromous, non-anadromous fluvial, and resident lacustrine populations ranging from Prince William Sound in southern Alaska to the Eel River in Northern California. In addition, Behnke (1965) recognized an inland complex of subspeices native to the Great Basin and intermountain drainages on both sides of the Continental Divide. Currently, different subspecies are recognized in most independent drainages and basins (Figure 3).

Because there is neither a description of an Asian counter part to S. clarki (Behnke, 1966) nor adequate fossil evidence, the origin of the North American cutthroat trout is uncertain. However, the extensive distribution of the inland subspecies is evidence that S. clarki was probably widely distributed in North America prior to most recent Pleistocene glaciation. Moreover, both volcanism and glaciation during the Pleistocene are believed to have influenced the distribution and differentiation of the extant subspecies (Behnke, 1972; Loudenslager and Thorgaard, 1979; Loudenslager and Kitchin, 1979; Loudenslager and Gall, 1980).

2. Sampling and Analysis

Samples from populations currently recognized as the Lahontan, S. c. henshawi; the Utah, S. c. utah; the Colorado, S. c. pleuriticus; the Yellowstone, S. c. bouvieri; and the west-slope, S. c. lewisi subspecies were analyzed electrophoretically at 36 loci. The enzymes examined, their tissue distribution, and the number of loci scored from each are listed in Table 2.

Figure 3. Sample locations and approximate distribution of cutthroat trout subspecies.

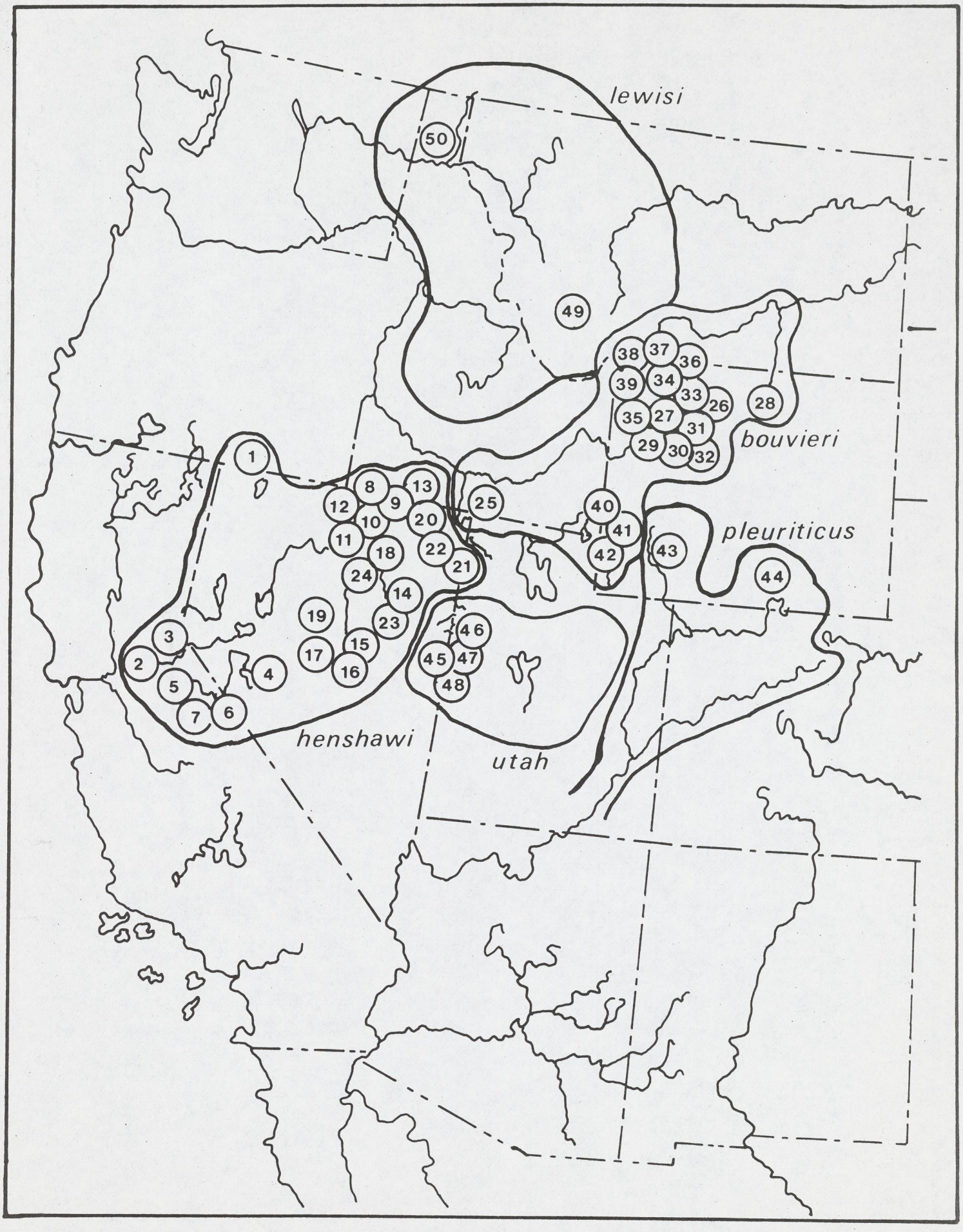

lewisi
50
49
38
37
36
39
34
33
26
28
35
27
31
29
30
32
bouvieri
1
8
13
12
9
25
40
10
20
41
11
22
42
43
pleuriticus
18
21
24
44
14
19
23
46
3
15
17
45
47
2
16
4
48
5
7
6
henshawi
utah

TABLE 1. CUTTHROAT TROUT POPULATIONS SAMPLED

Sample #	Basin/Drainage	Collection Site	# Indvs.	Hetero-zygosity
S. c. henshawi				
1.	Lahontan/Summit Lake	Summit Lake, NV.	42	0.004
2.	Lahontan/Carson River	East Carson River, CA.	23	0.000
3.	Lahontan/Carson River	Poison Flat Creek, CA.	21	0.014
4.	Lahontan/Walker River	Walker Lake Hatchery Stock, NV.	40	0.050
5.	Lahontan/Walker River	By-Day Creek, CA.	9	0.000
6.	Lahontan/Walker River	Dunderberg Creek, CA.	11	0.038
7.	Independent Drainage	O'Harrel Creek, CA.	25	0.004
8.	Lahontan/Humboldt River	Gance Creek, NV.	41	0.015
9.	Lahontan/Humboldt River	California Creek, NV.	12	0.012
10.	Lahontan/Humboldt River	Fraizer Creek, NV.	14	0.011
11.	Lahontan/Humboldt River	S. Fork Little Humboldt River, NV.	28	0.008
12.	Lahontan/Humboldt River	N. Fork Little Humboldt River, NV.	6	0.000
13.	Lahontan/Humboldt River	N. Fork Humboldt River, NV.	43	0.018
14.	Lahontan/Humboldt River	Conrad Creek, NV.	47	0.024
15.	Isolated Central Basin	Shoshone Creek, NV.	31	0.045
16.	Lahontan/Humboldt River	Tierney Creek, NV.	21	0.040
17.	Lahontan/Humboldt River	Washington Creek, NV.	38	0.025
18.	Lahontan/Humboldt River	Coyote Creek, NV.	37	0.027
19.	Lahontan/Humboldt River	Marysville Creek, NV.	12	0.008
20.	Lahontan/Humboldt River	T Creek, NV.	32	0.007
21.	Bonneville Basin	Donner Creek, UT.	21	0.010
22.	Lahontan/Humboldt River	Marys River, NV.	10	0.007
23.	Lahontan/Humboldt River	Long Canyon Creek, NV.	24	0.022
24.	Lahontan/Humboldt River	Rock Creek, NV.	39	0.021

Sample #	Basin/Drainage	Collection Site	# Indvs.	Hetero-zygosity
S. c. bouvieri				
25.	Snake River	Big Goose Creek, NV.	40	0.023
26.	Yellowstone River	Sylvan Lake, WY.	38	0.024
27.	Yellowstone River	Thumb Creek, WY.	14	0.016
28.	Powder River	South Paint Rock Creek, WY.	41	0.007
29.	Snake River	Hart Lake, WY.	13	0.007
30.	Snake River	Sheridan Lake, WY.	27	0.019
31.	Yellowstone River	south east arm-Yellowstone Lake, WY.	16	0.014
32.	Yellowstone River	south arm Yellowstone Lake, WY.	16	0.008
33.	Yellowstone River	Yellowstone River, WY.	29	0.011
34.	Yellowstone River	Allum Creek, WY.	15	0.008
35.	Yellowstone River	Bear Creek, WY.	35	0.012
36.	Yellowstone River	Soda Butte Creek, WY.	22	0.028
37.	Yellowstone River	Pebble Creek, WY.	30	0.017
38.	Yellowstone River	Cascade Creek, WY.	25	0.019
39.	Yellowstone River	upper Slough Creek, WY.	21	0.019
40.	Bonneville Basin/Bear River	Giraffe Creek, WY.	50	0.029
41.	Bonneville Basin/Bear River	Raymond Creek, WY.	22	0.013
42.	Bonneville Basin/Bear River	Alice Lake, WY.	37	0.015
S. c. pleuriticus				
43.	Green River	Colorado River Brood Stock, Daniel Hatchery, WY.	50	0.000
44.	Yampa River	Ted Creek, WY.	30	0.000
S. c. utah				
45.	Isolated Central Nevada Basin	Pine Creek, NV.	41	0.074
46.	Isolated Central Nevada Basin	Goshute Creek, NV.	40	0.030
47.	Bonneville Basin	Hendries Creek, NV.	40	0.053
48.	Bonneville Basin	Trout Creek, UT.	31	0.075
S. c. lewisi				
49.	Missouri River	Cougar Creek, WY.	41	0.019
50.	upper Columbia River	Kings Lake Brood Stock, ID.	46	0.023

TABLE 2. ENZYMES SYSTEMS STUDIED IN SALMO

Enzyme	Abbreviation	Tissue	# of loci
Aspartate aminotransferase	AAT	Muscle	2
Alcohol dehydrogenase	ADH	Liver	1
Para albumin	PALB	Serum	2
Creatine kinase	CK	Muscle	2
Diaphorase	DIA	Liver	1
Fumarase	FUM	Muscle	2
Glycerol phosphate dehydrogenase	AGPDH	Muscle	2
Isocitrate dehydrogenase	IDH	Liver	2
Lactate dehydrogenase	LDH	Liver/muscle/eye	5
Malate dehydrogenase	MDH	Heart	4
Malic enzyme	ME	Liver	1
Phospho-hexose-isomerase	PH1	Muscle	3
G-phospho glucouate dehydrogenase	GPGD	Liver	1
Phospho-manose-isomerase	PMI	Liver	1
Peptidase[1]	PEP	Eye/muscle	4
Phosphoglucomutase	PGM	Muscle/liver	1
Superoxide dismutase	SOD	Liver	1
Sorbitol dehydrogenase	SDH	Liver	2

[1]Substrates for pep-1&2 is glycyl-lucine; for pep-3 is leucyl-glycyl-glycine; and for pep-4 is phenylalanyl-proline.

Collection localities and sample sizes are presented in Table 1 and Figure 3. The analysis included 24 henshawi and 18 bouvieri populations whereas only 4 utah, 2 pleuriticus, and 2 lewisi populations were examined. This sampling scheme facilitated a thorough examination of intra-subspecific variation as well as inter-subspecific comparisons.

3. Results

Individual protein systems differ in their geographic pattern of variation and their contribution to estimates of differentiation among subspecies (Loundenslager and Gall, 1980). Eight loci, ME, IDH-3, PHI-3, SDH-1 and -2, PEP-1, PEP-3, and PEP-4 effectively differentiate the inland subspecies of S. clarki. Examples of the patterns of variation are presented in figure 4,5 and 6 for PEP-1, PEP-3 and IDH-3. Since electrophoretic data is recorded as allele frequencies for each genetic locus, it is convenient to summarize this information into a single index of either genetic divergence or genetic similarity among populations.

The index proposed by Nei (1972), the normalized genetic identity (I), defines the similarity between two populations. For a single locus j, it is defined as:

$$I_j = \Sigma x_i y_i \Big/ (\Sigma x_i^2 \, \Sigma y_i^2)^{1/2}$$

where x_i and y_i represent the frequencies of the i^{th} allele at locus j in the two populations X and Y. Summarized for all loci sampled, including monomorphic loci, the overall genetic identity index of populations X and Y is defined as

$$I_{xy} = J_{xy} \Big/ (J_{xx} J_{yy})^{1/2}$$

where J_{xx}, J_{yy} and J_{xy} are the arithmetic means, for all loci, of

Figure 4. Geographic variation at the peptidase-1 locus.

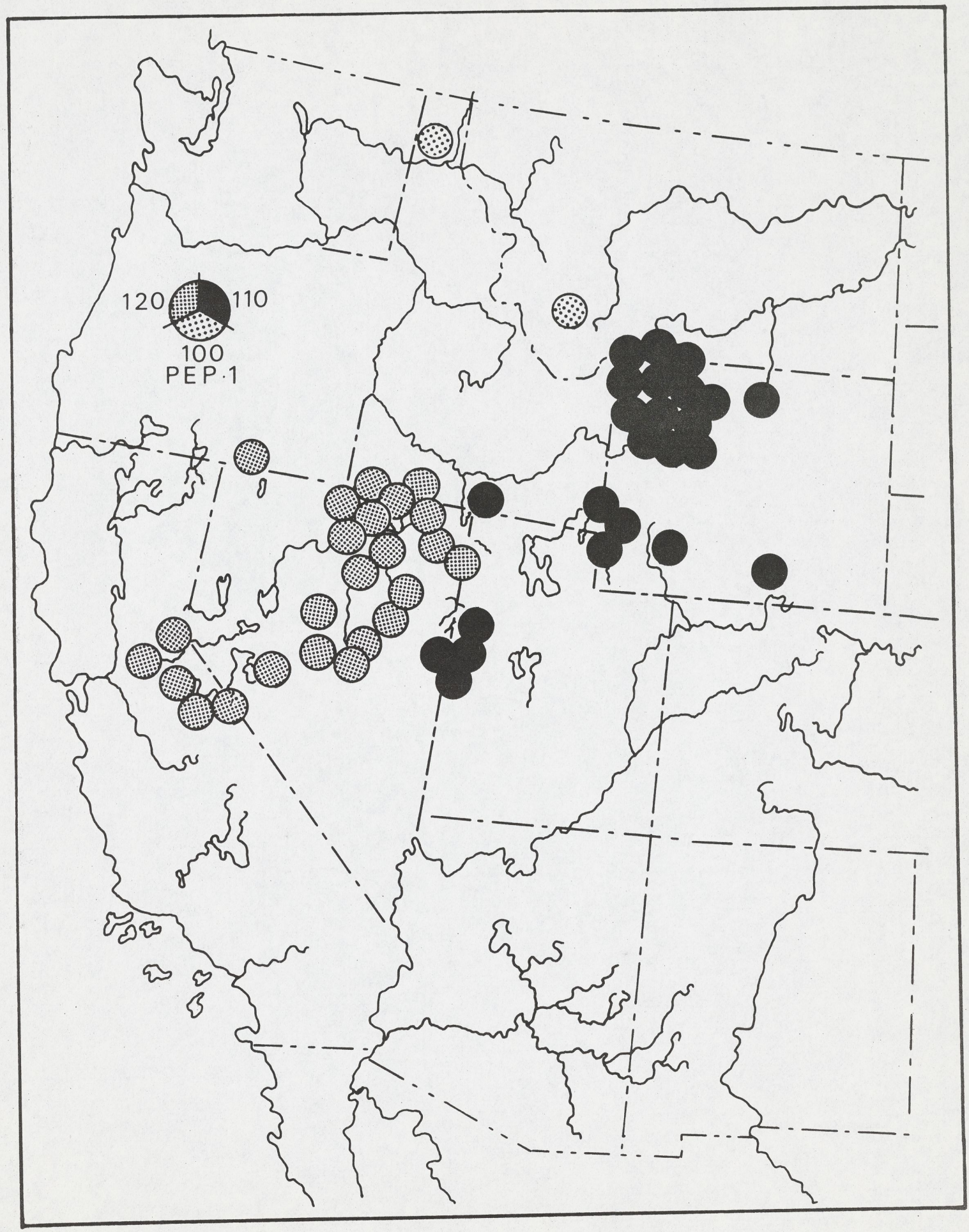

120
110
100
PEP·1

Figure 5. Geographic variations at the peptidase-3 locus.

