FINAL REPORT

SECTION 6

ENDANGERED SPECIES ACT

FEDERAL AID PROJECT E-29

Status of Threatened and Endangered Fishes in Oklahoma

MAY 1, 1994 - DECEMBER 31, 1998

FINAL REPORT

STATE: OKLAHOMA PROJECT NUMBER: E-29

PROJECT TITLE: Status of Threatened and Endangered Fishes in Oklahoma

STUDY TITLE: Reintroduction of the Arkansas River Speckled Chub and Taxonomic Status and Genetic Structure of the Speckled Chub in the Arkansas and Red River Drainages

PERIOD COVERED: 1 May 1994 to 31 December 1998

ABSTRACT

This report is divided into two parts. Part A describes an attempt to reintroduce a member of the speckled chub complex (cf. *Macrhybopsis aestivalis*) into the Medicine Lodge and Salt Fork of the Arkansas rivers upstream of Great Salt Plains Reservoir. In May of 1994, one site on the Medicine Lodge River and one on the Salt Fork received, respectively, 591 and 1,340 specimens from the Arkansas River below the dam on Kaw Reservoir. Subsequent collections made at 17 sites in the two rivers in 1994 and 1995 failed to produce speckled chubs. Unusually low water flows in the Salt Fork during the 1994 breeding season might explain the failure of the reintroduction effort. Subsequent to this effort, a morphologically based review of the systematics of speckled chubs (Eisenhour, 1997) divided what was considered a single species into several species and suggested that the speckled chub that historically occupied the area of attempted reintroduction might have been a separate species (*M. tetranema*) from the population (*M. hyostoma*) that was serving as the source stock. Any subsequent reintroduction effort should focus on *M. tetranema*, a species now restricted to the Ninnescah River of Kansas and the South Canadian River in New Mexico and Texas Panhandle.

In Part B of this report, we use protein electrophoresis of the allozyme products of 22 gene loci to address the question of whether there are three species in the Arkansas and Red river basins. Eisenhour (1997) recognized *M. tetranema* and *M. australis* as endemics to, respectively, the Arkansas and Red river basins, and a widespread species, *M. hyostoma*, in both basins. The analysis included the following samples (number of samples in parentheses): *M. tetranema* (3), *M. australis* (7), *M. hyostoma* (17 from the Red and Arkansas River basins and 7 from sites widely distributed across the range of the species). For added insight into systematics, we also included samples of the remaining species of the speckled chub complex, *M. marconis* from the San Marcos River in Texas and *M. aestivalis* from the Pecos River in New Mexico. The monophyly of both *M. australis* and *M. tetranema* was supported, although somewhat weakly because of a remarkably high level of genetic similarity among these two species and *M. hyostoma*. The results are consistent with speciation and subsequent genetic introgression after secondary contact between *M. hyostoma* and each of the two regionally endemic species. There was little evidence of geographic pattern in genetic variation within either *M. australis* or *M. tetranema*.

REPORT CONTENT

OBJECTIVES:

Part A:

1. To capture Arkansas River speckled chubs from areas where the species is known to be common and relocate them to the Medicine Lodge and Salt Fork of the Arkansas rivers upstream of Great Salt Plains Reservoir.

Part B:

1. Use protein electrophoresis to examine how many species of speckled chub occur in the Arkansas and Red river basins in Oklahoma.

2. Collect baseline data on population genetics that would be useful in future management of the speckled chub

PART A

ATTEMPTED REINTRODUCTION OF THE ARKANSAS RIVER SPECKLED CHUB

I. INTRODUCTION:

Transplantation of species to previously occupied sites within their native range (repatriation) is popular with conservation biologists (Booth, 1988; Brown, 1988; Conway, 1988; Griffith et al., 1989; Wikramanayake, 1990; Hendrickson and Brooks, 1991) and is recommended in most recovery plans for endangered fishes (Williams et al., 1988). Attempts at repatriation have been particularly common in the southwestern United States (Hendrickson and Brooks, 1991). Williams et al. (1988) and Hendrickson and Brooks (1991) emphasized the need for documentation and publication of such attempts to aid future researchers. In this paper, we report an attempt to repatriate speckled chub (Cyprinidae: cf. *Macrhybopsis aestivalis*) to the Salt Fork of the Arkansas (= Salt Fork) and Medicine Lodge rivers of Oklahoma and Kansas. We examine habitat and streamflow conditions and discuss additional factors that may have contributed to the failure of this effort.

