The Journal of Heredity 67:330–335. 1976.

Biochemical genetic variation in populations of golden trout, *Salmo aguabonita*

Evidence of the threatened Little Kern River golden trout, *S. a. whitei*

G. A. E. GALL, C. A. BUSACK, R. C. SMITH, J. R. GOLD, AND B. J. KORNBLATT

SINCE the original study and description of a group of brilliantly colored trouts of the southern Sierra Nevada, California, known collectively as the golden trout, *Salmo aguabonita*, concern has been expressed both by fisheries management agencies and sportsmen regarding the status, fate, and preservation of these unique trout[8,12,33]. This concern derives principally from the extremely narrow range and distribution of the species[13], the disturbance by man of the fragile golden trout environment[14,33], and the very real possibility that hybridization of endemic goldens with rainbow trout introduced into significant portions of the golden's range has diluted and contaminated the native golden stocks[11,18,20]. Previous scientific studies of these trout have been confined largely to descriptions of morphological and meristic characters[10,34], and the comparison of the golden trout with other species of Western North American *Salmo*[24]. Gold and Gall[19] reported a chromosome number of $2n = 58$ with 104 chromosome arms for *S. aguabonita*. Variability in chromosome number within six of the populations reported on in this paper permitted the identification of two distinct population distributions, one believed to represent an introgressed group and the other the normal *S. aguabonita* karyotype. Studies of biochemical-genetic variation in *S. aguabonita* have included only a brief description of gene duplication at the malate dehydrogenase locus[6].

The model of the experiment under which the sampling was carried out was presented in detail by Gold and Gall[18]. It was postulated that a comparison of populations of *S. a. aguabonita* should define the degree of naturally occurring diversity expected among golden trout. Also, it was expected that the relative diversity among *S. a. aguabonita* populations would be less than that between populations of the *S. a. whitei* subspecies and populations of introgressed fish, and that *S. a. whitei* populations should be more closely related to their sister subspecies. *S. a. aguabonita*, than to introgressed fish from adjoining waters. Three populations from the eastern Kern River area were sampled to represent the rainbow-trout free, geographically isolated, golden trout subspecies *S. a. aguabonita*. Soda Springs Creek and Deadman Creek were chosen as the sites where several small, partially isolated populations of the golden trout, *S. a. whitei*, endemic to the Little Kern River were thought to exist. It was postulated that samples from these creeks, which were similar to eastern Kern River populations, could be considered to belong to the *S. aguabonita* species, whereas samples that differed could be considered to represent introgressed populations of rainbow and golden trout as proposed by Dill[11].

The objectives of the investigation were to examine the amount of genetic variability in natural golden trout populations, to identify polymorphic loci with variability unique to golden trout, and to utilize this uncovered variability to further define the systematics and racial origin of the trout in the Little Kern River basin.

Materials and Methods

Golden trout were collected in the summer and fall of 1973 and 1974 from eight locations. Six of the populations were described by Gold and Gall[18]. Three from the Soda Springs Creek area of the Little Kern River basin were referred to as Little Kern River (LKR), lower Soda Springs Creek (LSSC) and upper Soda Springs Creek (USSC). Three from the eastern Kern River area, sampled to represent *S. a. aguabonita*, were referred to as Golden Trout Creek (GTC), Cottonwood Creek (CWC), and South Fork Kern River (SFK). Two additional populations not described by Gold and Gall[18] were sampled from middle Soda Springs Creek (MSSC), approximately 2.4 km below the USSC location, and from Deadman creek (DMC), a stream tributary to Soda Springs Creek at MSSC. A rainbow trout sample, included for comparative purposes, was obtained from the domestic Mt. Shasta stock (RTS) and was kindly provided by the State Fish

Dr. Gall is associate professor of animal science, and principal investigator, Fisheries Biology Research Facility; C.A. Busack, R.C. Smith, and B.J. Kornblatt are research assistants; Dr. Gold was post-doctoral fellow, Department of Animal Science, University of California, Davis CA 95616. Dr. Gold is presently assistant professor, Department of Plant Science, Texas A&M University, College Station. The authors wish to express their appreciation to the California Department of Fish and Game for collecting the specimens and to Boyd Bentley and Robert Pipkin for their expert technical support. The study was supported by Federal Aid to Fish Restoration funds as Dingell-Johnson Project F-28-R.