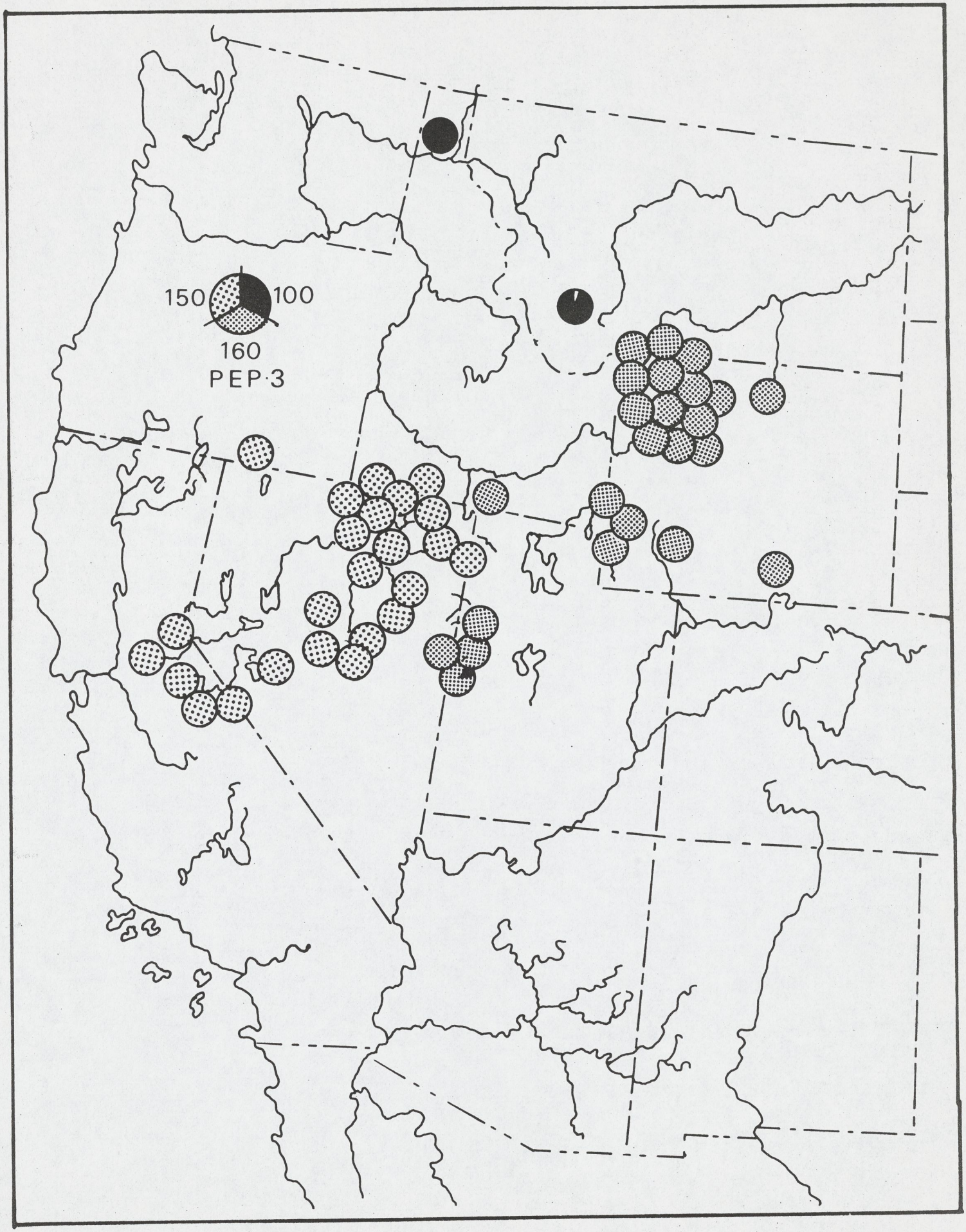
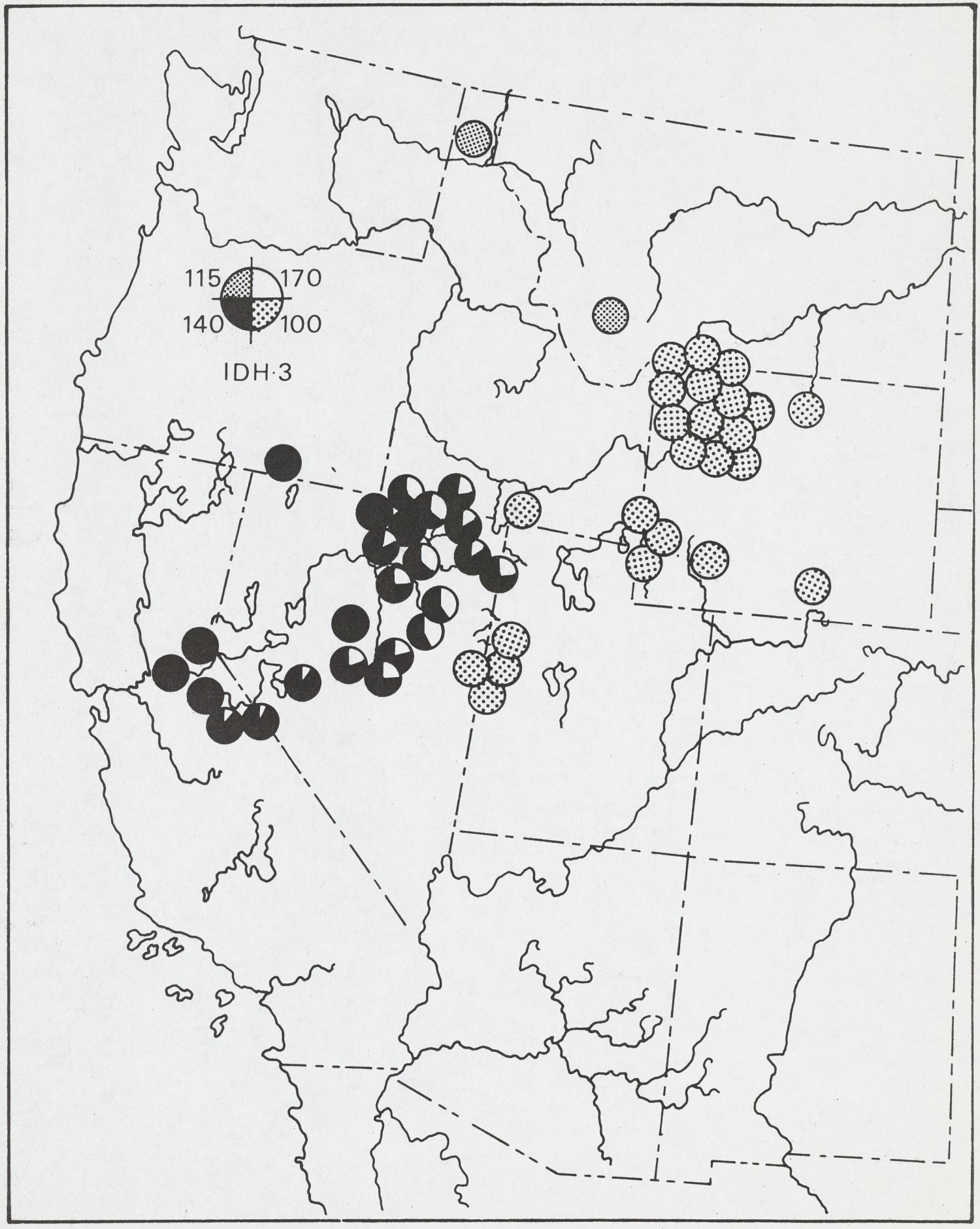

150
100
160
PEP·3

Figure 6. Geographic variations at the isocitrate dehydrogenase-3 locus.


115
170
140
100
IDH·3

Σx_i^2, Σy_i^2 and $\Sigma x_i y_i$, respectively.

Thus, the genetic identity index is an estimate of the proportion of sampled alleles which are electrophoretically identical in pairs of populations relative to the homozygosity expected within the populations. A value of I = 1.0 indicates that all alleles and their frequencies are identical in the two populations whereas a value of I = 0 indicates that each population possess a completely different set of alleles.

Pairwise comparisons among populations were used to summarize the average genetic identity between populations within subspecies (diagonal, Table 3) and contrast it to the average identity between populations from different subspecies (off diagonal, Table 3).

Populations within individual subspecies all had high average genetic identities, ranging from 1.00 to 0.984. Nevertheless, some populations within the two extensively sampled subspecies henshawi and bouvieri did exhibit substantial differentiation. Within henshawi, Shoshone and Marysville Creeks were divergent, having an identity of only 0.953 with the remainder of the Lahontan Basin populations which had an identity of 0.995 amongst themselves. Within bouvieri, Bear Creek, a thermally isolated population, was divergent with an identity of 0.968 with the other populations. However, identity among the remaining bouvieri populations, which includes samples from several independent drainages was 0.993.

Identity between subspecies pairs ranged from 0.969 for utah and pleuriticus to 0.737 for lewisi and utah, while the average identity among subspecies was 0.852. The average identity among populations of utah, bouvieri, and

TABLE 3. MATRIX OF GENETIC IDENTITY AMONG CUTTHROAT TROUT SUBSPECIES AND AVERAGE GENETIC IDENTITY (diagonal) AMONG POPULATIONS WITHIN EACH SUBSPECIES

	bouvieri	pleuriticus	utah	henshawi	lewisi
bouvieri	.991	.909	.894	.858	.820
pleuriticus		1.00	.969	.810	.770
utah			.984[+]	.783	.737
henshawi				.989	.777
lewisi					.993

[+]includes only the four Snake Valley populations.

pleuriticus was 0.920 indicating that they form a closely related subspecies complex. The Lahontan basin subspecies, henshawi, had an average genetic identity of 0.880 with the utah-bouvieri-pleuriticus complex. Salmo c. lewisi was divergent having an identity of only 0.779 with the other subspecies.

In summary, the genetic identity comparisons demonstrate substantially greater divergence among subspecies pairs than between pairs of populations within subspecies. There is, however, a considerable range in the genetic identity of subspecies pairs. And, there can be isolated populations within subspecies that exhibit nearly as much differentiation as closely related subspecies.

Genetic relationships among all the populations are summarized in a UPGMA dendogram (Figure 7) based on genetic identity indexes. The evolutionary relationships among the subspecies are presented as an unrooted Wagner network (Figure 8). Uniform rates of amino acid substitution are assumed throughout the phyletic lines when evolutionary relationships are inferred from a UPGMA dendogram. However, the unrooted Wagner network allows for different ratios of substitution in different lineages as well as reversibility of character states (Sneath and Sokal, 1973).

The UPGMA dendrogram demonstrates well defined subspecies. Populations of all subspecies form tight clusters. Even the divergent populations of henshawi and bouvieri cluster well within the sampling error. Both the UPGMA dendrogram and Wagner network suggest similar evolutionary relationships, which are consistent with cutthroat trout zoogeography.

4. Discussion

Cutthroat trout are distributed throughout an area of western North America that has undergone considerable topographical change which may have influenced

Figure 7. UPGMA dendrogram of 50 cutthroat trout populations using Nei's Index of genetic identity.