Members of the speckled chub complex are small cyprinids ($\leq$ 76 mm SL) that live about 1.5 years (Becker, 1983; Starrett, 1951). They apparently spawn during spring and summer spates, and the eggs drift downstream suspended in current until they hatch in 24 to 28 hours (Bottrell et al., 1964). Larvae drift downstream as they develop and later life-stages apparently disperse upstream.

The last known collection of speckled chub in the Salt Fork River drainage upstream of Great Salt Plains Reservoir was made in 1964, over two decades after completion of Salt Plains

Dam in 1941 (Luttrell, 1997). Extirpation of the species appears associated with unusually low flows during the May-August spawning periods in 1964, and 1966-1968 (Luttrell, 1997). The presence of Salt Plains Dam would have prevented recolonization from downstream. Similar circumstances explain the disappearance of speckled chub from the upper North Fork of the Red River in southern Oklahoma (Winston et al., 1991).

In 1994, we attempted to reintroduce speckled chub into the Medicine Lodge and Salt Fork rivers upstream of Great Salt Plains Reservoir. Qualitative observations indicated that habitat conditions in these streams were adequate, and we assumed that the high reproductive potential of speckled chub (Starrett, 1951; Becker, 1983) would allow rapid establishment of a population.

In an ongoing study of speckled chub systematics, and subsequent to our attempted reintroduction, D. Eisenhour (pers. comm.) concluded that several nominal subspecies of *M. aestivalis* should be elevated to species status. His work indicates that two species of speckled chub formerly occurred in the Salt Fork River drainage; *M. tetranema* in the Medicine Lodge and Salt Fork rivers upstream of Great Salt Plains Reservoir and *M. hyostoma* in the Salt Fork River downstream of the reservoir. The two species co-occurred only immediately downstream of the reservoir. Thus, without knowing of Eisenhour's work, our efforts to reintroduce speckled chub into stream reaches upstream of Great Salt Plains Reservoir resulted in the release of *M. hyostoma* in an area previously occupied only by *M. tetranema*.

II MATERIALS AND METHODS:

In March of 1994, we explored the Salt Fork and Medicine Lodge rivers upstream of Great Salt Plains Reservoir to locate suitable habitat for speckled chub. We chose two sites (Fig. 1), one on the Salt Fork River near Alva, Woods Co., Oklahoma (T27N R13W, R14W S13, S18) and a second on the Medicine Lodge River near Lake City, Barber Co., Kansas (T31S R14W S14). These sites were chosen based on former occurrence of speckled chub, presence of pea-sized gravel substrata required by members of the complex (Luttrell, 1997), and stream accessibility by automobile.

During May 1994, four collections of speckled chub (now known to have been *M. hyostoma*) were made from an unusually dense aggregation immediately downstream of the Kaw Reservoir spillway on the Arkansas River, Kay\Osage Co., Oklahoma (Fig. 1). Following capture with seines, individuals were counted, placed in an aerated 94.6-liter insulated tank, transported to one or the other of the release sites, thermally acclimated to within 2°C of the receiving water temperature by addition of stream water to the hauling-tank, and released. All fish rapidly swam away following release. All releases occurred within four to six hours of initial capture. Thermal acclimation periods ranged from one-half to two hours with temperature changes ≤ 3°C per hour.

About 1340 specimens of *M. hyostoma* were released at the Lake City site on the Medicine Lodge River, 600 on 17 May and 740 on 18 May 1994. The Alva site on the Salt Fork River received about 591 specimens, 400 on 19 May and 191 on 25 May 1994. A sample (OSUS 26784; n = 143) from the capture site on 26 May ranged from 37 to 67 mm in SL ($\bar{x}$ = 48 mm, SD = 3.87) and contained 65 males and 78 females. The fish released at the reintroduction sites were adults or sub-adults that should have spawned in 1994 (Starrett, 1951), and releases were made in mid-May near the start of a May-August breeding season (Cross, 1967). Many of the released females were visibly

gravid, and released eggs with slight pressure to their distended abdomens. Introduction efforts were limited to May 1994 to prevent depletion of speckled chub at the capture site. We were unsuccessful in efforts to collect sufficient numbers of the species, for subsequent introduction attempts, elsewhere in the drainage.

In July and August 1994, we made habitat measurements and attempted to collect speckled chub at the two release sites and, for comparative purposes, at two sites on the lower Salt Fork River (downstream of Great Salt Plains Reservoir) where the speckled chub had not been extirpated. Habitat measurements and attempts to collect the species were made at four additional sites, two on each stream, upstream of Great Salt Plains Reservoir in August 1995 (Fig. 1).