Hatchery, California Department of Fish and Game, Mt. Shasta, California.

All specimens were transferred live to the Fisheries Biology Research Facility and fin-clipped to identify their origin. Procedures used for the collection and processing of tissues basically followed Bailey et al.[6], and Utter et al.[38]. Horizontal starch-gel electrophoresis followed the procedures of Kristjannson[23] and Utter et al.[39]. Three buffer systems were used: 1) a discontinuous lithium-borate (pH 8.2), tris-citrate (pH 8.6) system described by Ridgway et al.[30]; 2) a continuous tris-borate NaEDTA system (pH 8.6) described by Markert and Faulhaber[25]; and 3) a continuous citrate-phosphate system (pH 6.4) described by Bailey and Wilson[5]. Alcohol dehydrogenase (*ADH*), esterase (*EST*), phosphoglucomutase (*PGM*), tetrazolium oxidase (*TO*), and transferrin (*TFN*) were resolved using Ridgway's discontinuous system. Malate dehydrogenase (*MDH*) was resolved using the citrate-phosphate system and 6-phosphogluconate dehydrogenase (*6PGDH*) with the tris-borate NaEDTA system. Three protein systems, *6PGDH*, *PGM* and *MDH*, were studied in extracts of muscle tissue, *ADH*, *TO* and *EST* analyses utilized liver tissue, and *TFN* analysis was performed with blood. Staining procedures for all proteins were after Shaw and Prasad[35]. The gels were incubated in a 37°C, dark incubator until staining was complete and then fixed in a 5:4:1 solution of water: methanol: acetic acid.

Stringent restrictions were imposed on what constituted genetic variation since breeding studies in golden trout have not been available. A genetic basis was assumed only if: 1) the array of phenotypes fit a simple Mendelian model, and 2) a genetic basis had previously been determined for a closely related species. Furthermore, protein patterns were considered genuine only if repeated subsampling of the same tissue of an individual produced identical patterns.

The system of nomenclature follows that of Richmond[29]. Each locus was named using an abbreviation of the protein name and when duplicate loci were observed the loci were numbered according to the migration rate of the protein products, i.e., the form with the slowest migration was assigned the numeral one, the next fastest form the numeral two, and so on. When allelic variation was found, the most common allele was assigned a migration distance of 1.0 and all other alleles were assigned migration distances that represented their migration rate relative to the most common allele.

Results and Discussion

Protein systems

The results of electrophoresis revealed that all of the protein systems except *ADH* and *6PGDH* showed variability in at least five populations. The migration patterns and migration rates observed for each protein system were identical in both golden trout and the domestic rainbow trout. All of the alleles identified for rainbow trout systems were also observed in golden trout. Golden trout exhibited two additional alleles in the *TO* system, and one allele for *EST*-2 that were not observed in the rainbow trout studied.

Alcohol dehydrogenase: Two invariant, cathodally migrating bands were observed in all populations (371 fish). However, the fastest migrating band showed very intense staining relative to the slower migrating band. Since *ADH* has been shown by Allendorf[2] to be a dimeric protein controlled by a single locus, we interpreted *ADH* to be monomorphic in all populations. The existence of the slower migrating, faintly staining band was attributed to storage or tissue extraction procedures.

6-Phosphogluconate dehydrogenase: A single, clear, anodally migrating band of *6 PGDH* activity was observed in all of 319 fish tested. A single, monomorphic locus was assumed, as reported by Utter et al.[39].

Phosphoglucomutase: Two anodally migrating bands were observed for *PGM* in a single specimen from each of four of the golden trout populations and in four rainbow trout specimens. The remaining individuals exhibited a single-band phenotype with a migration rate equal to the fast band of the two-band phenotypes. These two phenotypes were assumed to represent the homozygous and heterozygous forms for a monomeric protein controlled by a single locus with two codominant alleles. This interpretation is in agreement with Roberts et al.[31] and Utter et al.[39].