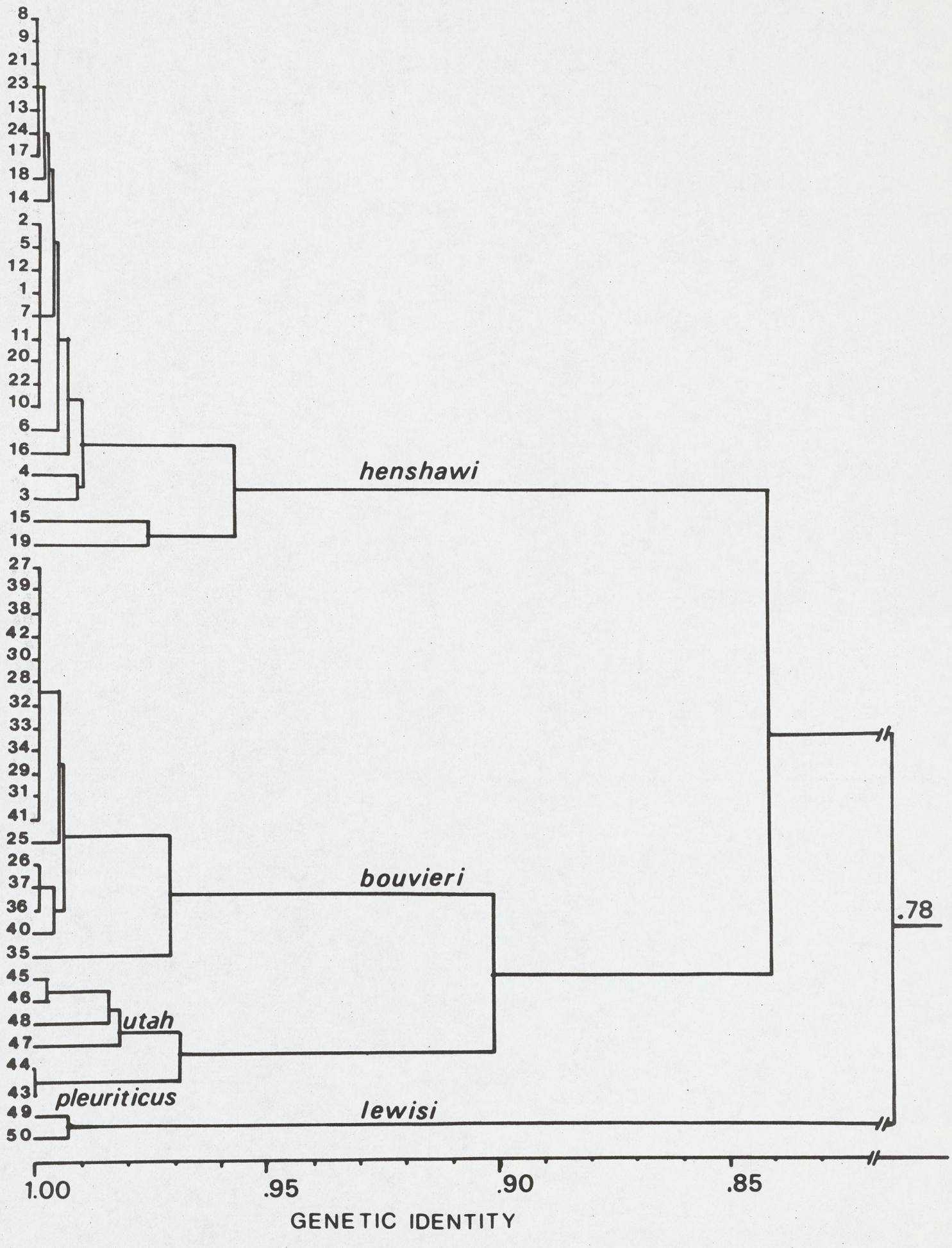
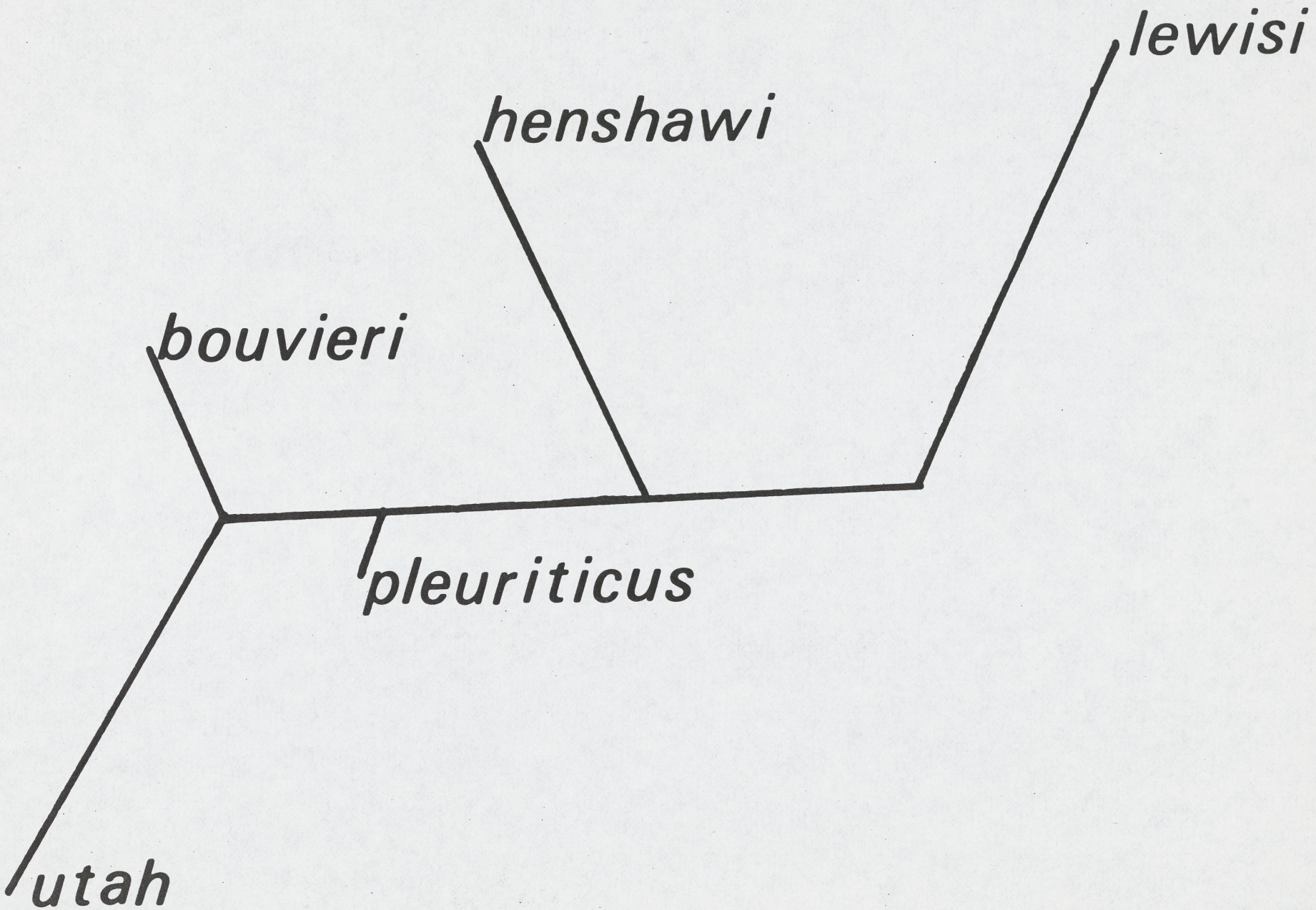

8
9
21
23
13
24
17
18
14
2
5
12
1
7
11
20
22
10
6
16
4
3
15
19
27
39
38
42
30
28
32
33
34
29
31
41
25
26
37
36
40
35
45
46
48
47
44
43
49
50
henshawi
bouvieri
utah
pleuriticus
lewisi
.78
1.00
.95
.90
.85
GENETIC IDENTITY

Figure 8. Wagner network of cutthroat trout subspecies


lewisi
henshawi
bouvieri
pleuriticus
utah

subspeciation in S. clarki. Fluctuating glacial and pluvial periods isolated and reconnected portions of the basins and drainage systems during the Pleistocene (Behnke, 1972). In addition, continual tectonic and volcanic activity has lead to increased isolation of independent basins and drainage systems (Morrison, 1965). The isolation of the physical habitat has reduced colonization and dispersal, while the unstable environmental conditions have increased extinction of western fishes (Smith, 1978). A precise chronology to account for the present distribution of the S. clarki subspecies would be imprecise and speculative; however, there are examples of how Pleistocene geological events may have influenced the isolation and subdivision of S. clarki populations.

The analysis of electrophoretic relationships suggests that the inland complex of cutthroat trout can be divided into three major subspecies groups consistent with their zoogeography: a Lahontan Basin subspecies group consisting of henshawi and S. c. seleniris, (Paiute cutthroat) a subspecies indistinguishable from henshawi electrophoretically (Busack, 1977); an upper Snake River-Colorado River-Bonneville Basin subspecies group which contains at least the utah, bouvieri and pleuriticus subspecies; and finally an upper Columbia-Missouri River subspecies group consisting of only the lewisi subspecies. Two other subspecies, S. c. stomias (Greenback cutthroat) and virginalis (Rio Grande cutthroat) could potentially form part of the upper Snake River-Colorado River-Bonneville Basin group.

The genetic identities among subspecies suggest further that the Lahontan Basin and Snake-Colorado-Bonneville groups are more closely related to each other than they are to the Columbia-Missouri River group. This conclusion is supported by observations on chromosome number; henshawi (Gold et al., 1977),

utah (Loudenslager, unpublished data), bouvieri (Loudenslager and Thorgaard, 1979) and pleuriticus (Loudenslager, unpublished data) all have a karyotype of 2n=64 with NF=104 (NF=chromosome arm number), whereas lewisi has a karyotype of 2n=66 with NF=104 (Loudenslager and Thorgaard, 1979). In addition, all of the inland subspecies can be karyotypically differentiated from coastal cutthroat trout (S. c. clarki) which have a 2n=68-70 with NF=104-106 karyotype (Simon, 1964; Gold et al., 1977).

Although closely related electrophoretically and chromosomally, henshawi is easily distinguishable from the utah-bouvieri-pleuriticus group at the IDH-3, MDH-2, SDH-2, PEP-1, PEP-3, and PEP-4 loci. The lack of integradation of allele frequencies at these loci indicates that recent transfers, with successful colonization, are unlikely to have occurred between the Lahontan and Bonneville Basins. This observation is in close agreement with geological and biogeographic evidence. First, the Lahontan basin has an unbroken rim (Hubbs and Miller, 1948; La Rivers, 1962) indicating that the basin never filled and discharged into contiguous drainages during the later pluvial periods. Second, Smith (1978) in a study of the biogeography of intermountain fishes examined the efficiency of barriers among many western basins and developed a barrier index which ranged from 1 (total isolation) to 0 (no isolation). The barrier index between the Lahontan and Bonneville Basins was .92 which provides further evidence of the low probability of colonization via headwater capture across the divide.

Salmo c. utah, bouvieri, and pleuriticus are closely related subspecies. Although they presently inhabit independent drainage systems, historical connections between the Bonneville Basin and the upper Snake River are well documented. The Bear River, which is now tributary to the Great Salt Lake, was

previously tributary to the upper Snake River and was diverted into the Bonneville Basin by lava flows (Malde, 1965). Subsequent to the transfer of the Bear River, the Bonneville Basin filled and discharged into the Snake River flood plain (Bright, 1963, in Malde, 1965). These geological events could have provided a transfer of cutthroat trout from one system to the other. The barrier index between the upper Snake River and the Bonneville Basin is only 0.32 (Smith, 1978) supporting the hypothesis that fish species within these drainage systems should be closely related. Connections between either the upper Snake River or tributaries to the Bonneville Basin and the Green River drainage in Wyoming are unknown. However, dispersal via headwater capture remains the best hypothesis to account for the presence of S. clarki in the Colorado River system.

On the basis of zoogeographic, chromosomal, and electrophoretic evidence, the Lahontan Basin and the Snake-Colorado River-Bonneville Basin subspecies groups probably shared a common ancestor. Dispersal followed by barrier formation and differentiation could account for the present subspecies distributions of cutthroat trout. However, systematic relationships between the subspecies groups with 64 chromosomes and lewisi which has 66 chromosomes and clarki which has 68-70 remain enigmatic.

III. CUTTHROAT SYSTEMATICS - SOME PROBLEMS

Systematic relationships among cutthroat trout subspecies inferred from morphological (Behnke, 1979), electrophoretic and chromosomal evidence are largely concordant, however, there remains several nomenclatural controversies.

1. The Snake River Drainage

Within the Snake River drainage in Wyoming, between Jackson Lake and Palidades Reservoir, there are two morphological varieties of cutthroat trout: the large-spotted S. c. bouvieri and a fine-spotted morph. Behnke (1965) noted that several drainages in western United States were inhabited by large- and fine-spotted populations and that intergradation was present. Later, Behnke (1972) proposed that the fine-spotted morph in the Snake River warranted subspecific recognition, but has not provided such a description because of uncertainties about the fine-spotted morph's taxonomic affinities. It was unclear if the fine-spotted cutthroat represented divergence from a large-spotted ancester within the Snake River, or if it represented the invasion of a differentiated subspecies (lewisi or clarki) into the upper Snake River.

Two populations of the fine-spotted morph were analyzed electrophoretically at 24 loci by Loudenslager and Kitchin (1979) and compared with large-spotted Snake River cutthroat. No loci were found to distinguish between the fine- and large-spotted forms and the estimated genetic identity between the two forms was 0.995. Time since divergence between two taxa can be estimated as $t=D/cn_T a$, where t is the period of time since the populations diverged, D is the genetic distance between the populations ($D=-\ln I$), c is the proportion of amino acid substitutions detectable electrophoretically, n_T is the number of codons involved in the synthesis of each protein, and a is the rate of amino acid substitution per polypeptide per year (Nei, 1971). Applying this statistic to

protein identities for cutthroat trout populations and using Nei's (1971) values of c=0.30, N_T=800, and a =2.1 x 10^{-9}. Loudenslager and Kitchin (1979) estimated that divergence between the large and fine-spotted cutthroat trout occurred 20,000 years ago. The significance of this estimation is that it places the origin of the fine-spotted Snake River cutthroat within the post-glacial period, not preglacial. Loudenslager and Kitchin (1979) further concluded that the fine-spotted Snake River cutthroat trout was derived from a bouvieri like ancester within the Snake River. Because of the high genetic identity and morphological intergradation observed within the Snake River (Loudenslager, unplublished) we believe that the Snake River fine-spotted cutthroat trout should be considered as part of a morphologically and ecologically variable S. c. bouvieri, not a new subspecies.

2. The Bonneville Basin

A) Snake Valley and Bear River

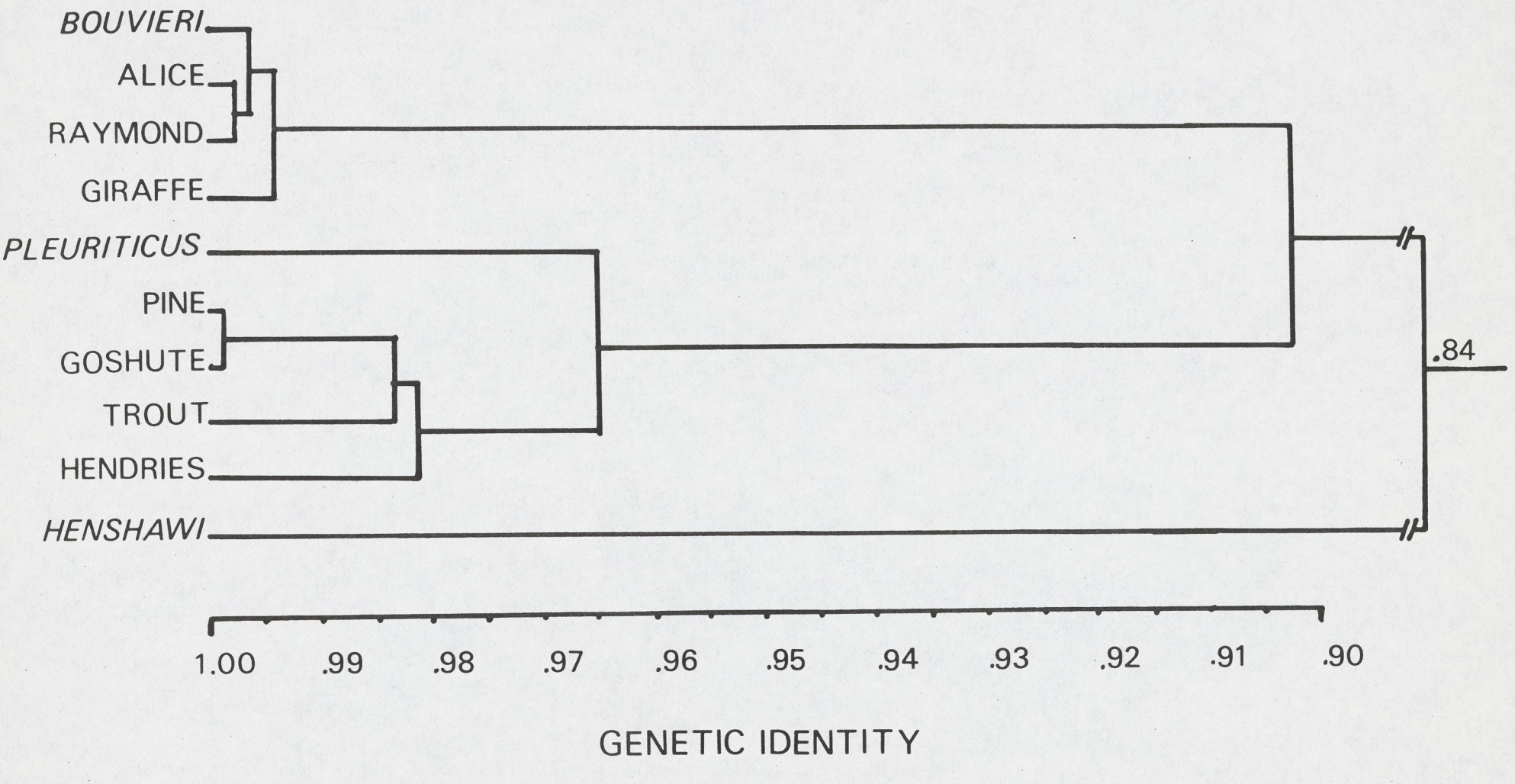
Three morphologically differentiated groups of cutthroat populations are currently recognized within the Bonneville Basin (Hickman and Duff, 1978; Behnke, 1979). They are associated with the Snake Valley region on the Nevada-Utah border, the Bear River drainage in Wyoming, Idaho, Utah, and the Bonneville Basin proper. Behnke (1979) proposed that, although slightly differentiated from each other, these three groups of populations were consubspecific and represented differentiation from a common ancester which gained access to the Bonneville Basin contemporaneously with the transfer of the Bear River from the Snake River drainage to the Bonneville Basin approximately 30,000 years B.P.