Sampling consisted of 10 downstream seine-hauls (1.8-m by 7.6-m seine with 3.2-mm mesh) covering 1,000 to 1,520 m^2 in, or adjacent to, the main channel within a 1.5- to 2.0-km stream reach. Main channel habitats were distinguished as the cross-sectional stream portion with the highest evident surface velocity; in prairie streams these areas usually have the deepest waters and coarsest substrata (pers. observ.). For each seine-haul, area (m^2) seined was measured, and depth (cm), velocity (cm/s), substratum compaction (cm), and percent composition of seven substratum particle sizes were recorded at five or six points within the area seined. Measurements were made at five points (four corners and center) when seine-haul area was < 150 m^2 and six points (four corners and two near center) when the area was > 150 m^2.

A metered wading-rod (3-cm diameter) was used to measure depth and substratum compaction. The rod was field-calibrated with a Pygmy-Gurley current meter to approximate current velocity at 0.60 depth from the surface. Difference in water height (cm) on the upstream and downstream sides of the rod was converted to velocity in cm/s using the following regression equation ($n = 60$, $r^2 = 0.59$, $P < 0.01$, Standard Error of Estimate = 0.085): Velocity in cm/s = 0.138386 + 0.068903 • difference in water height. Substratum compaction was recorded as the distance the rod penetrated the substratum when a 10-kg force was applied.

Substrata were assessed by scooping into the stream bottom with a wide-mouthed 470-ml jar until it was full, then covering the jar and raising it to the surface and pouring out the contents. Percent abundance of fines (<1 mm), small-sand (1-2 mm), coarse-sand (2-5 mm), and pea-sized gravel (5-9 mm) were then visually estimated (Luttrell, 1997). Abundance of pebble\cobble (9-250 mm), boulder (≥ 250 mm), and bedrock were estimated from six hand-grab samples made within a 0.75-m^2 area centered around the rod at each sample point measurement; i.e., 30 or 36 grab samples per seine-haul. For analytical purposes, each seine-haul was characterized by the mean for each habitat variable.

In addition to collection efforts associated with habitat measurements (six collections at six sites), we made 19 presence-absence collections at 17 sites, 10 in the upper Salt Fork River and seven in the Medicine Lodge River, to document presence-absence of speckled chub (Fig. 1). At each site, six to eight 20- to 30-m seine-hauls (1.8-m by 7.6-m seine with 3.2 mm mesh) were made in the downstream direction, in, or adjacent to, the main channel.

Arcsin (for percentages) and natural log (all other data) transformations improved normality and homoscedasticity of habitat data. To compare habitat differences among streams we used analysis-of-variance (ANOVA; $\alpha = 0.01$) with hierarchically nested effects (i.e., seine-haul within site within stream) to account for within-stream variation in testing among-stream differences (Steel and Torrie, 1980). Multiple comparison of stream means were performed using Tukey's HSD ($\alpha = 0.01$). The objectives of this analysis were to determine if habitat conditions in the Medicine Lodge

and upper Salt Fork rivers differed from those in the lower Salt Fork and Cimarron rivers where speckled chub still occur. Cimarron River habitat data (Luttrell, 1997) were obtained as described for the study area.

Detection of species absence with confidence is often problematic. We used Reed's (1996) equation, $N = [\ln(\alpha \text{ level})] \cdot [\ln(1 - P)]^{-1}$, to determine number of site visits (N) needed to conclude with 95% confidence ($\alpha = 0.05$) that speckled chub were absent upstream of Great Salt Plains Reservoir. We considered the entire study area as one site, and each visit to the area was treated as a site-visit. We assumed that the species was sufficiently rare that species detectability (P) was 0.15; i.e., the species would be collected in 15 out of 100 visits to the study area.

United States Geological Survey daily streamflow data from two stations, one on the Medicine Lodge River and one on the upper Salt Fork River, were analyzed. Flow data from a site on the Medicine Lodge River near Kiowa, Kansas (station number 07149000) and a site on the upper Salt Fork River near Alva, Oklahoma (station number 0714800) were used to evaluate flow conditions surrounding the 1994 reintroduction of speckled chub (Fig. 2).