Malate dehydrogenase: Either three or six anodally migrating bands of NAD-dependent *MDH* activity were expressed in all individuals studied. The three bands of the invariant form of *MDH* showed symmetrical staining when extracted from heart tissue but differential, asymmetric staining was observed for extracts of liver and muscle tissue of the same fish. The differential staining and the existence of three bands is consistent with the hypothesis that *MDH* is a dimeric protein encoded by more than one locus[5,6,28,39].

Two sets of loci coding for electrophoretically different proteins, *MDH-1* and *MDH-2* (the A and B forms, respectively, of Bailey and Wilson[5]) are proposed. As indicated by the asymmetrical staining, the *MDH-1* loci were more active than *MDH-2* loci in liver extracts, less active than *MDH-2* in muscle extracts and showed equal activity in heart extracts.

Only one homodimeric band was observed for *MDH-1*, whereas *MDH-2* was variable in six of the nine populations. The *MDH-2* variant forms displayed asymmetric staining patterns similar to those first described in rainbow trout by Bailey and Wilson[5] and in golden trout by Bailey et al.[6]. In agreement with their interpretation, it is clear that at least the *MDH-2* form of the protein is coded for by two loci, *MDH-2a* and *MDH-2b*, with each locus containing alleles that code for proteins with identical electrophoretic mobilities. The single variant form produced a homodimeric protein that migrated more slowly than the common *MDH-2* homodimer, but more rapidly than the heterodimer composed of both *MDH-1* and *MDH-2* forms. The two additional heterodimers expected for the variant were observed, one migrating midway between the two *MDH-2* homodimeric forms and one migrating midway between the variant *MDH-2* and the *MDH-1* homodimers.

As pointed out by Numachi et al.[28], when two loci possess identical alleles such that a variant form could arise whenever one of the four alleles is mutant, it is not possible to determine which of the loci is polymorphic. We shall refer to a set of two or more such loci as *isoqualitic* loci. The low frequency of the variant allele observed in the populations sampled (range of 0.01 to 0.09) indicated that it is unlikely the allele existed at

Table I. Observed (O) and expected (E) distributions of phenotypes for five polymorphic protein systems examined in eight sampled golden trout populations and one domestic rainbow trout stock (RTS)

Protein / Population	Sample size	Phenotype						Gene frequency
		O	E	O	E	O	E	
Phosphoglucomutase		1.0/1.0		1.0/0.89		0.89/0.89		
LKR	58	58						1.00
LSSC	36	35	35	1	1			0.99
MSSC	39	39						1.00
USSC	71	70	70	1	1			0.99
DMC	36	36	35	1	1			0.99
GTC	38	37	37	1	1			0.99
CWC	26	26						1.00
SFK	39	39						1.00
RTS	20	16	16	4	4			0.90
Malate dehydrogenase-2a		1.0/1.0		1.0/0.9		0.91/0.9		
LKR	60	59	59	1	1			0.99
LSSC	39	38	38	1	1			0.99
MSSC	39	39						1.00
USSC	74	73	73	1	1			0.99
DMC	36	36						1.00
GTC	37	34	33	3	3	0	1	0.98
CWC	26	26						1.00
SFK	40	37	36	3	3	0	1	0.98
RTS	20	13	13	7	6	0	1	0.91
Esterase-1		1.0/1.0		1.0/0.94		0.94/0.94		
LKR	53	30	26	15	22	8	5	0.71
*LSSC	36	30	27	3	8	3	1	0.88
*MSSC	37	18	14	10	17	9	5	0.62
*USSC	66	52	46	7	18	7	2	0.84
DMC	36	31	31	5	5			0.93
GTC	37	25	24	10	11	2	2	0.81
*CWC	25	15	10	2	12	8	3	0.64
SFK	40	23	20	11	16	6	4	0.71
RTS	20	6	8	12	9	1	3	0.63
Esterase-2		1.0/1.0		1.0/0.94		1.0/0.94		
LKR	55	36	33	14	19	5	3	0.78
LSSC	37	25	22	8	13	4	2	0.78
*MSSC	37	21	18	9	16	7	3	0.69
*USSC	66	52	44	4	20	10	2	0.82
DMC	36	25	24	9	11	2	1	0.82
GTC	37	25	23	9	12	3	2	0.80
CWC	25	21	21	4	4			0.92
SFK	40	30	29	9	10	1	1	0.86
RTS	20	20						1.00
Transferrin		1.0/1.0		1.0/0.9		0.9/0.9		
*LKR	33	11	14	21	15	1	4	0.65
*LSSC	22	5	8	16	10	1	4	0.59
*MSSC	39	18	21	21	15	0	3	0.73
*USSC	51	12	20	39	24	0	7	0.62
DMC	36	35	35	1	1			0.99
GTC	27	12	14	15	11	0	2	0.72
CWC	14	5	6	9	6	0	2	0.68
SFK	33	15	16	17	14	1	3	0.71
RTS	20	0	2	12	8	8	10	0.30