Seven Bonneville Basin populations were sampled during our present study: Giraffe Creek (#40), Raymond Creek (#41), Alice Lake (#42), from the Bear River drainage and Pine Creek (#45), Goshute Creek (#46), Hendries Creek (#47) and

TABLE 4. GENETIC IDENTITIES AMONG BONNEVILLE BASIN CUTTHROAT TROUT POPULATIONS AND PLEURITICUS, BOUVIERI, AND HENSHAWI.

		2	3	4	5	6	7	8	9	10
1	Alice	.998	.996	.997	.900	.913	.879	.907	.914	.862
2	Raymond		.996	.994	.895	.912	.875	.901	.915	.864
3	Giraffe			.997	.896	.904	.875	.907	.907	.854
4	bouvieri				.899	.904	.879	.907	.911	.860
5	Trout					.983	.983	.986	.977	.787
6	Goshute						.976	.997	.969	.801
7	Hendries							.979	.957	.764
8	Pine								.972	.798
9	pleuriticus									.810
10	henshawi									-

Figure 9. UPGMA dendrogram of Bonneville Basin cutthroat trout populations and representative populations of *S. c. bouvieri*, *pleuriticus*, and *henshawi*.


BOUVIERI
ALICE
RAYMOND
GIRAFFE
PLEURITICUS
PINE
GOSHUTE
TROUT
HENDRIES
HENSHAWI
.84
1.00
.99
.98
.97
.96
.95
.94
.93
.92
.91
.90
GENETIC IDENTITY

Trout Creek (#48) from the Snake Valley area. Genetic identities, based on 36 loci, were calculated and comparisons made among populations within and between the Snake Valley and Bear River drainages as well as with pleuriticus, bouvieri, and henshawi (Table 4). A mean genetic identity of 0.997 was found for populations within the Bear River drainage and an equally high value of 0.984 was found among populations from the Snake Valley region. These values are in sharp contrast to the average genetic identity of only 0.888 observed between the Bear River and Snake Valley groups. In addition, the Bear River populations were most similar to bouvieri (I = 0.996) and the Snake Valley populations most similar to pleuriticus (I = 0.969). The two groups of populations are clearly more similar to these two subspecies than they are to each other. A UPGMA dendogram of the genetic identities shows three distinct clusters (Fig. 9): one contains only henshawi; a second consists of pleuriticus and Pine, Goshute, Trout and Hendries Creeks, representing the Snake Valley populations; the third is made up of bouvieri and Alice Lake, Raymond Creek and Giraffe Creek, populations from the Bear River drainage.

These taxonomic relationships, inferred from the genetic identity dendrogram, are discordant with the current classification (Hickman, 1978). Moreover, the genetic relationships suggest that perhaps the cutthroat trout populations in the Bonneville Basin are the result of multiple invasions of ancestral cutthroat trout.

The close genetic relationship between bouvieri and Bear River drainage cutthroat populations supports previous hypotheses that bouvieri was recently transferred into the Bonneville Basin (Hubbs and Miller, 1948; Behnke, 1965, 1969; Hickman, 1978; Loudenslager and Thorgaard, 1979; Loudenslager and Gall, 1980). The similarly close relationship between Snake Valley cutthroat

populations and pleuriticus suggests a recent common ancester between these forms. If this interpretation accurately reflects cutthroat trout cladistic relationships, then exchanges of trout between the Bonneville Basin and Colorado River drainage is implied. Identifying this transfer is difficult since there is no geological documentaiton of major stream transfers between the Colorado River drainage and the Bonneville Basin. Smith (1978) demonstrated that colonization across the divide was rare. Ancestral cutthroat trout may have gained access to the Colorado River drainage via either direct transfer from the upper Snake River to the upper Green River, Wyoming, or indirectly by transfer from the upper Snake River to The Colorado River drainage via the Bonneville Basin.

B) Salmo clarki utah

Although genetic evidence suggests that Bonneville Basin cutthroat trout are polyphyletic, there still remains several unresolved questions with regard to the systematics of S. c. utah. Both Behnke (1979) and Loudenslager and Gall (1980) demonstrated a close taxonomic relationship between bouvieri, utah, and pleuriticus and proposed that these subspecies diverged from a "bouvieri like" ancester which dispersed from the upper Snake River. With the exception of the transfer of bouvieri into the Bonneville Basin, the details of the dispersal and subsequent differentiation of these subspecies is obscure. The available data is insufficient to determine whether pleuriticus and Snake Valley utah, differentiated from a "bouvieri like" ancester within the Bonneville Basin or within the Colorado River drainage. It is also impossible to determine if several reciprocal exchanges took place among these basins and drainages.

Our interpretation of a polyphyletic origin of the Bonneville Basin cutthroat trout causes problems in determining the taxonomic status of S. c. utah.

Suckley (1874) first described Salmo utah from Utah Lake, a population which is now extinct. Thus, there is no way of determining if the type locality was a population of Bear River utah or whether it was a Snake Valley utah. This makes it nearly impossible to determine which type of Bonneville Basin cutthroat actually represent S. c. utah. Further, Behnke and Miller (in Hickman and Duff, 1978) both suggested that the Snake Valley populations represented an undescribed subspeices. Later, Behnke (1979), after analysis of additional samples, concluded that all Bonneville Basin populations were consubspecific.

The objective of our classification is to provide adequate designations for the Snake Valley and Bear River drainage population groups. The problem is to determine if these two groups should receive their own taxonomic designiations or whether they should be classified with the subspecies to which they are genetically most similar. Because of the morphological differences between pleuriticus and Snake Valley utah, and because Snake Valley utah exhibits a number of genetic polymorphisms that are distinctive to this region, we suggest that the Snake Valley populations retain the utah designation. Because of the high genetic identity and lack of diagnostic morphological criteria (Murphy, 1974; Behnke, 1979) we conclude that the Bear River cutthroat trout populations should be recognized as a subgroup of bouvieri.

The major drawback to our classification is that it makes the task of managing the Bear River drainage populations more difficult: utah is recognized as a dwindling resource and as such receives priority in land management policy decisions whereas bouvieri, which is abundant in Yellowstone National Park, does not receive such attention. What is really needed, however, is not new subspecific names for every isolated population but rather a management policy that is flexible enough to recognize the need to maintain populations in different

drainages whenever a unique resource, such as the Bear River bouvieri cutthroat, is being managed. Bending the taxonomic rules to accomodate fish management decisions will only lead to a continued proliferation of additional names. For example, it is conceivable that bouvieri would need to be subdivided into five subspecies to protect its present distribution: one in Big Goose Creek, Nevada, a second for the upper Snake River, a third for the Bear River drainage, a fourth for the Yellowstone drainage, and a fifth for the population in South Paint Rock Creek, Wyoming!

IV. HYBRIDIZATION BETWEEN CUTTHROAT AND RAINBOW TROUT

1. Background

Cutthroat and rainbow trout are largely allopatric but closely related species. Where sympatric along the Pacific Coast and in the upper Columbia River drainage, ecological isolating mechanisms maintain species identity. In many coastal streams only one of the two species is present with the coastal cutthroat, S. clarki clarki, exhibiting a preference for low gradient, small streams while the rainbow trout, S. gairdneri, has a preference for large steep gradient streams. When found within the same drainage cutthroat usually inhabit and spawn in small side tributaries and the headwaters of major drainage systems while rainbow inhabit and spawn in mainstream tributaries.

The S. clarki subspecies that inhabit interior drainges historically were isolated from secondary contact with S. gairdneri. However, because of the frequent introduction of rainbow trout for recreational angling, many of the once isolated cutthroat populations have been exposed to artificial secondary contact. Behnke (1965, 1971) and Miller (1950) have suggested that there are no ecological and ethological barriers to hybridization between the inland cutthroats and S. gairdneri. They further suggested that hybrid swarms were the typical outcome when rainbow are introduced into an isolated population of cutthroat. However, there has been only circumstantial evidence and anecdotal documentation of hybridization between these species until recently (Busack and Gall, 1980).

Since S. gairdneri was repeatedly introduced into diverse environments throughtout the entire range of S. clarki, there now exists a unique opportunity to examine the extent and the dynamics of hybridization between S. c. henshawi, utah, bouvieri, and the congener S. gairdneri.

2. Methods of Analysis

Any analysis attempting to identify wild caught, putative hybrids requires the establishment of a priori criteria for the identification of the parental species and at least F_1, F_2 and backcross hybrids. Because of the inherent variability among populations of a species, reliable criteria can only be established after a large geographic sampling of the parental species has been undertaken. It is also highly desirable to examine hybrids of known ancestry in order to confirm the nature of the hybrid phenotype. Attempting to identify wild caught, putative hybrids using an analysis of the variability in the putative hybrids themselves results in a circular argument. For example, it is often assumed that hybrids exhibit morphological characters intermediate between the parental forms. When such individuals are observed they are classified as hybrids, and used as evidence that hybrids exhibit intermediate characters!

Discriminant function and principal component analyses of meristic and morphometric characters, electrophoretic analysis of protein systems and analyses of chromosome number and morphology all provide potentially valid approaches to the identification of hybrids. We strongly urge that all three methods be used in the development of fish management programs whenever suitable data can be obtained. As an example, Busack and Gall (1981) compared electrophoretic and meristic analyses as they apply to Paiute cutthroat - rainbow hybridization. Their report demonstrates the utility and application of the two methods of analysis. This report considers only the results of electrophoretic analysis.

In our survey of western Salmo, analysis of 2,129 specimens from 78 populations representing 9 recognized taxa have been completed to date. In addition, several rainbow broodstocks have been examined (Busack et al., 1979).

These studies indicated that 6 loci controlling the expression of 4 enzymes (peptidase (PEP-1&3), malic enzyme (ME), creatine kinase (CK-1&2) and isocitrate dehydrogenase (IDH-3&4)) could be useful in distinguishing S. c. bouvieri, henshawi, utah and pleuriticus from their hybrids with S. gairdneri.

We used three criteria in selecting electrophoretically detectable loci for use in the analysis of putative hybrid populations: 1) the locus must be fixed, or nearly so, for alternate alleles in the different species or subspecies; 2) F_1 hybrid individuals must exhibit a combination of the parental banding patterns and; 3) the protein system and the genetic model must be sufficiently simple so that both parental types and the hybrid can be unambiguously identified.

Of the 6 loci considered, CK-2 was eliminated because of difficulty in identifying heterozygotes reliably and thus they could be mistaken for parental phenotypes. IDH-3&4 were not used because this duplicated locus exhibits so much allelic variability in rainbow brookstocks that we were not able to assign the variation to a specific locus. The remaining three loci, ME, PEP-1, and PEP-3 appeared to meet all the criteria and were established as markers for cutthroat hybridization. Confirmation that hybrids exhibited a combination of parental banding patterns was obtained from examining progeny of a cross between the Heenan Lake stock of S. c. henshawi and the Junction Kamloops stock of S. gairdneri.

Twenty-one populations were sampled for analysis of hybridization (Table 5). They were selected on the basis of having been planted with rainbow trout as indicated by state or federal planting records, or by having been classified as containing trout that phenotypically exhibit the coloration and spotting patterns expected for putative hybrids. Individuals in each population were

TABLE 5. POPULATIONS WITH PUTATIVE HYBRIDS

Population	Subspecies	Planting record for S. gairdneri		Status
		#	Date	
Long Canyon Creek, NV.	henshawi	400,000	1920-1969	pure cutthroat & pure rainbow no hybrids
Conrad Creek, NV.	henshawi	60,000	1895-1955[1]	pure cutthroats
Coyote Creek, NV.	henshawi	20,000	1936-1947	pure cutthroats
N. F. Humboldt River, NV.	henshawi	400,000	1920-1969	pure cutthroats
California Creek, NV.	henshawi	7,000	1920-1953[1]	pure cutthroats
Gance Creek, NV.	henshawi	8,500	1926-1955	pure cutthroats
Tea Creek, NV.	henshawi	13,000	1913-1941	pure cutthroats
Marys River, NV.	henshawi	30,000	1896-1952	pure cutthroats
Heenan Lake Stock, CA.	henshawi	hybridized while in upper Blue Lake, CA.		introgressed cutthroat
Dunderberg Creek, CA.	henshawi			? introgressed[2]
East Fork Desert Creek, CA.	henshawi			? introgressed[2]
Juniper Creek, CA.	henshawi			pure rainbow
Silver King Creek, CA.	seleneris	5,050	1949	(2 populations present) a. introgressed cutthroats b. pure cutthroats[3]
Trout Creek, UT.	utah			introgressed cutthroats
Hendries Creek, NV.	utah			pure cutthroats
Soda Butte Creek, WY.	bouvieri	105,000	1937	introgressed cutthroats
Lower Slough Creek, WY.	bouvieri			hybrid swarm
Upper Slough Creek, WY.	bouvieri			pure cutthroats

Population	Subspecies	Planting record for S. gairdneri #	Date	Status
Buffalo Fork Creek, WY.	bouvieri			hybrid swarm
Rose Creek, WY.	bouvieri	70,600	1938[1]	hybrid swarm
Wolf Lake Outlet, WY.	600,000 1,500,000 (in Gibbon	S. c. bouvieri, S. gairdneri River and Grebe Lake)		pure rainbow
Big Goose Creek, NV.	bouvieri	36,000	1926-1952	pure cutthroats

[1]Includes plants into adjacent tributaries;

[2]Small sample size precludes definitive description - hybrids are present;

[3]Busack and Gall, in prep.;

[4]All cutthroat trout populations listed in section 2 and not discussed in this section are electrophoretically pure native populations.

classified as a cutthroat, a rainbow or a hybrid on the basis of electrophoretic phenotype; the hybrids were identified as either F_1 hybrids or as backcross hybrids, the latter including second, third, and later generation hybrids. An individual exhibiting the homozygous parental phenotype was classified as either a cutthroat or a rainbow. An individual heterozygous for all 3 discriminating loci was classified as an F_1 hybrid. An individual homozygous for either a cutthroat or rainbow allele at one locus, but either homozygous or heterozygous for the alternate allele at another locus was classified as a backcross hybrid.

3. Results

One of the most interesting findings of the study was the extensive variation observed in the extent of cutthroat-rainbow hybridization. Almost every conceivable outcome of the mixing of cutthroat and rainbow trout was observed. In 12 of the 22 populations examined, no evidence of hybridization could be demonstrated: nine of these populations contained cutthroat while two contained rainbow trout. Several of the cutthroat populations which exhibited no hybridization are from tributaries to the Humboldt River, Nevada. One of these populations, the North Fork Humboldt River, was planted with nearly 500,000 rainbow trout from 1900 to 1970. On the other extreme, Wolf Lake (Gibbon River headwaters) in Yellowstone National Park was planted with cutthroat (bouvieri) in about 1910 (Varley, per comm) and records indicate that a self perpetuating population was established. Rainbow trout were then planted in the lake as well as the Gibbon River about 1925. Based on our electrophoretic analysis, the lake presently contains only rainbow trout. In this lake, it appears that rainbow trout have successfully replaced the cutthroat population. In another case, Long Canyon Cr., Nevada, the trout population consisted of cutthroat (henshawi) and rainbow trout in nearly equal

numbers, but no hybrids. The last recorded plant of rainbow trout was in 1947 suggesting that sympatry without hybridization is stable in this population.

The remaining populations exhibited evidence of either rainbow alleles in what otherwise appeared to be cutthroat trout (Introgressed Populations) or a mixture of all parental and hybrid forms (Hybrid Swarms).

A) Introgressed Populations

Introgression is the incorporation of alleles from one species into the gene pool of another through hybridization with subsequent backcrossing. The trout populations classified as introgressed consisted of predominantly cutthroat trout, but also contained a number of individuals with rainbow trout alleles in an otherwise cutthroat genome, presumably as the result of past hybridization. These populations were found in Soda Butte Creek, Yellowstone National Park (bouvieri), Trout Creek, Utah (utah) and Heenan Lake, California (henshawi). No rainbow trout or F_1 hybrids were observed and the proportion of backcross hybrids ranged from about 10% in Trout Creek to 30% in Heenan Lake. The latter fish were usually heterozygous for a single rainbow allele.