III. RESULTS:

Sampling efforts at the release sites in August 1994, June 1995, January 1997 and at 15 other sites in 1995, 1996, and 1997 (Fig. 1) failed to produce speckled chub in the area of attempted introduction. Similar efforts at two sites on the lower Salt Fork River (Fig. 1) produced 27 speckled chub at one site and two at the other. If the species had been present at a detectability (P) of 0.15, 19 site-visits (collection attempts) would be needed to conclude with 95% confidence that speckled chub were absent from the study area; 25 site-visits were made in this study, corresponding with a probability of 0.02 that the species was present at a detectability of 0.15 and went undetected as a result of sampling error.

The ANOVA revealed two statistically significant differences ($\alpha = 0.01$) between streams where *M. hyostoma* was present and those where they were absent (Table 1). Mean depth was significantly lower ($P < 0.01$) and pebble\cobble substrates were more abundant ($P < 0.01$) in the Medicine Lodge and upper Salt Fork rivers than in the Cimarron and lower Salt Fork of rivers.

IV. DISCUSSION:

The introduction of *M. hyostoma* upstream of Great Salt Plains Reservoir was either unsuccessful or resulted in a population that was undetectable. The former seems more likely, given the extent of sampling and the expected high reproductive potential of this small cyprinid. One of us (GRL) was present during all visits and have had extensive experience sampling speckled chubs throughout the Arkansas River Basin (Luttrell, 1997). Further, all sampling focused specifically on main-channel habitats most likely to produce the species. Thus, it seems unlikely that the species was present and went completely undetected during 25 separate visits to sites in the study area.

In Iowa and Wisconsin, populations referable to *M. hyostoma* (D. Eisenhour, pers. comm.) apparently reproduce and die within 16 months of hatching (Starrett, 1951; Becker, 1983); Oklahoma populations are probably similar in life history. Thus, the individuals released in 1994, all

of which were ≥ 1 year of age, probably would not have survived into 1995. If they had spawned in the first spring and summer after release, their progeny would have reached reproductive age by June 1995 (Starrett, 1951). If the introduction had been a success, the 1996 population would have included third-generation offspring from the original founders. Little is known about reproductive potential in speckled chub, but, with three years of successful reproduction, there probably would have been a detectable population. In Wisconsin, individual females can contain several hundred eggs (Becker, 1983), and Starrett (1951) observed that a limited population of speckled chub can produce a dense population by the following year, implying high reproductive potential. Further, within one year's time, a recolonizing population of *M. hyostoma* became sufficiently abundant that it was detected at 11 sites over a 191-km stretch of the Cimarron River in Oklahoma (Luttrell, 1997).

In a review of attempts to introduce small, short-lived species into areas of previous occurrence, in the western United States, Hendrickson and Brooks (1991) found that only about 26% of 406 attempts were successful (39% of 49 attempts with cyprinids). Reasons for failure were generally unknown, but marginal habitats and the presence of non-endemic predators and competitors were considered important factors. Effects of non-endemic species seems unlikely as an explanation for failure of our attempted introduction of speckled chub. All species found in the study area coexist with speckled chub elsewhere in the Arkansas River drainage (pers. observ.).

So far as is known, habitat requirements of speckled chub include pea-gravel substrata (Luttrell, 1997) and spring or summer floods for spawning purposes (Bottrell et al., 1964). Our analysis indicated that pea-gravel is no less abundant in the area of attempted introduction than it is in riverine situations that consistently support *M. hyostoma*. Discharge records for the Medicine Lodge and Salt Fork of the Arkansas rivers (Fig. 2) indicate that insufficient flooding in 1994 may explain failure of the introduction of this short-lived species. However, the intensity of flooding needed for successful spawning of speckled chub is unknown.

Sampling points in the streams of attempted introduction were shallower than those in stream reaches that support *M. hyostoma* elsewhere in the Arkansas River drainage. A more detailed analysis (Luttrell, 1997) indicates that speckled chub select different depths depending on the location of preferred substratum, and, as just mentioned, preferred substratum does not seem to be a limiting factor in the area of attempted introduction. *Macrhybopsis hyostoma* was historically absent from upstream areas of the Arkansas River drainage, including the area of attempted introduction (Luttrell, 1997). Instead, the upstream areas once supported *M. tetranema*, a species that may have been better adapted to smaller riverine habitats. Thus, reintroduction of *M. tetranema* might have a greater chance of success.

It appears that there are only two extant populations of *M. tetranema*, one in the Ninnescah and Arkansas rivers of Kansas and the other in the South Canadian River in northeastern New Mexico and the Texas Panhandle (Luttrell, 1997; D. Eisenhour, pers. comm.). Should one or the other of these two populations be lost or further depleted, reintroduction of the species upstream of Great Salt Plains Reservoir could become a high priority goal for conservation of the species.