* Significant deviation ($P < 0.05$) from expected frequencies

both the *MDH-2a* and *MDH-2b* loci. Therefore, the variant allele was arbitrarily assigned to the '*a*' locus and designated *MDH-2a(.9)*.

Esterase: Three different and independent systems of liver esterase activity were observed in all individuals. The systems were designated, from slowest to fastest anodal migration, as *EST-1*, *EST-2* and *EST-3*. The expression of three phenotypes was observed for each of *EST-1* and *EST-2* reflecting dimeric quatemary structure for both proteins, i.e., two single-band phenotypes and one three-band phenotype. These phenotypes fit the single locus, two codominant allele models proposed by Minvielle[26] for rainbow trout.

The *EST-3* phenotypes did not conform to any simple genetic model and their interpretation must await definitive breeding studies. Asymmetrical staining patterns coupled with the absence of single-band phenotypes suggested that *EST-3* is coded by more than one locus. Given these reservations concerning *EST-3* and difficulties encountered in recording *EST-1* and *EST-2* phenotypes, the possibility exists that the latter two systems may have a more complex inheritance than hypothesized here.

Transferrin: Two single-band phenotypes and one two-band phenotype were observed for the *TFN* system in blood, consistent with the disomic inheritance for a monomeric protein proposed by Utter and Hodgins[37] and Utter et al.[39] for rainbow trout. The slow migrating single-band phenotype was frequent in the rainbow trout sampled and was observed in one individual in each of only three golden trout populations. It must be noted that an excess of heterozygous phenotypes was found in all nine populations (Table I) with five of the deviations being statistically significant ($P < 0.05$). In addition, it was suspected that differential staining observed for many two-band phenotypes was not due to variations in technique. Therefore, it is possible that the transferrin proteins in golden and rainbow trout are controlled by two *isoqualitic* loci similar to the duplicate locus system observed for *MDH-2*.

Tetrazolium oxidase: The phenotypes observed for *TO* in blood could be completely described using the model for a dimeric protein encoded by a single locus proposed by Utter and Hodgins[37]. For golden trout, the four codominant alleles observed were symbolized *TO(1.4)*, *(1.2)*, *(1.0)* and *(0.6)*. Two of these alleles, *(1.4)* and *(1.0)*, were also found in rainbow trout. It is also important to note that identical migration rates and staining patterns were observed in muscle, liver, and blood extracts from all single individuals studied. This result contradicts the interpretation of Cederbaum and Yoshida[7] that liver and blood *TO* phenotypes are coded by different loci in rainbow trout and supports the contention of Utter et al.[38] that there is only one *TO* locus in salmonid fish.