B) Hybrid Swarms

Populations consisting of hybrid swarms are characterized by having a distribution of more than one hybrid type with backcross hybrids being most abundant. Hybrid swarms were found between bouvieri and rainbow in lower Slough, Rose, and Buffalo Fork Creeks in Yellowstone National Park, and Busack and Gall (1980) reported the case between S. c. seleniris and rainbow in Silver King Creek, California.

In lower Slough Creek, cutthroat trout followed the backcross hybrids in abundance, although F_1 hybrids and rainbow trout were present in low numbers. A barrier to fish migration (a water fall) exists on Slough Creek and a pure

population of bouvieri inhabits the stream above the barrier. The presence of a substantial number of pure bouvieri below the barrier may indicate the regular recruitment of cutthroat into the hybrid swarm from above the barrier. In Rose and Buffalo Fork Creeks, cutthroat trout were present in the sample in low numbers. Rainbows were present in both populations and F_1 hybrids were found in Buffalo ForK Creek. The large proportion of backcross hybrids in all these populations is compelling evidence that the hybrids are fertile and reproducing.

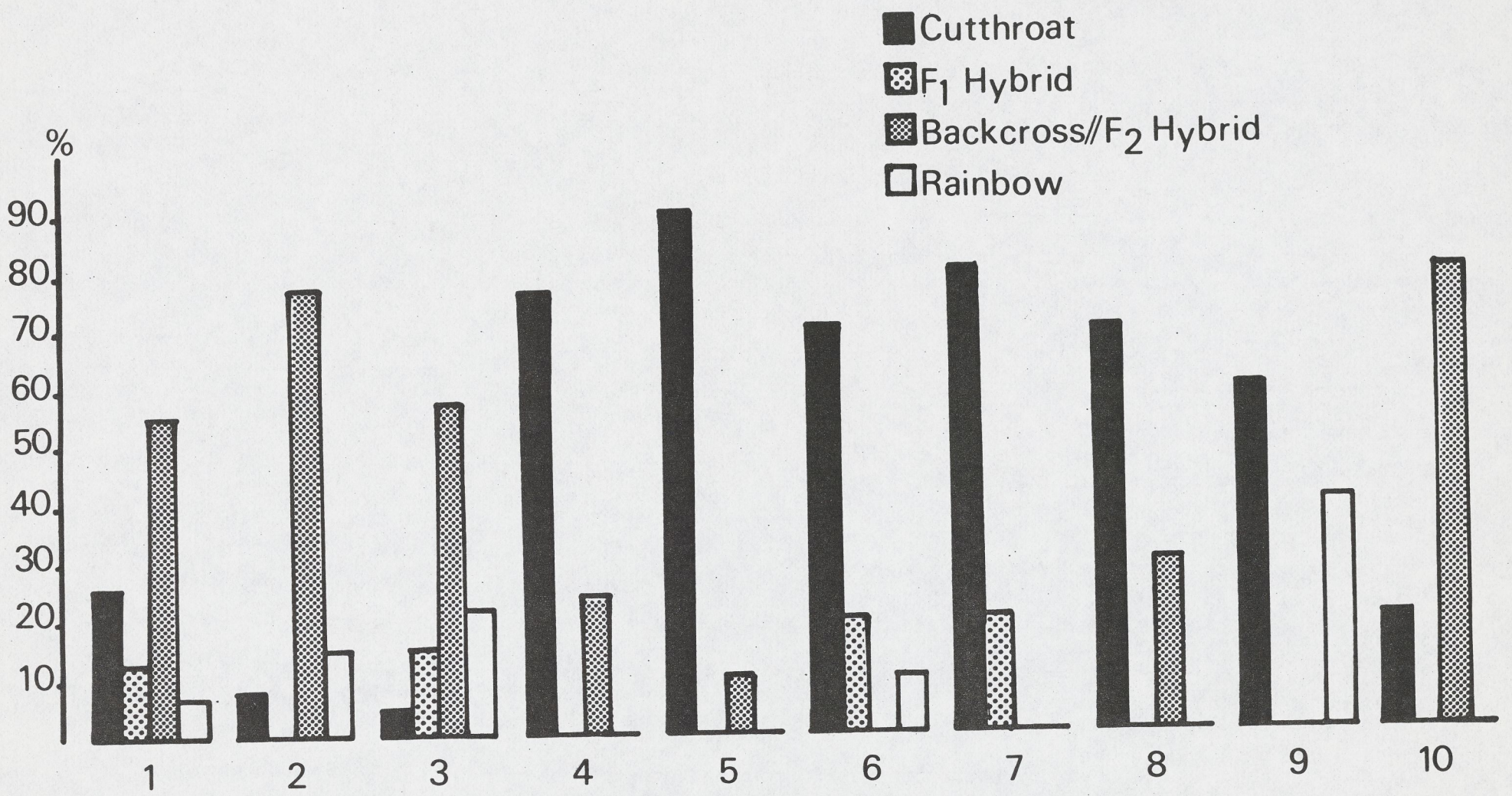
The fish in Silver King Creek appeared to represent two separate populations; one was a population of pure, or nearly pure, seleniris while the other was a hybrid swarm between seleniris and planted rainbow (Busack and Gall, 1981). The hybrid swarm consisted of primarily backcross hybrids with rainbow trout being absent. Busack and Gall (1981) discuss the dynamics and the interaction of these populations.

In Dunderberg and East Fork Desert Creeks, tributaries to the East Walker River, California, the populations consisted of cutthroats (henshawi) and F_1 hybrids but there were no backcross/F_2 hybrids in the sample (one rainbow was found in the E. F. Desert Cr. sample). Because of the small number of fish sampled, we cannot determine whether these popualtions represent examples of recent hybridization or incompletely sampled hybrid swarms.

4. Discussion

The electrophoretic examination of the putative hybrid populations provides insight into the dynamics of hybridization between cutthroat and rainbow trout. Evidence from both the hatchery production of rainbow-cutthroat hybrids and our studies indicate a lack of prezygotic isolating mechanisms. Thus, the variation in the extent of hybridization observed is probably not due to a lack of genetic compatability between these species. Rather, it suggests that the outcome of

Figure 10. Distribution of individuals within ten populations which exhibited rainbow phenotypes. 1. lower Slough Creek; 2. Buffalo Fork Creek; 3. Rose Creek; 4. Soda Butte Creek; 5. Trout Creek; 6. East Fork Desert Creek; 7. Dunderberg Creek; 8. Heenan Lake; 9. Long Canyon Creek; 10. Silver King Creek. Samples 1, 2, 3, and 10 are hybrid swarms; populations 4, 5 and 8 are introgressed; classification of 6 and 7 is unclear because of a low sample size, which sample 9 contains populations of cutthroat and rainbow trout without hybridizations.


Cutthroat
F1 Hybrid
Backcross//F2 Hybrid
Rainbow
%
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10

secondary contact between rainbows and cutthroats is influenced by ecological variables. Behnke (1979) proposed that cutthroat trout populations do not hybridize with rainbows in marginal trout habitats where native populations are adapted to the harsh conditions. The underlying determinant with this model is that the extent of hybridization is dependent on the ability of the introduced hatchery fish to establish a reproducing population. This model may account for the lack of hybridization observed within the Humboldt River drainage, Nevada. However, this hypothesis can not account for the sympatry observed in Long Canyon Creek. Genetic techniques are clearly useful in analysing the structure of hybridized populations. In addition, ecological studies are needed to further understand the outcome of mixing these congeners.

IV. GENETIC VARIATION IN CUTTHROAT TROUT POPULATIONS

1. Background

A fundamental concept in our present understanding of population biology is that heritable variation is a necessary prerequisite if populations are to adapt to changes in the environment, either physical (temperature, salinity) or biological (competition, predation). Prior to the use of electrophoresis, direct measurements of genetic variability within populations were difficult. Estimates of the extent of genetic variability could be made, in some cases, for heritable, continuously distributed characters and for morphological polymorphisms when the Mendelian basis of the polymorphisms could be readily demonstrated. At present, genetic variation within wild populations is best estimated using electrophoretic data, although there is a need to recognize that these are minimum estimates. There are numerous DNA base substitutions which will not result in a change in the amino acid sequence of the protein as well as large classes of amino acid substitutions which do not alter a protein's electrophoretic mobility. Current studies by several research groups (Johnson, 1975, 1976, 1977; Coyne et al., 1978) are examining methods of detecting and studying the inheritance of additional, biochemically detectable, allelic variation.

2. Method of Analysis

The objective of the analysis was to assess the level of genetic differentiation among populations of cutthroat trout using electrophoretic allele frequencies as the basic data set. Since cutthroat populations generally represent geographical isolates of the subspecies of Salmo clarki, genetic differentiation is potentially identifiable at three levels of organization; the local population, the subspecies and the species. However, a

single three-level analysis was not considered since the present study did not include extensive sampling of all five subspecies. Instead, three, two-level analyses were undertaken. Two of these evaluated the genetic differentiation among populations of two subspecies, S. c. henshawi (24 populations) and S. c. bouvieri (18 populations). The third analysis included all populations sampled for the five subspecies and was used to estimate the genetic differentiation among cutthroat populations ignoring the presence of subspecific population organization. The significance of subspecific differentiation was then assessed qualitatively by contrasting the results observed for the two subspecies with those found for all populations in the third analysis.

Each analysis followed the method described by Nei (1973, 1975) and discussed by Hartl (1980) and had the following general characteristics. The method is based on the notion that heterozygosity estimated from observed allele frequencies provides an unbiased measure of genetic variation. Heterozygosity, a measure of genetic variability, is defined as the average proportion of individuals heterozygous at each locus. The heterozygosity expected if all populations are considered to represent a single unit is referred to as "total genetic diversity". The total, in turn, can be partitioned into components representing genetic variation within populations and variation due to differences among populations. Consequently, total genetic diversity, H_T, is equal to the sum of estimated genetic diversity among populations D_{ST} and the heterozygosity observed within populations H_S, that is, $H_T = D_{ST} + H_S$.

In our analyses, total genetic diversity was calculated from the average allele frequencies of all populations included in an analysis. The within population component was estimated as the unweighted average of the heterozygosity observed for each population (Table 1). An estimate of the

enetic diversity among populations was then obtained as the difference (D_{ST}) between the total and the within population values. The magnitude of the genetic differentiation among populations relative to the total was estimated as the ratio D_{ST}/H_T and is referred to as the coefficient of gene differentiation, G_{ST}. The latter coefficient provides an index of heterogeneity in allele frequencies and facilitates comparisons between groups of populations with different levels of total genetic diversity.

The rationale for the analyses was as follows. If the populations used in each analysis represented samples from a single randomly mating population, then the heterozygosity observed within populations should be approximately equal to the total genetic diversity. If, on the other hand, the populations represented non-interbreeding units, then the within population variability would be low relative to the total and the among population component provides a measure of local differentiation. The relative magnitude of the among population diversity, G_{ST}, estimates the extent of reproductive isolation between population. It must be noted that in the analysis of all S. clarki populations sampled, the among population component will contain differences due to subspecies differentiation as well as differentiation among populations within subspecies. However, the analysis of henshawi and bouvieri provide clear estimates of among population differentiation.

3. Population Variability

Estimates of average heterozygosity for individual cutthroat populations (Table 1) were highly variable, ranging from zero in several populations to as high as 0.075 for Pine Creek, one of the utah subpopulations. The genetic diversity observed within populations of each subspecies was also significantly variable. The values of H_S were: henshawi, 0.016; bouvieri, 0.016, utah,

0.063; pleuriticus, 0.000; and lewisi, 0.021. (The reader is reminded that the utah populations include only those from the Snake Valley region; the Bear River populations are treated as bouvieri.) The within population genetic diversity for all 50 populations sampled was 0.019. However, the unweighted average of the subspecies values was 0.023, an estimate which assumes there are equal numbers of populations of each subspecies.

The results of the analyses of the henshawi and bouvieri populations and all cutthroat populations are summarized in Table 6. In all three analyses, the total genetic diversity, H_T, was larger than the within population genetic diversity, H_S, suggesting that the populations studied did not represent samples from a single, large randomly mating population. The total genetic diversity observed for all cutthroat populations was about five times greater than that found in the separate analyses of the two subspecies. However, the within population diversity was similar in all three analyses. Consequently, most of the differentiation observed among cutthroat populations can be attributed to differences among the subspecies rather than to populations within subspecies. Based on the coefficient of genetic differentiation, G_{ST}, for all populations, approximately 80 percent of the allelic heterogeneity found in cutthroat trout can be assigned to differentiation among populations while only about 20 percent represents heterogeneity within populations.

There were, however, clear differences between the two subspecies, henshawi and bouvieri, in the relative level of allelic heterogeneity among populations. The G_{ST} of 0.41 observed for henshawi was nearly twice as large as the estimate of 0.27 for the bouvieri populations. The high GST found for henshawi indicates that the Lahontan Basin populations have undergone more extensive subdivision than the bouvieri populations. This conclusion is further

TABLE 6. PARTITIONING OF TOTAL GENETIC DIVERSITY, H_T, IN POPULATIONS OF *SALMO CLARKI*, INTO WITHIN POPULATION, H_S, AND AMONG POPULATION, D_{ST}, COMPONENTS OF GENETIC DIVERSITY. G_{ST} IS THE RATIO D_{ST}/H_T.

Analysis Taxa	H_T	H_S	D_{ST}	G_{ST}
1. *S. c. henshawi*	.027	.016	.011	.41
2. *S. c. bouvieri*	.022	.016	.006	.27
3. All *S. clarki* populations	.118	.019	.099	.84

substantiated by the observation that significant heterogeneity of allele frequencies existed among the Lahontan Basin populations at all of the four most polymorphic loci: IDH-3, PH1-1, SDH-1 and SDH-2 ($P<0.005$).

4. Discussion

Table 7 summarizes estimates of heterozygosity in four Salmonidae genera. The estimates reported for species of Salmo and Oncohynchus demonstrate the large range in values observed within species as well as the high degree of variability observed among average values for different species. Selander (1976; see also Hartl, 1980) summarized a large number of studies and arrived at an estimate of 0.078 for the average heterozygosity of 14 species of fish.

The mean estimate of heterzygosity of 0.019 observed for the 50 inland populations of cutthroat trout is similar to the mean estimate of 0.018 for sockeye (Onchorhyncus nerka) and 0.015 for coho (O. kisutch) salmon but less than the 0.080 for rainbow trout (S. gairdneri) and the average pf 0.040 for other Onchorhyncus species found by Allendorf and Utter (1978). Merrit et al. (1978) compared the heterozygosity of several species of fish and found that Pacific salmon were significantly less variable than the average of 0.058 observed for all fish species in their analysis. A Kruskal-Wallis test demonstrated that the inland S. clarki populations analyzed in the present study were significantly less heterozygous ($P<0.005$) than the 41 populations of S. gairdneri analyzed by Allendorf and Utter (1978), the only other Salmo species extensively sampled. A similar test of heterozygosity estimates among the cutthroat subspecies showed highly significant differences; utah exhibited the highest estimate (0.063) and pleuriticus exhibited the lowest estimate (0.00).