PART B

Taxonomic Status and Genetic Structure of the Speckled Chub in the Arkansas and Red River Drainages

I. INTRODUCTION:

In this study, we used protein electrophoresis to examine the taxonomy and genetic structure of members of the speckled chub complex (Cyprinidae: cf. *Macrhybopsis aestivalis*) in the Red and Arkansas river basins of Oklahoma, Texas, and Kansas. Until recently, speckled chubs were considered a single, wide-ranging, geographically variable species, *M. aestivalis*. It had been suggested, however, that the species name might represent a complex of species (Miller and Robison, 1973; Page and Burr, 1991). Correspondingly, a recent morphological analysis of the complex recognized five species, including three from the area of concern in this study (Eisenhour, 1997; in press): *M. tetranema* and *M. australis*, which are endemic to, respectively, the Arkansas and Red river basins, and a wide-ranging form, *M. hyostoma*, which occurs in both basins and in other streams from the upper Mississippi River drainage south into the Sabine and Brazos rivers of Texas. The distinguishing characteristics of the three species are given in Appendix C.

In both the Red River and the Arkansas River, the wideranging form, *M. hyostoma*, is morphologically intermediate between the *M. hyostoma* morphotype seen in other basins and the endemic species (Eisenhour, 1997, in press); i.e., *M. tetranema* in the Arkansas and *M. australis* in the Red. This pattern might be explained as a result of genetic introgression resulting from past or ongoing hybridization, or it might represent non-genetic (ecophenotypic) or genetic (ecotypic) morphological convergence in the absence of genetic introgression. Our purpose was to use genetic data to evaluate these hypotheses. Specifically, we asked the following questions: 1) Are there any genetic markers that diagnose the three species? 2) Does the pattern of geographic variation indicate genetic introgression? 3) Is there evidence of genetic isolation in areas of contact between endemic species and *M. hyostoma*? 4) And lastly, do the various species represent separate, monophyletic groups as expected if they have had genetically separate evolutionary histories?

Knowledge of geographic patterns of genetic variation may be important in future management of the various species of the speckled chub complex. For example, *M. tetranema* has disappeared from about 95% of its historic range, and now occupies two widely disjunct areas, the Ninnescah River in Kansas and the South Canadian River in eastern New Mexico and the western panhandle of Texas (Luttrell, 1997; Luttrell et al., submitted). There is some potential for repatriation of the species into areas such as the Cimarron River and the Salt Fork of the Arkansas River (Luttrell et al., submitted), and the choice of which existing population should serve as the source of stock for repatriation is critically dependent upon the pattern of genetic variation.

II. MATERIALS AND METHODS:

We made 31 samples of the speckled chub complex from throughout most of its distribution (Fig. 3, Appendix A). The samples, with collection-site numbers as shown in Figure 3,

included *M. tetranema* from the Ninnescah River (8) and the South Canadian River (15,16) in the Arkansas River Basin, *M. australis* from the upper Red river Basin (18-24), *M. hyostoma* from both the Arkansas (9-14) and Red (17, 23-26) river basins. The analysis also included *M. hyostoma* from widely separated localities outside the Red and Arkansas River basins, as follows: upper Mississippi River drainage (1-7) in Illinois, Indiana, Missouri and Kansas, and the Sabine (29), Angelina (30), and Brazos (28) river systems in Texas. To test the monophyly of the three species in the Arkansas and Red River basins, we included samples of *M. marconis* from the San Marcos River in Texas (site 31) and *M. aestivalis* from the Pecos River in New Mexico (site 27); these, together with *M. tetranema*, *M. australis*, and *M. hyostoma*, represent all five species that Eisenhour (1997) recognized from his morphological analysis of the western members of the speckled chub complex. Finally, we used a sample of *M. gelida* from Missouri (site 32) as the outgroup for the phylogenetic analysis.

The samples (n = 10 to 35) were collected by seining, frozen immediately on dry ice or in liquid nitrogen, transported to the laboratory, and stored at -76 C or lower. For each fish, a sample of epaxial muscle and a mixture if eye and brain were homogenized separately in distilled water, centrifuged (4,000X gravity) for 15 sec, and stored at -76 C prior to protein electrophoresis. Standard methods of horizontal starch-gel electrophoresis (Murphy et al., 1996) were used to examine the products of 22 presumptive gene loci (Table 2). Locus nomenclature follows (Buth, 1983). Alleles were assigned letters alphabetically in order of decreasing anodal mobility.