Observed variability

The phenotypic (genotypic) distributions and estimated gene frequencies for five systems controlled by disomic loci are summarized in Table I. The most striking contrast among the five systems is that two, *PGM* and *MDH-2*, showed a very low level of variablity, whereas the remaining three, *EST-1*, *EST-2*, and *TFN* exhibited a high degree of genetic variability. This clear grouping based on variability followed that proposed by Gillespie and Kojima[16]. They reported that enzymes involved in energy metabolism in *Drosophila* showed less genetic variability than nonspecific enzyme systems such as esterases. This notion is further supported by the lack of variability in *MDH-1*, *ADH*, and *6PGDH* in the present study and the high variability found for *TO* (Table II). A second general conclusion was that the rainbow trout stock tended to show more variability, in terms of more intermediate gene frequencies, than most of the wild populations sampled. The latter observation may of course be confounded with domestication and population size as has been suggested by Wright[40].

The extremely low variability observed for *PGM* and *MDH-2* precludes any discussion of the distributions of phenotypes for these systems. *EST-1* and *EST-2* (Table I) both consistently showed a deficiency of heterozygotes and an excess of both homozygotes, although deviations from expected were not significant in all cases. The observed frequencies of heterozygotes pooled over the eight golden trout populations were 0.19 and 0.17 compared to the pooled expected frequencies of 0.33 and 0.40 for *EST-1* and *EST-2*, respectively. Such genotypic distributions represent the classical expected effects of small population size and /or nonrandom mating[9].

The distributions of phenotypes observed for the *TFN* system (Table I) were in direct contrast to those observed for *EST*. Five of the eight golden trout populations showed significant deviations from expected based on a one-locus, two-allele model. All populations appeared to have an excess frequency of heterozygotes and a deficiency of both homozygotes. When pooled over the eight populations, the observed frequency of heterozygotes was 0.55 compared to an expected frequency of 0.38. Interestingly, a similar excess of heterozygotes was found in the rainbow trout stock despite the fact the allele frequencies were reversed from that seen in golden trout. The observed disequilibrium may have been the result of small sample sizes or it may reflect the effects of selection at a locus showing overdominance[32]. An excess of heterozygotes was also reported for *TFN* in skipjack tuna, *Katsuwonus pelamis*, by Fujino and Kang[15], and there is evidence that the *TFN-A* allele is lethal when homozygous in eastern brook trout, *Salvelinus fontinalis*. However, further studies of brook trout failed to provide evidence of differential fitness of

Table II. Frequency of four alleles observed for the multiple allelic tetrazolium oxidase system in eight sampled golden trout populations and one domestic rainbow trout stock (RTS)

Population	Sample size	Alleles			
		1.0	*1.4*	*1.2*	*0.6*
LKR	57	0.68	0.07	0.01	0.24
LSSC	37	0.66		0.02	0.32
MSSC	39	0.56			0.44
USSC	66	0.90	0.02		0.08
DMC	36	0.96			0.04
GTC	37	0.86	0.05		0.09
CWC	25	0.88	0.08	0.04	
SFK	40	0.91	0.03		0.06
RTS	20	0.80	0.20		

the *TFN-A* allele[22,40]. A decision on the nature of *TFN* inheritance in salmonid fish will be reserved until breeding studies can be completed because an excess of heterozygotes will always be observed for a one-locus, two-allele model if in fact inheritance is controlled by two *isoqualitic* loci as we suspect.

Of the four alleles observed for the *TO* system, two were found in very low frequency (Table II) and only two alleles, *TO*(*1.4*) and *TO*(1.0), were seen in the rainbow trout stock. The distributions of phenotypes could not be tested for goodness-of-fit due to the large number of empty classes, but the observed frequencies did not appear to deviate from the expected for a one-locus model. It should be noted that the golden trout sampled from LKR, LSSC and MSSC had a frequency of the *TO*(0.6) allele that may have been derived from planting rainbow trout with a high frequency of this allele.

Population diversity

The genetic variability of a population is usually measured by the average heterozygosity per locus[27]. Estimates are given in Table III calculated as the unweighted average frequency to remove the effect of variable numbers of observations per locus. With the exception of the DMC population, all samples gave estimates of from 21.0 to 28.2 percent heterozygotes for the six polymorphic loci or from 12.6 to 13.9 percent if the four invariant loci are included in the estimates. The DMC population is thought to be small in size, which probably accounts for the low genetic variability. The eight golden trout populations were polymorphic at from four to six of the 10 loci examined (assuming *MDH-1* to be a single locus) or an average of 51 percent of the loci. These estimates of genetic variability are high when compared to other species[3,21,36,39]. The high estimates may be due to the limited number of loci examined or may be a function of the particular loci chosen for study. However, it seems reasonable to conclude that despite their limited geographical distribution, these populations have maintained a significant level of genetic variability, possibly caused by fluctuating environmental conditions as suggested by Gillespie and Langley[17].