Efforts to explain differences in heterozygosity have lead to hypotheses which relate genetic variation to biological and ecological characteristics of

TABLE 7. ESTIMATES OF AVERAGE HETEROZYGOSITY $\overline{H}$ AND THE RANGE IN HETEROZYGOSITY REPORTED FOR FOUR SALMONIDAE GENERA

Species	Common Name	$\overline{H}$	Range	Source
Salmo T				
S. clarki	Cutthroat Trout	0.019	0.00 - 0.090	present study
S. gairdneri	Rainbow Trout	0.080	0.020 - 0.103	Allendorf and Utter, 1978 (present study)
S. apache	Arizona Trout	0.0186	0.008 - 0.029	present study
S. aguabonita	Golden Trout	0.049	0.019 - 0.073	Smith (1981)
S. gilae	Gila Trout	0.034	0.026 - 0.049	present study
S. salar	Atlantic Salmon	0.024	0.020 - 0.028	Allendorf and Utter, 1978
Onchorhyncus				
O. gorbusha	Pink Salmon	0.039	0.032 - 0.047	Allendorf and Utter, 1978
O. keto	Chun Salmon	0.045	0.043 - 0.048	Allendorf and Utter, 1978
O. kisutch	Coho Salmon	0.015	0.00 - 0.025	Allendorf and Utter, 1978
O. nerka	Sockeye Salmon	0.018	0.008 - 0.024	Allendorf and Utter, 1978
O. tsawytscha	Chinook Salmon	0.035	0.024 - 0.052	Allendorf and Utter, 1978
Coregonus				
C. clupeaformis	Lake Whitefish			
	normal	.077	0.071 - 0.084	Kirkpatrick and Selander, 1979
	dwarf	.064	0.063 - 0.065	Kirkpatrick and Selander, 1979
Thymallus				
T. arcticus	Artic Grayling	.034		Lynch & Vise, 1979

pecies. Selander and Kaufman (1973) proposed that heterozygosity is an adaptive feature and have explained variation in heterozygosity in terms of Levins (1968) model of evolution in changing environments. Another explanation is that heterozygosity is related to population size and gene flow between adjacent populations (Soule', 1976; Patton and Yang, 1977). According to this model heterozygosity is reduced in small isolated populations.

There is no simple explanation for the low but variable heterozygosity observed for inland cutthroat trout. The difference between rainbow and cutthroat trout can be explained using a model based on effective population size and structure. Many of the rainbow trout populations studied by Allendorf and Utter (1978) are anadromous populations consisting of relatively large numbers of fish with the potential for gene exchange among conspecific populations. In contrast, cutthroat populations, although variable in size, are frequently isolated above falls or within desicating basins which reduces the opportunity for gene exchange. The rationale, however, fails to explain the differences among the S. clarki subspecies. For example, the utah populations we sampled inhabit desert streams which are subject to periodic drought and flash-flooding resulting in restricted habitable area and population sizes. Yet, these were the populations which yielded the highest heterozygosity estimates. In contrast, populations of S. c. bouvieri in Yellowstone Lake exhibit less variability even though spawning runs in the tributary streams consist of several thousand individuals and there is extensive opportunity for gene flow among populations.

The analyses of henshawi and bouvieri populations demonstrated greater differentiation among populations of henshawi. The differences in allelic heterogeneity observed for these two subspecies are not surprising in view of

their different evolutionary histories. Salmo c. henshawi was derived from an ancestral interior cutthroat trout that gained access to the Lahontan Basin prior to its isolation from contiguous drainages. During pluvial periods this basin was occupied by a large, contiguous body of water the size of present day Lake Erie (La Rivers, 1962). Water levels in the ancient lake fluctuated according to available precipitation, isolating portions of the basin several times in the last 50,000 to 100,000 years. Since the final desiccation of pluvial Lake Lahontan (8,000 B.P.), the cutthroat trout populations have inhabited large lakes and headwater tributaries of the Humboldt, Truckee, Carson, and Walker Rivers which are presently isolated from one another. In contrast, the bouvieri populations we examined all inhabit previously glaciated regions and are thus of relatively recent origin. Even though the bouvieri populations are distributed in presently isolated drainages recent connections are known.

V. SYSTEMATICS OF WESTERN NORTH AMERICAN *SALMO*

1. Background

A major objective of systematics is to provide a classification for organisms within an inclusive set. The theoretical problem is establishing a classification in which groups of similar taxa (genera, species, subspecies, etc.) can be arranged by their similarities into heirarchies. These similarities are established using morphological, chromosomal, biochemical-genetic, zoogeographic, and breeding data. In practice, the problem involves analyses of extant populations and inferring whether they represent full species, complexes of sibling species, or subspecies of polytypic species.

The classification system should provide information both on which taxa resemble each other and on which taxa evolved from the most recent common ancesters to be of any utility to nonsystematists. Unfortunately, taxanomic methods which provide classifications, based on the resemblance of extant taxa, called phenetic classifcations, and classifications using methods which provide branching sequences to indicate ancestral origin, called cladistic classifications, (Hull, 1970) are frequently incompatable. Moreover, classifications based on different sets of characters (biochemical genetics, chromosomes, meristics, osteology) are frequently different for a given set of taxa.

There are no established criteria on which to judge one classification as more appropriate than any other. In addition, there is no accepted *a priori* level of differentiation which can be used to determine whether populations represent subspecies, sibling species, or full species; thus, there must be many "best effort" classifications. Any proposed classification of *Salmo* should incorporate all available information on life history, ecology, genetics, and

morphology and even though the result may not solve all the controversies concerning western Salmo, the classification should be based on principals of systematic zoology, not on management needs for individual populations. Legislatures and agencies responsible for enacting and implementing the rare and endangered species act must learn to work within the best available taxonomic guidelines to manage all rare resources.

A major difficulty in Salmo systematics is that many populations from different drainages are morphologically or genetically different from each other. The problem is to delineate the number of evolutionary lineages of extant western Salmo remaining, the relationships among the lineages, and the level of taxonomic recognition to be provided for the different forms within the lineages. Because all available evidence suggests that various taxa of Salmo evolved as allopatric groups of populations that can and will hybridize, information on reproductive isolation is of little utility in establishing relationships. Under these circumstances, only estimates of similarity (or dissimilarity) using karyotypic, morphological, and electrophoretic data can be used to establish relationships.

2. Current Status

As many as 33 nominal species were recognized by Jordan, Everman, and Snyder (reviewed by Miller, 1950); Miller (1950) was the first to attempt a systematic summary of the species of western Salmo. Miller recognized two major evolutionary lineages which he referred to as the cutthroat series and the rainbow series. The rainbow series contained seven species: S. gairdneri (7 subspecies); S. smaragdus Snyder (emerald trout); S. regalis Snyder (royal silver trout); S. aguabonita Jordan (South Fork golden trout); S. whitei Everman (Soda Creek golden trout); S. roosevelti Everman (Volcano Creek golden trout);

and S. gilae Miller (gila trout). The cutthroat series contained two species S. clarki (11 subspecies) and S. evermanni Jordan and Grinnell (San Gorgonia trout). Needham and Gard (1959) followed Miller in recognizing the cutthroat and rainbow series. They however, felt that attempting subspecies designations for S. gairdneri was inappropriate because of environmental influence on phenotypes. Further, they reduced S. gilae, S. smaragdus, and S. regalis to synonomy with S. gairdneri. They put off any judgement regarding the three golden trout species until further studies were completed.

Schreck and Behnke (1971) and Legandre et al. (1972) made a radical departure from these earlier studies. They suggested that there were three major evolutionary lineages of western Salmo consisting of the cutthroat and rainbow series plus a "golden trout complex" consisting of the California golden trout (S. whitei, S. aguabonita, and S. roosevelti reduced to S. aguabonita aguabonita and S. aguabonita whitei), gila trout (S. gilae), perhaps the Mexican golden trout (S. chrysogaster), and a newly recognized form, the red-banded trout (as yet unnamed). They based their classification on a novel proposal that the "golden trout complex" was a monophyletic assemblage of recent species having it's origin in the lower Colorado River system and that this complex diverged from the cutthroat series. Previous analyses (Miller, 1950; Needham and Gard, 1959) suggested that these forms were closely related to rainbow trout.

Miller (1972) added yet another species, S. apache (Arizona trout) to the golden trout complex and his analysis provided an alternative hypothesis to that of Schreck and Behnke (1971). He argued that the traits common to all species in the golden trout complex were retained, primative characters and thus, not valid indicators for inferring phylogenetic relationships. He followed by

suggesting a polyphyletic origin for what was recognized as the golden trout complex. Suggesting the most parsimonius solution, he hypothesized that S. aguabonita and red-band trout were closely related and derived from an ancestral form from the north (Pacific northwest); he recognized their similarities to both the cutthroat and rainbow series, but did not hypothesize evolutionary affinities. The Arizona trout (S. apache) was thought to have been derived from the cutthroat trout in the lower Colorado River system and recently transferred to the Gila River drainage whereas the Gila trout, (S. gilae) was considered to have been derived from the Mexican golden trout. Thus, his phylogeny involved four to five evolutionary lineages.

Chromosome number and morphology had been incorporated into the studies of Schreck and Behnke (1971) and Miller (1972) based partially on data from Simon (1964) and Simon and Dollar (1963). Gold (1977) using karyotypic information from both his work (Gold and Gall, 1975; Gold et al., 1977) and other workers (Miller, 1972; Wilmot, 1974) suggested a phylogeny based on chromosome number (2N) and chromosome arm number (NF). California golden trout (2N=58; NF=104) and red-band trout (2N=58; NF=104) were considered closely related, and more distantly related to rainbow trout (2N=60; FN=104). He hypothesized that cutthroat trout (2N=64-70; NF=104) and rainbows were both derived from an ancester with 104 chromosome arms, while Arizona trout with an arm number of 106 represented a second major evolutionary lineage. More recent studies (Thorgaard, 1976; Thorgaard, 1979; and per comm) demonstrated that rainbows actually exhibit a range of chromosome numbers from 58 to 64 and thus, totally intergrade from the golden/red-band chromosome number to the inland cutthroat chromosome number. However, the 64/104 rainbow karotype and the 64/104 cutthroat karyotype are not identical in chromosome morphology.

Diagramatic representations of the phylogenies of Schreck and Behnke (1971), Miller (1972), and Gold (1977) are presented in Figure 11.

3. Biochemical-Genetic Analysis

Our analysis of western Salmo included 78 populations representing five subspecies of cutthroat trout, California golden trout, Gila trout, Arizona trout, rainbow trout, and trout from the Rio Mayo, Mexico which are presently unclassified. The populations studied in addition to cutthroat trout are listed in Table 8. Nineteen enzymes encoded by 36 genetic loci were used to examine both phenetic and cladistic relationships. Phenetic relationships involved clustering individual populations into a UPGMA dendogram using Nei's genetic identity as the similarity index. Cladistic relationships were determined from a Wagner network. For the network, individual populations were grouped into 11 taxa: five cutthroat, S. clarki, subspecies; S. gilae; S. apache; S. aguabonita; Rio Mayo trout; Goose Lake Basin trout (Lassen, Davis, Thomas, Buck, and Cranele Creeks); and S. gairdneri (steelhead, resident rainbows and various hatchery populations). In the latter analysis, alleles were coded as being present (1) in a taxa if present in a frequency of greater than 0.05 when averaged across all populations, otherwise as absent (0).

The dendrogram formed by UPGMA is depicted in Figure 13. The points at which populations, or groups of populations, join together to form a cluster do not imply evolutionary branch points but rather, levels of overall similarity. There are several important features to this dendogram. Within the currently recognized taxa of Salmo, genetic identity is uniformly high ($I>0.95$) among populations within the taxa. With the exclusion of S. c. lewisi, cutthroat subspecies exhibit greater genetic identity to each other than they do to other taxa. Salmo c. lewisi is approximately as divergent from other cutthroat

Figure 11. Classification of western Salmo: A. Schreck and Behnke (1971) based on morphology; B. Gold (1977) based on morphology; C. Gold (1977) based on chromosomes; D. Summarized from Miller (1972) based on morphology, zoogeography, and chromosomes. Dashed lines in D indicate areas where Miller (1972) only tenatively suggested relationships.

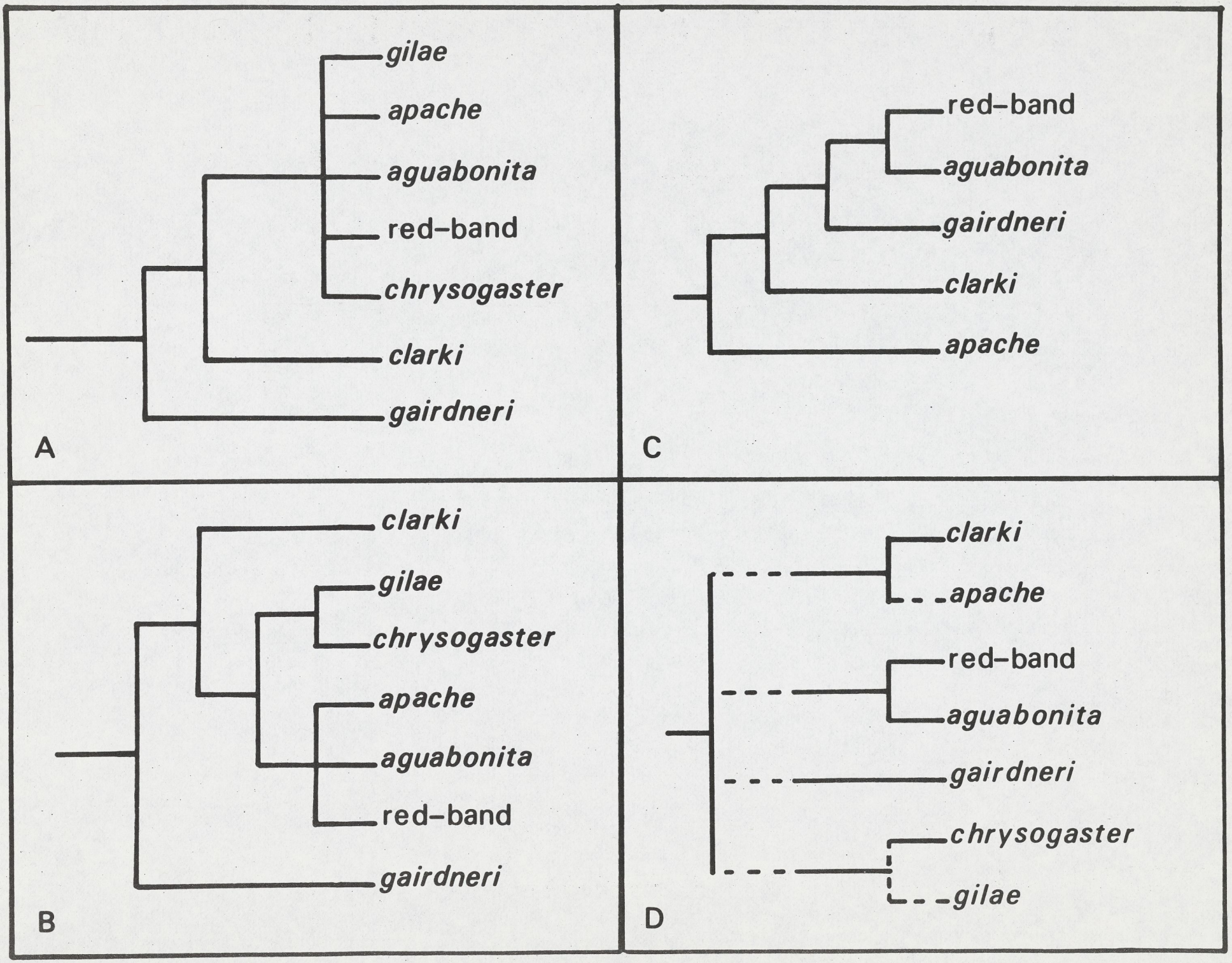

gilae
apache
aguabonita
red–band
chrysogaster
clarki
gairdneri
A
red–band
aguabonita
gairdneri
clarki
apache
C
clarki
gilae
chrysogaster
apache
aguabonita
red–band
gairdneri
B
clarki
apache
red–band
aguabonita
gairdneri
chrysogaster
gilae
D

Figure 12. Sample locations and approximate distribution of species of western Salmo. Numbers refer to locations listed in tables 1 and 8.

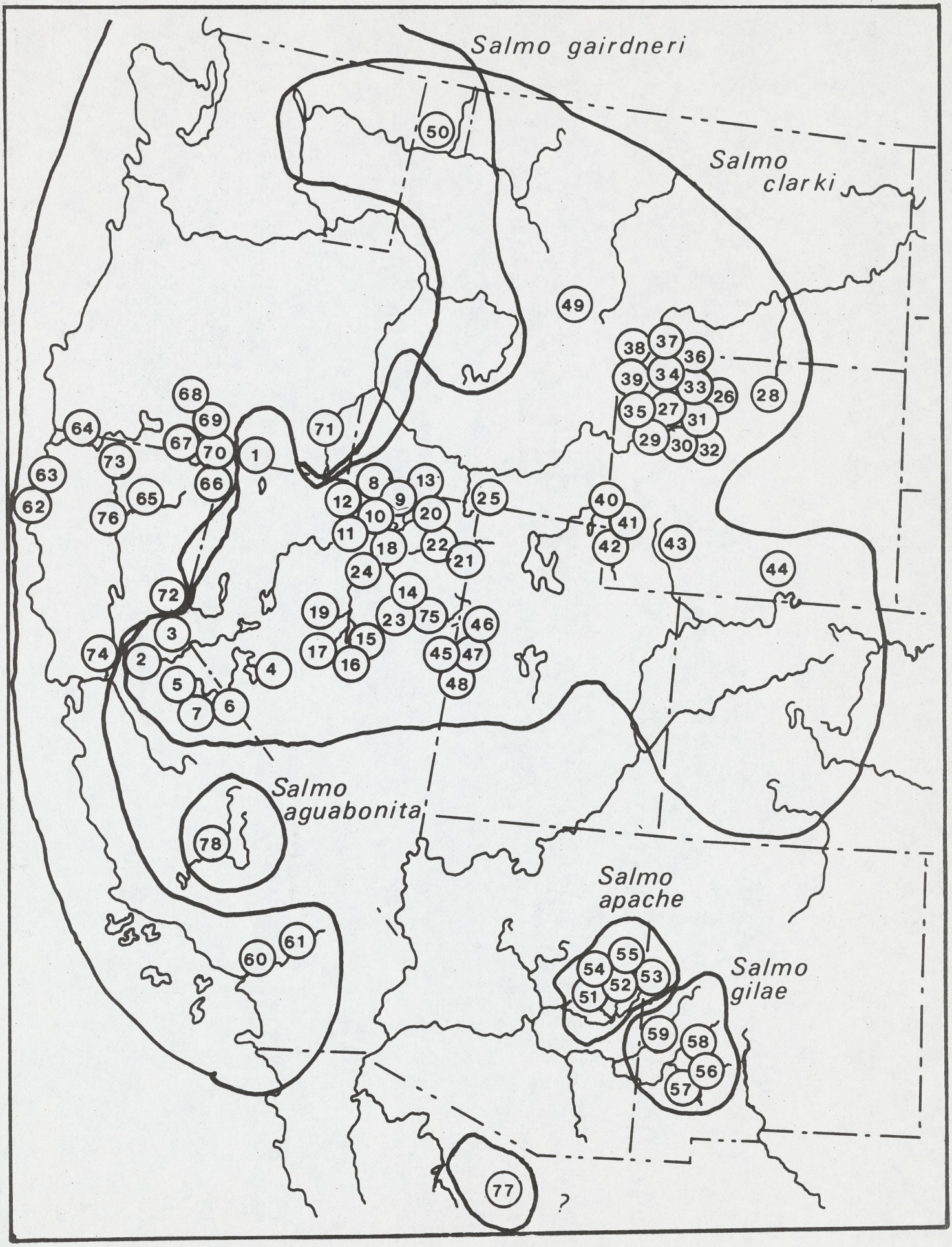

Salmo gairdneri
Salmo clarki
Salmo aguabonita
Salmo apache
Salmo gilae
?

TABLE 8. SUPPLEMENTAL POPULATIONS OF *SALMO* SAMPLED FOR BIOCHEMICAL-GENETIC ANALYSIS

Population #	Taxa	Location	# of Individuals
51	*S. apache*	Squaw CreeK, AZ.	16
52	*S. apache*	Crooked CreeK, AZ.	15
53	*S. apache*	Flash CreeK, AZ.	19
54	*S. apache*	Christmas Tree Lake, AZ.	28
55	*S. apache*	East FK White River, AZ.	18
56	*S. gilae*	Diamond CreeK, NM.	16
57	*S. gilae*	South Diamond CreeK, NM.	15
58	*S. gilae*	Iron CreeK, NM.	15
59	*S. gilae*	Spruce CreeK, NM.	14
60	*S. gairdneri*	Pauma CreeK, CA.[1]	17
61	*S. gairdneri*	San Louis Rey River, CA.[1]	23
62	*S. gairdneri*	Gaulala River, CA.[2]	57
63	*S. gairdneri*	Eel River, CA.[2]	47
64	*S. gairdneri*	Butte CreeK, CA.[3]	31
65	*S. gairdneri*	McGill CreeK, CA.[3]	35
66	*S. gairdneri*	Lassen Creek, CA.[3,5]	62
67	*S. gairdneri*	Davis Creek, CA.[3,5]	22
68	*S. gairdneri*	Thomas Creek, CA.[3,5]	19
69	*S. gairdneri*	Buck Creek, CA.[3,5]	22
70	*S. gairdneri*	Crane Creek, CA.[3,5]	20
71	*S. gairdneri*	Chino CreeK, NV.[3]	40
72	*S. gairdneri*	Juniper CreeK, CA.[4]	32

Population #	Taxa	Location	# of Individuals
73	S. gairdneri	Shasta Hatchery Stock, CA.[4]	26
74	S. gairdneri	Davis Hatchery Stock, CA.[4]	12
75	S. gairdneri	Long Canyon CreeK, NV.[4]	18
76	S. gairdneri	North FK Little Squaw CK, CA.[1]	37
77	S. sp	Rio Mayo, Mexico	14
78	S. aguabonita	lower Wet Meadows CreeK, CA	21

[1]Resident coastal rainbow trout.

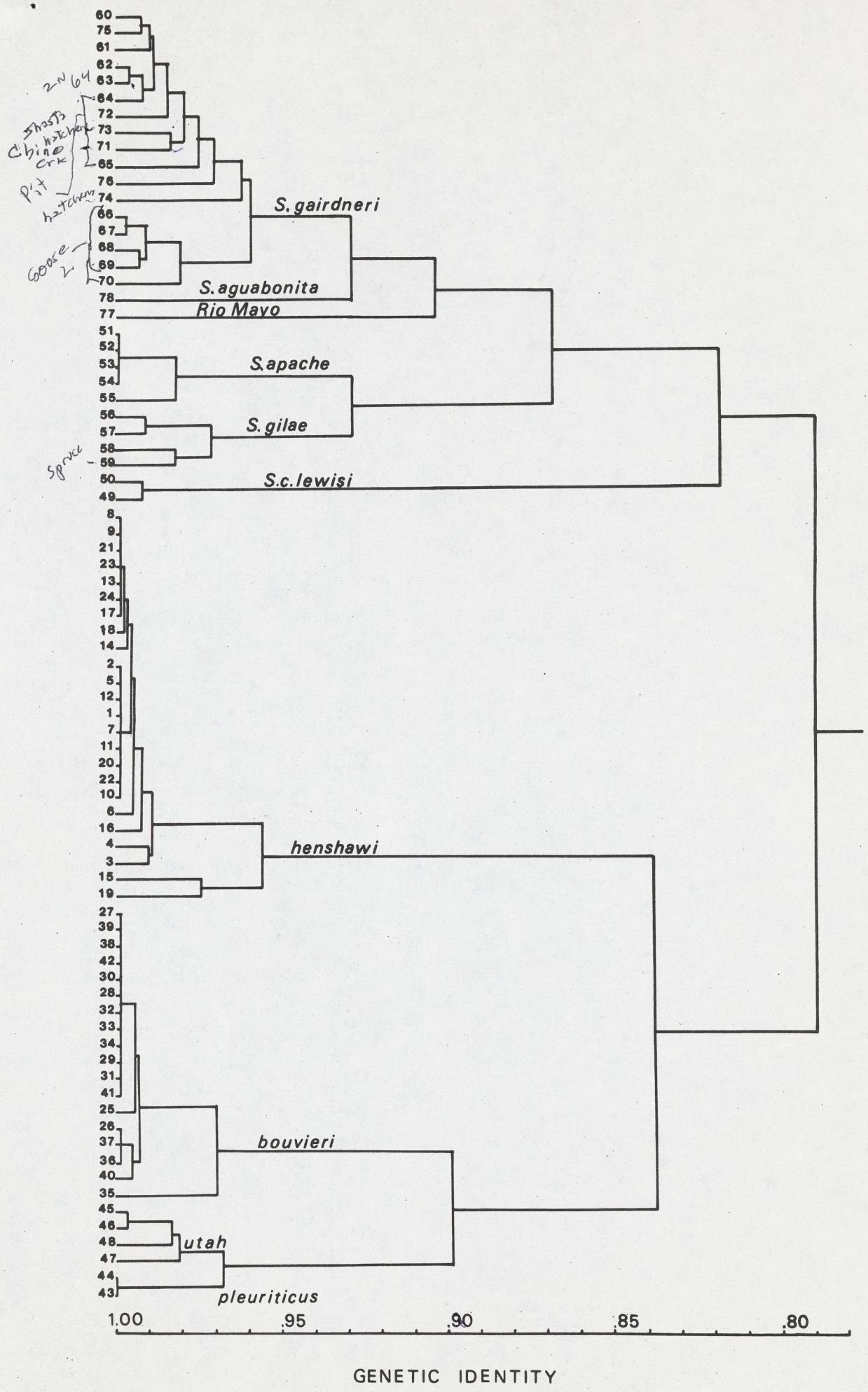
[2]Steelhead rainbow trout.

[3]Isolated interior rainbow trout (red banded trout, Behnke).

[4]Hatchery rainbow trout.

[5]Goose Lake Basin trout.

Figure 13. UPGMA dendrogram of 78 populations of western Salmo representing nine recognized taxa.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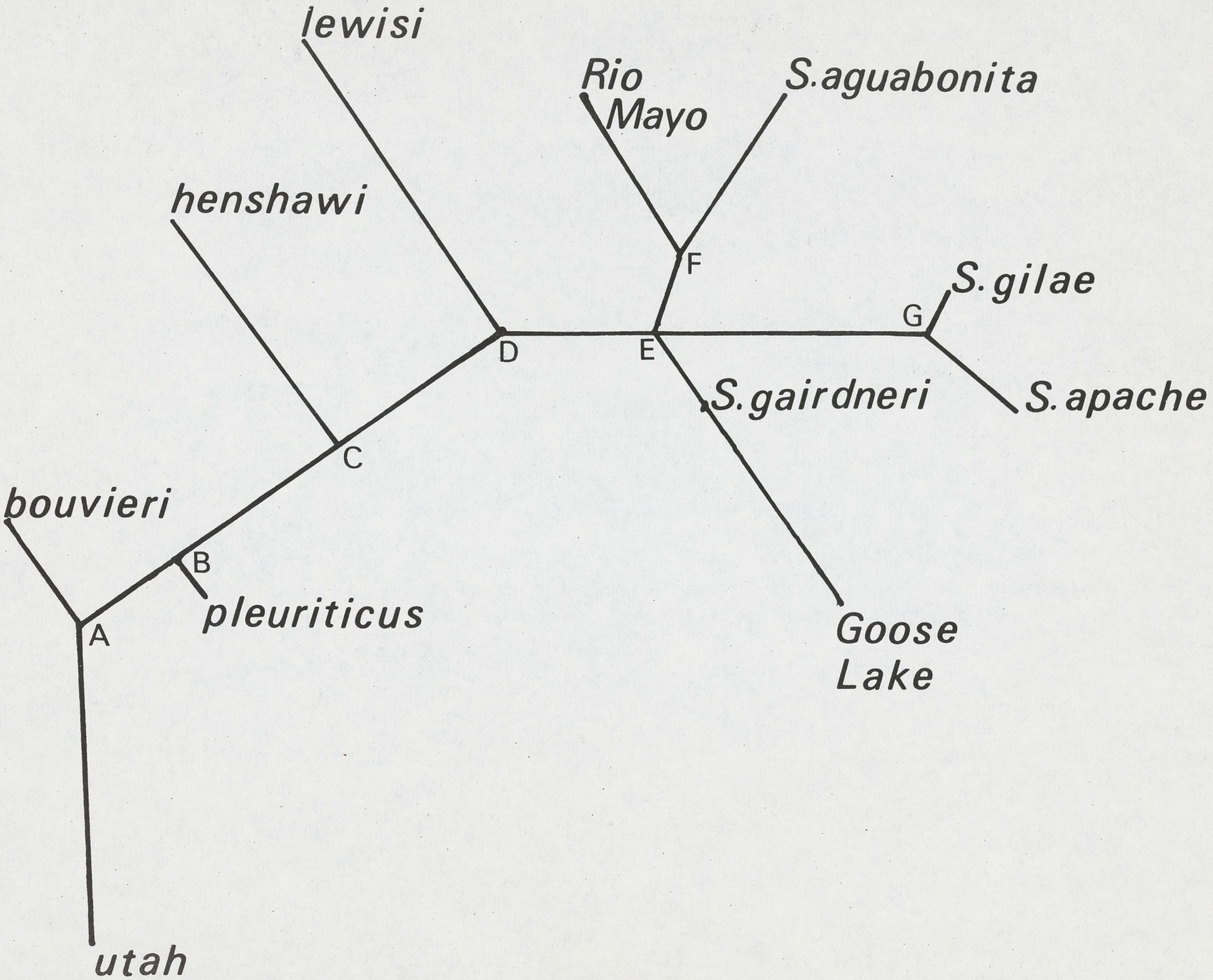
48
47
44
43
S. gairdneri
S. aguabonita
Rio Mayo
S. apache
S. gilae
S.c. lewisi
henshawi
bouvieri
utah
pleuriticus
1.00
.95
.90
.85
.80
GENETIC IDENTITY

Figure 14. Wagner network of western Salmo. S. gairdneri includes steelhead. hatchery rainbows. Putative red-band, and coastal rainbow trout. Goose Lake, represented by samples 66-69, is a divergent group of rainbow trout presently without taxanomic recognition.


lewisi
Rio
Mayo
S.aguabonita
henshawi
F
S.gilae
G
D
E
S.gairdneri
S.apache
C
bouvieri
B
pleuriticus
A
Goose
Lake
utah

subspecies as it is from all other trout. Gila trout and Arizona trout are genetically more similar to each other than to any other taxa. This is in agreement with their zoogeographic proximity to each other, but differs from Miller's (1972) interpretation. Salmo apache and S. gilae, along with S. aguabonita and the Rio Mayo trout, appear to be more similar to S. gairdneri than to any of the cutthroat subspecies. Several populations included in the S. gairdneri group (Butte Creek, Chino Creek, McGill Creek) have been designated by Behnke (1979) as red-band trout. These populations exhibit an extremely high similarity to hatchery, steelhead, and recognized inland populations of rainbow trout. Finally, the currently recognized full species S. aguabonita, S. gairdneri, S. gilae, and S. apache, as well as the Rio Mayo trout exhibit genetic identities among each other which are very similar to the genetic identities observed among the cutthroat subspecies S. c. bouvieri, S. c. utah, S. c. henshawi, S. c. pleuriticus, and S. c. lewisi.