Three of the loci examined also shed some light on the question of the possibility of hybridization of the endemic Little Kern River basin golden trout with introduced rainbow trout, the latter having occurred during the years 1931–41[11]. The gene frequency of *EST-1*(*1.0*) was similar in two populations (LKR and MSSC) most likely to have been planted with rainbow trout due to their accessibility by pack trail. These frequencies, although possibly coincidentally, also tend to be similar to that found in RTS rainbows. Three populations, LKR, LSSC, and MSSC, showed a gene frequency cluster for *EST-2*(*1.0*) that tends to be distinct from the remaining two Little Kern populations, USSC and DMC. The frequency of *TO*(*0.6*) in these same three populations was also different from that in USSC and DMC and may be typical of a rainbow stock. In addition, the gene frequencies at these three loci in samples from USSC and DMC are very similar to those observed for the geographically isolated populations of *S. a. aguabonita* sampled from GTC and SFK. The inconsistency of gene frequencies for CWC, a transplanted population of *S. a. aguabonita*, may be due to a "founder" effect since the population was originally founded by only 12–13 trout[13], environmental differences in habitat since CWC is over 1000 feet higher in elevation than the GTC and SFK sites, or due to limited contamination from rainbow trout since the Cottonwood Lakes immediately above CWC are managed with golden trout hatched and reared in rainbow trout hatcheries.

The observation that the trout in upper Soda Springs Creek (USSC) and Deadman Creek (DMC) tend to be genetically distinct from the trout populations a short distance downstream (MSSC, LSSC, and LKR) and genetically similar to samples of the geographically distant *S. a. aguabonita* confirms the hypothesis of Gold and Gall[18,19,20], based on meristic and cytogenic evidence, that USSC and DMC trout represent vestige populations of the original Little Kern golden trout, *S. a. whitei*. Impassable barriers[18] separating trout in upper Soda Springs Creek and Deadman Creek from those downstream has prevented contamination from the introgressed *S. a. whitei*–rainbow population considered to be present in the Little Kern River.

Table III. Average heterozygosity (H) calculated as the unweighted average frequency of heterozygotes at the six loci showing variability. Estimates including the four invariant loci are six-tenths of the values shown[27]

Population	No. genomes	H	*se*
LKR	316	0.282	0.088
LSSC	207	0.258	0.084
MSSC	230	0.297	0.095
USSC	394	0.202	0.071
DMC	216	0.090	0.045
GTC	213	0.228	0.060
CWC	141	0.210	0.083
SFK	232	0.217	0.070
RTS	120	0.279	0.069
Combined	2069	0.218	—

Summary

Eight wild populations of the High Sierra golden trout, *Salmo aguabonita*, and one domestic stock of rainbow trout, *Salmo gairdneri*, were examined for biochemical-genetic variation in eight protein systems. Variation within the eight systems was determined by at least 10 loci in both golden and rainbow trout and all the alleles identified in rainbow trout were observed as electrophoretically identical phenotypes in golden trout. Variation was observed at an average of 51 percent of the loci in the golden trout samples and for five of the 10 loci in the rainbow trout. Average heterozygosity ranged from 12.6 to 13.9 percent for seven of the golden trout populations with one showing a low value of 5.4 percent. A comparable estimate of 12.1 percent was found for the rainbow stock.