In interpreting the Wagner network (Figure 14), it must be remembered that direction of branching is only implied because there is no way to determine whether allelic states are primative or derived characters. The network is also a parsimonius tree, i.e., it gives minimum possible distance between endpoints of the branches. The distance between two points indicates the minimum number of mutations that differ between taxa at the 36 loci examined. The end points are termed operational taxonomic units (OTU's) and are extant species or populations. The internodes are termed hypothetical taxonomic units (HTU's) and represent presumed ancesters that gave rise to the extant taxa.

The Wagner network (Figure 14) is similar to the UPGMA demondrogram. The analysis indicates that all cutthroat subspecies have shared at least one common ancester (HTU-D) since the cutthroat and rainbow series diverged. The extant

species S. aguabonita, S. gilae, S. apache, S. gairdneri, the Goose Lake Basin population, and the Rio Mayo trout all share a common ancester (HTU-E). As with the dendogram, there is no greater separation among these full species than there is among cutthroat subspecies.

The phenetic and cladistic analyses of the electrophoretic data provide some important insights into the evolutionary relationships among the extant Salmo taxa. Moreover, these relationships are generally concordant with relationships hypothesized from other evidence. However, they do not inherently contain a classification and must be interpreted in light of all information on zoogeography, genetics, ecology and morphology. The available data indicates that there are two superspecies groups of western Salmo: a cutthroat trout and a rainbow trout. Each group contains semispecies which have not received consistent classification. What are currently referred to a cutthroat trout subspecies exhibit as much genetic differentiation as full species in the rainbow trout group. These conclusions are based not only on the overall estimates of genetic similarity, but upon the sharp zones of demarcation among the cutthroat trout subspecies; natural zones of intergradation among the subspecies are unknown. Further, we observed that populations in independent drainages with documented connections during the post-Pleistocene do not exhibit substantial genetic divergence. On the other hand, populations in drainages and basins that have been isolated since the middle or late Pleistocene exhibit extreme genetic subdivision. This provides evidence that the electrophoretically delineated subspecies groups of cutthroat trout are possibly of greater antiguity than some of the rainbow series species.

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p. 15 = p. fig 7-8 Wagner dendrogram depicting
evol. relationships - assume
amino acid substitutions uniform!

recognize selenisis - even though electrophoretically indistinguishable from lewisi
Cactually closer to Carson drainage henshawi - [illegible] - Truckee -
- consistent than orth- / elect- [illegible] selenisis Carson :: Carson .

- p. 38 - Snake R. - 2 morphological variants large-spotted.
fine-spotted morphs - time of divergence estimated
.996 - 20,000 yrs. -- because of high genetic identity &
morphological intergradation observed within Snake R.
(Loudenslager, unpublished) -- part of bouvieri not a new subsp.
GI scores see Table 4 p. 40

Snake Valley, .969 to pleuriticus

Bear R. .996 to bouvieri - The two groups are
clearly more similar to these two subsp. than to each other
p. 43 -- (Not in coloration, scale count, spotting)
- why not make pleuriticus syn. = utah - fig. 9 clearly shows this

-

p. 45 = * type locality -- Utah L. --- trib to Grt S. L.
Provo, Weber, Ogden, drainages
- utah = S. Valley - Sevier ... [illegible]

* - Bear R. =. subgroup of bouvieri
- simply, G.I. scores should not be sole criteria for
subsp. recognition - -- Kornfield - S. alpinus 2 S. a. o. more distinct than [illegible]
Considerable diff
rakers 7-8 gill
2-3 vert.

98 - strongly urge all 3 methods used
to id hybrids - Bos.

54 - Paiute - 2 pop.

— humboldtensis — 21 vs 24 - spots

20-30 for sam.

but trenchant evol. dim.

- mgt. implications ... - nothing in your paper

67 - classif. system

- phenetic & cladistic

- based on principles syst. zool. not mgt. need of individual sep. -

68 - not all Salmo. = subgenus Parasalmo

71 clearly state - dendogram - does not imply evolutionary branch pts - but rather levels of overall similarity.

15 – Pleistocene glacial (most recent)

15 – 36 loci

28 – 4yrs between subsp. – .969 utah – pleuriticus

.737 lewisi – utah

(X) x .852 for all subsp.

p.? – dendogram gent. ident. is a phenogram (Sokal –) not cladogram or phylo – yet make phyl. groups Tx. – fine spotted S.R.

–

p.35 – stomias – virginalis . could potentially form part of bouvieri – utah – pleuriticus group – not potential – either do or don't but no logical way except via Colo. R. – (pleuriticus) – bright color group.

p 36 henshawi – distinctive at 6 gene loci – .

p.38 – S.R. fine-spotted morph. – morph = polymorphism ?

– 24 loci. fine-spotted large-spotted – no loci distinguish. no evidence of divergence

– estimated time divergence – .995 = 20,000 yrs

? but Bear Crk – Turbid L. – .968 = 120,000 (only 5000)

Carson Valley Big Smoky – Shoshone – H.M. – Marys River Crk – Eshanten – .953 = 200,000 yrs..?

– x but ... – ... area – 2 distinct phenotypes

if make dif. .953 subsp w/ henshawi

+ intergradation fine spotted (unpublished) – why not publish here –

should be considered morphol. variable S.c. bouvieri

(X) Pine – Goshute 20 yrs – .997 = 10,000 yrs ?

... – .90 – higher surprise

thus ...

– two – Snake Valley + pleuriticus – only on few loci

Bear R + bouvieri – pl

p. 43 ~ clearly more similar to each other – implies phylogeny –

– problems – of taxonomy w/ polyphyletic subsp. – .

p.45 S.c. utah . type loc. Utah L. – extinct – thus no way determining if pop. of type loc. = pop. of Bear R. utah or Snake Valley utah. – can't be either it was pop. of Utah L.

– p.47 – only circumstantial and anecdotal evidence for rainbow x cutt hybrid. – 40-50 reports of tax. character (Glacier Park w/ Allendorf) – no parental species present only hybrids – how circumstantial –

p.48 – Paiute hybrids – why all work needed to prove?

rainbows. strain spots upper ... scales ... – so on – no more pure Paiutes –

fish. mgt. really doesn't have to prove!

6 loci controlling expression of 4 enzymes

PEP 1&2 , ME , CK 1&2 , IDH 3&4

for cutt x rainbow hybrids.

- Allendorf Glacier Peak - slight hybrid influence can't detect.

~~- can't detect Heenan L. rainbow influence~~

p.50 Long Canyon #23 Humboldt Crk. NV - 1920-1969 - 400,000 RB stocked

now pure cutt, pure RB ! -

- would like to see tax. characters - spotting

p.53 Trout Crk. Utah - 10% of fish had rainbow type gene at a 1 of 3 enzymes. 30% Heenan L.

p.82 - no way to determine if alleles prim. or derived -

- Oncorhynchus. - - - - - shared prim. - - -

* need data on chrysogaster

- not adequately handle diff morphol - regulation vs. - metabolic structural genes.

Dr. Eric Loudenslager — make 3 copies
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Dear Eric:

Thanks for a copy of the trout manuscript to review. Of course, I have some comments for you.

My major point is that you are making phylogenetic interpretations and taxonomic decisions based on phenetic similarities or phenograms. You clearly recognize this point on page 71 when you wrote that the dendogram does not imply evolutionary branching points. This problem is particularly acute when dealing with a group such as the subgenus Parasalmo where it now seems very evident that the regulatory genomes (and karyotypes) governing morphology, ecology and behavoir have been ~~speciating~~ differentiating at a much more rapid rate than the structural genome governing the relatively few genes that you are able to sample with your technique. Also, Jukes, in an issue of Science, ~~pointed~~ demonstrated the relatively enormous amount of change that can take place ~~in~~ in codons that can not be detected by electrophoresis (silent substitutions). I would also suggest reading the review of the Willi Hennig Society meeting in the most recent issue of Systematic Zoology (p. 76-81) concerning criticisms of the logical basis for using Nei's genetic identity or distance scores and making "evolutionary clock" interpretations. You should be fully aware of the logical flaws involved in the data on which you are making phylogenetic interpretations.

You should also be fully aware that ~~as~~ classification and nomenclature of salmonid fishes is much more

avidly used in nonsystematic literature than in the systematic literature. Thus, an element of practicality should be considered before proposing any changes. For example, I recently received specimens of Salmo mykiss from Kamchatka. They are identical to coastal rainbow trout. If you had the opportunity to study them electrophoretically, I'm sure you would reach the same conclusion. Phylogenetically, they are of recent separation and definitely should be classified as the same species. However, because of the great confusion that would result, I would not propose to make S. gairdneri a synonym of S. mykiss as I would if I were dealing with a group of gobies from the Indian Ocean of interest mainly to ichthyologists.

I would ~~also~~ not separate stomias from pleuriticus nor utah from bouvieri if these names were not already established with these names forming the basis for state and federal programs. Thus, I am opposed to your the transfer of the Bear River cutthroat trout from S. c. utah to S. c. bouvieri. ~~The~~

Two weeks ago a hearing and field trip was held in Cokeville, Wyoming, concerning the listing of S. c. utah as a "threatened species." Even if not listed, the awareness and concern generated by the proposed listing will be ~~beneficial~~ a positive factor to protect and improve the habitat and the prospects for survival of this rare trout. Several agencies and groups are opposed to the listing (the Bear River drainage - courses the Overthrust Belt). If those opposed

to the listing had access to your ms, they would make much over your conclusions that the Bear River cutthroat is not S. c. utah after all.

I would point out a recent article in the Canadian Journal of Fisheries and Aquatic Sciences by Dr. Kornfield and others on an extensive electrophoretic study of eastern North American Salvelinus alpinus. They found greater electrophoretic dissimilarity between two local Maine populations of blueback charr (S. alpinus oquassa) than between the New England charr in general and an Arctic Ocean population from the Northwest Territories. My studies on these same groups of charr show the Northwest Territories charr have 6 to 8 more gillrakers and 2 to 4 more vertebrae. Clearly, the electrophoretic evidence is not in accord with evolutionary reality. ~~These authors also concluded that~~

The ~~complete~~ evidence regarding S. c. utah is far from complete. You found the Bear River samples to be more similar (in electropherograms) to bouvieri samples than to the samples originating in the Snake Valley drainage of the Bonneville basin, and that the Snake Valley samples are more similar to pleuriticus (+o be logically consistant, you should then propose to classify Snake Valley cutthroat as S. c. pleuriticus). You do ~~not, however,~~ mentioned that Hickman and I have pointed out that the Snake Valley cutthroat is a differentiated form of Bonneville cutthroat and that we recognize three differentiated groups of Bonneville cutthroat -- Snake Valley, Bear River, and Bonneville basin proper. The type locality of

the name utah is Utah Lake (Bonneville basin proper). You have no samples from this group of trout. You make a statement on p. 45: "Thus, there is no way of determining if the type locality was a population of Bear River utah or whether it was a Snake Valley utah. This makes it nearly impossible to determine which type of Bonneville basin cutthroat actually represent S. c. utah."

~~It~~ is no more "impossible" to arrive at a characterization of utah than any other taxa you discuss in the ms. ~~Not~~ You ~~have~~ do not have samples from the type localities of a single taxa, yet you didn't find it "impossible" to electrophoretically characterize these taxa. All that needs to be done is to get samples from extant populations of S. c. utah from the Bonneville basin proper. Until this is done, you are wrong to arbitrarily assign the name utah to Snake Valley cutthroat trout when revising the classification. Morphologically, S. c. utah specimens of the type locality are more similar to Bear River cutthroat than they are to Snake Valley cutthroat. ~~Only~~ Until a few thousand years ago when the Great Salt Lake became a barrier due to salinity, "Utah Lake cutthroat" and Bear River cutthroat could freely mix. This was not true of Snake Valley that is almost litterally a "bottleneck" situation. Thus, if you want to revise subspecies on your data, it is more likely that the Snake Valley trout would be named a new subspecies as Miller and I suggested many years ago - but you should propose no changes until you have data on ~~cutt~~ cutthroat native to

V

the Bonneville basin proper.

Fisheries biologists in general are naive in their understanding matters of taxonomy and electrophoresis, particularly when the data are presented as a computer output of complex mathematical formulation. Many will accept your phenograms as the ultimate and final word on western trout taxonomy and may find themselves in embarassing positions in regards to native trout restoration programs. To my mind, it is counterproductive to propose classifying the Bear River cutthroat trout as S. c. bouvieri at this time, and it is scientifically incorrect to do so without data on the cutthroat native to the Bonneville basin proper.

You consider the fine-spotted Snake River cutthroat as a "morph" of S. c. bouvieri, which and This has erroneous implications. This completely ignores the fact that the fine-spotted and large-spotted populations coexist in the Snake River drainage without massive hybridization. There is evolutionary and ecological unity to the two groups -- They are not simple "morphs" of a polymorphic population comparable to color "morphs" of garter snakes and tree frogs. You also give a mathematical formula to derive a separation time of 20,000 years for These two groups. Actually, I agree with you that this is a "good" approximate time, but the aura of scientific respectability given this dating is based on a false premise of quantifying time from nonmetric data. When I look over some of the GI scores given, the flaws in "molecular clock" dating becomes apparant. For example, the scores for Pine and Goshute creeks would indicate about 10,000 years or more of separation when in fact, the Goshute Creek

UL

population was stocked from Pine Creek about 20 years before you obtained your sample. Also I →

You state that you have unpublished data concerning intergradation between the fine-spotted and large-spotted Snake River cutthroat trout. Why not publish it here?

Why don't you formally propose to synonymize seleniris with henshawi to be consistant? The electrophoretic (and morphological) evidence ~~sho~~ agrees that Carson R. henshawi and seleniris are more closely related to each other than Carson henshawi is to other Lahontan basin henshawi. Again, this is a very practical matter. S. c. seleniris can be distinguished from henshawi by the spotting pattern just as can be done with fine-spotted and large-~~spotted~~ Snake River cutthroat trout. ~~However~~, Without its unique taxonomic designation, seleniris would ~~not~~ have been saved from ~~distinction~~ extinction.

It is mentioned that Long Canyon Creek (Lahontan basin, Nevada) has both pure rainbow trout and pure cutthroat trout with no hybrids. This is a most unusual situation that I would like to learn more about. Did you collect the specimens personally? Were the two species found together or is there a barrier separating them?

I hope you are able to continue with your work. ~~to date~~ Data on S. chrysogaster would be most interesting. It may be possible to discuss primitive and derived states for alleles at a locus if comprehensive data could be obtained on other Salmo and particularly the species of Oncorhynchus. This would be critical ~~to convert~~ information

→ would interpret a separation of about 120,000 years of the Bear Creek cutthroat population in Yellowstone Park, when its ~~actual~~ actual separate isolation from Yellowstone Lake occurred in the past few thousand years.

That would allow converting phenograms to cladograms and provide a basis for phylogenetic and taxonomic conclusions.

There are many other interesting aspects where your work could help answer some questions that aren't entirely apparant from my studies, such as the origin of the native cutthroat trout in the John Day River drainage, Oregon. I classify them as lewisi, but the large spots suggest some bouvieri influence. Did the Alvord basin cutthroat (Willow and Whitehorse creeks) come from the Lahontan basin or the Snake River system? With the several distinct loci in kershawi, ~~your~~ electrophoresis should be able to settle this question. How do the "redband" trout of the Oregon desert basins compare with the Goose Lake basin trout?

Thus, there is ~~quite a bit~~ considerable work to be done ~~and~~ I caution against taxonomic revisions until we ~~have~~ can make more authoratative conclusions.

Sincerely,