On the basis of genetic variation and allele frequencies at three loci, the eight golden trout populations were divided into two distinct groups. Three populations sampled from the Little Kern River basin tended to be genetically distinct from two additional Little Kern River basin populations and from three geographically distinct

populations sampled from the eastern Kern River area. The former three populations were hypothesized to be of a recent rainbow–golden hybrid origin. Trout in the other two Little Kern River basin populations, sampled in headwaters of a stream tributary to the Little Kern River, were considered to be the threatened Little Kern golden trout, *S. a. whitei* Evermann, due to their high degree of genetic similarity to the geographically distinct subspecies *S. a. aguabonita* sampled from the eastern Kern River area. The finding of substantial genetic variation in the wild golden trout populations indicates that this threatened species is not at present genetically impoverished and thus does not appear to be in immediate danger of extinction through lack of adaptive capability.

Literature Cited

1. ALLENDORF, F.W. Genetic variation, inheritance, and preliminary population distribution of some proteins of *Salmo gairdneri*. M.S. Thesis, University of Washington, Seattle. 1973.
2. ———. Genetic variability in a species possessing extensive gene duplication: genetic interpretation of duplicate loci and examination of genetic variability in populations of rainbow trout. Ph.D. Thesis, University of Washington, Seattle. 1975.
3. AVISE, J.C. and R.K. SELANDER. Evolutionary genetics of cave dwelling fishes of the genus *Astyanax*. *Evolution* 26:1–9. 1972.
4. AYALA, F.J., J.R. POWELL, and M.L. TRACEY. Enzyme variability in the *Drosophila willistoni* group. V. Genic variation in natural populations of *Drosophila equinoxialis*. *Genet. Res.* 20:19–42. 1972.
5. BAILEY, G.S. and A.G. WILSON. Homologies between isozymes of fishes and those of higher vertebrates. *J. Biol. Chem.* 243(22):5843–5853. 1968.
6. ———, ———, J.E. HALVER, and C.L. JOHNSON. Multiple forms of supernatant malate dehydrogenase in salmonid fishes. *J. Biol. Chem.* 245(22):5927–5940. 1970.
7. CEDERBAUM, S.D. and A. YOSHIDA. Tetrazolium oxidase polymorphisms in rainbow trout. *Genetics* 72:363–367. 1972.
8. CHRISTENSON, D.P. and R.C. SMITH. Status Report—Little Kern Golden Trout. California Department of Fish and Game. 11 p. 1974.
9. CROW. J.F. and M. KIMURA. An Introduction to Population Genetics Theory. Harper & Row. New York, Evanston, and London. 1970.
10. CURTIS, B. The golden trout of Cottonwood Lakes. *California Fish and Game* 21:102–109. 1935.
11. DILL, W.A. The Little Kern River Drainage, Tulare County. Administrative Report #45-29. California Department of Fish and Game. 19 p. 1945.
12. ———. A Report on the Golden Trout Fishery in California. Administrative Report #50-44. California Department of Fish and Game. 28 p. 1950.
13. ELLIS, S.L.N. and H.C. BRYANT. Distribution of the golden trout in California. *California Fish and Game* 6(4):141–152. 1920.
14. EVANS, W.A., R.C. SMITH, and M. BELL. A Reconnaissance Survey of The Fish Resources of the Little Kern River Drainage, California. USDA Forest Service, Region 5 and California Department of Fish and Game. Mimeo., 41 p. 1973.
15. FUJINO, K. and T. KANG. Transferrin groups of tunas. *Genetics* 59:79–91. 1968.
16. GILLESPIE, J.H. and K. KOJIMA. The degree of polymorphism in enzymes involved in energy production compared to that in nonspecific enzymes in two *Drosophila ananassae* populations. *Proc. Natl. Acad. Sci.* 61:582–585. 1968.
17. GILLESPIE, J.H. and C.H. LANGLEY. A general model to account for enzyme variation in natural populations. *Genetics* 76:837–884. 1974.
18. GOLD, J.R. and G.A.E. GALL. The taxonomic structure of six golden trout (*Salmo aguabonita*) populations from the Sierra Nevada, California (Pisces: Salmonidae). *Calif. Acad. Sci., Proc.* 4th Ser. XL:243–263. 1975.
19. ——— and ———. Chromosome cytology and polymorphism in the California High Sierra golden trout (*Salmo aguabonita*). *Can. J. Genet. Cytol.* 17:41–53. 1975.
20. ——— and ———. Further record of Little Kern golden trout, *Salmo aguabonita whitei*, in the Little Kern River basin, California. *California Fish and Game* 61:248–250. 1975.
21. GOTTLIEB, L.D. Gel electrophoresis: New approach to the study of evolution. *BioScience* 21:939–944. 1971.
22. HERSCHBERGER, W.K. Some physiochemical properties of transferrins in brook trout. *Trans. Am. Fish. Soc.* 99(1): 207–218. 1970.
23. KRISTJANSSON, F.K. Genetic control of two pre-albumins in pigs. *Genetics* 48:1059–1063. 1963.
24. LEGENDRE, P., C.B. SCHRECK, and R.J. BEHNKE. Taximetric analysis of selected groups of Western North American *Salmo* with respect to phylogenetic divergences. *Syst. Zool.* 21:292–307. 1972.
25. MARKERT, C.L. and I. FAULHABER. Lactate dehydrogenase isozyme patterns in fish. *J. Exp. Zool.* 159:319–332. 1972.
26. MINVIELLE, F.P.G. Esterase and tetrazolium oxidase polymorphisms in two populations of the rainbow trout (*Salmo gairdneri*). M.S. Thesis, University of California, Davis. 1973.
27. NEI, M. and A.K. ROYCHOUDHURY. Sampling variances of heterozygosity and genetic distance. *Genetics* 76:379–390. 1974.
28. NUMACHI, K., Y. MATSUMIYA, and R. SATO. Duplicate genetic loci and variant forms of malic dehydrogenase in chum salmon and rainbow trout. *Bull. Jap. Soc. Sci. Fish.* 38:699–706. 1972.
29. RICHMOND, R.C. Enzyme variability in the *Drosophila willistoni* group. III. Amounts of variability in the superspecies *D. poulistorum*. *Genetics* 70:87–112. 1972.
30. RIDGWAY, G.J., S.W. SHERBURNE, and R.O. LEWIS. Polymorphisms in the esterases of Atlantic herring. *Trans. Am. Fish. Soc.* 99:147–151. 1970.
31. ROBERTS, F.L., J.F. WOHNUM, and S. OHNO. Phosphoglucomutase polymorphism in the rainbow trout, *Salmo gairdneri*. *Experientia* 25:1109–1110. 1969.
32. ROBERTSON, A. Selection for heterozygotes in small populations. *Genetics* 47:1291–1300. 1962.
33. SCHNEEGAS, E.R. and E.P. PISTER. Habitat management plan for native golden trout waters. INYO National Forest Publication of the USDA Forest Service. 45 p. 1965.
34. SCHRECK, C.B. and R.J. BEHNKE. Trouts of the Upper Kern River Basin, California, with reference to systematics and evolution of western North American *Salmo*. *J. Fish. Res. Bd. Can.* 28:987–998. 1971.
35. SHAW, C.R. and R. PRASAD. Starch gel electrophoresis of enzymes: A compilation of recipes. *Biochem. Genet.* 4:297–320. 1970.
36. TRACEY, M.L., K. NELSON, D. HEDGECOCK, R.A. SCHLESER, and M.L. PRESSICK. Biochemical genetics of lobsters: Genetic variation and the structure of American lobster (*Homarus americanus*) populations. *J. Fish. Res. Bd. Can.* 32: 2091–2101. 1975.
37. UTTER, F.M. and H.O. HODGINS. Biochemical genetic variation at six loci in four stocks of rainbow trout. *Trans. Am. Fish. Soc.* 101:494–502. 1972.
38. ———, F.W. ALLENDORF, H.O. HODGINS, and A.G. JOHNSON. Letters to the Editor. *Genetics* 73:159. 1973.
39. ———, ———, and ———. Genetic variability and relationships in Pacific salmon and related trout based on protein variations. *Syst. Zool.* 22:257–268. 1973.
40. WRIGHT, J.E., JR. Polymorphisms for transferrin and LDH loci in brook trout populations. *Trans. Am. Fish. Soc.* 99(1): 179–192. 1970.