We used BIOSYS-1 (Swofford and Selander, 1981) to obtain average heterozygosity per individual (H, estimated from allele frequencies for each sample), within-sample polymorphism (P = proportion of loci with >1 allele), tests of conformance to Hardy-Weinberg expectations for genotypic frequencies (exact significance test with Levene's [1949]correction for small sample size), heterogeneity in allele frequencies across samples, and hierarchical analyses of the distribution of genetic diversity. To obtain a visual summary of overall genetic divergence among samples, we used principal components analysis (PCA) of the variance/covariance matrix of arcsine-transformed allele frequencies. The samples of *M. gelida*, *M. marconis*, and *M. aestivalis* and *M. hyostoma* from the Brazos river were excluded from the PCA; they were sufficiently divergent that their inclusion in the analysis resulted in little resolution of pattern for the remainder of the samples.

For phylogenetic analysis, we used the approach for allele frequency parsimony recommended by Berlocher and Swofford (1997). In this analysis, the BIOSYS-1 datafile was converted to the format for FREQPARS (a program for analysis of allele-frequency parsimony; Swofford and Berlocher, 1987) and imported into PAUP (vers. 4d60; written by D. Swofford). PAUP produced a matrix of pairwise Manhattan distances (MANOB metric) and the associated distance-based stepmatrix. This stepmatrix was then subjected to the heuristic search, generalized parsimony algorithm in PAUP, with *M. gelida* as the outgroup. *Macrhybopsis gelida* was chosen as the outgroup because previous analyses indicated that it is either sister to the speckled chub complex (Coburn and Cavender, 1992) or it is one of a pair of species forming the sister clade to the complex (Dimmick, 1993). We saved the 30 shortest trees derived with the simple addition sequence option and used FREQPARS to test each one for allele frequency parsimony, in which tree length is the sum of branch lengths expressed in units of a Manhattan distance metric (MANAD) similar to MANOB, but constrained such that allele frequencies of hypothetical ancestors sum to 1.0. All 32 samples were kept in the analysis of relationships with PAUP.

However, because of limitations imposed by the FREQPARS program, the number of samples was reduced to 20 in the tests of the 30 shortest trees. For these tests, we eliminated the relatively small samples of *M. hyostoma* from the Des Moines River and the Angelina River, and, based on geographic proximity and the results from PAUP, we combined several sets of samples into single samples.

III. RESULTS:

Two or more alleles occurred in 18 of the 22 loci examined (Appendix B). One locus, CBP-B was difficult to score consistently and was eliminated from the analysis. However, this locus is of interest because all samples from the Red River Basin (both *M. hyostoma* and *M. australis*) had, at moderately high frequencies (>0.50) an allele (CBP-B^c) that appeared absent elsewhere. None of the 291 individual chi-square tests indicated significant deviations from Hardy-Weinberg expectation after the Bonferroni correction for a Type I error of 0.05.

Genetic variability was highest in *M. australis* ($H = 0.13$; $P = 0.76$) and *M. hyostoma* ($H = 0.14$; $P = 0.86$) from an area where both species were taken together (sites 23 and 24 combined), suggesting the possibility of genetic mixing. However, high levels of variability also occurred in all other samples of both *M. australis* and *M. hyostoma* from the Red River Basin ($H = 0.11$ to 0.13; $P = 0.33$ to 0.57), and in samples of *M. hyostoma* from drainages south of the Red River--the Sabine, Angelina, and Brazos rivers in Texas ($H = 0.09$ to 0.11; $P = 0.33$ to 0.48). Samples from other areas generally had lower variability. In *M. tetranema*, variability was highest in the samples from the Ninnescah River ($H. = 0.08$; $P = 0.43$) and the South Canadian River in New Mexico ($H = 0.07$; $P = 0.43$) and was somewhat lower in the sample from the South Canadian River in the Texas panhandle ($H = 0.05$; $P = 0.38$). Genetic variability in samples of *M. hyostoma* from the Arkansas River Basin ($H = 0.07$ to 0.08: $P = 0.33$ to 0.48) was similar to that in samples from the upper Mississippi River Basin ($H = 0.07$ to 0.11; $P = 0.33$ to 0.67). Variability was relatively low in *M. aestivalis* from the Pecos River ($H = 0.04$; $P = 0.24$) and *M. marconis* from the San Marcos River ($H = 0.06$; $P = 0.24$) compared with the other members of the speckled chub complex.

Principal components I and II from the PCA analysis of allele frequencies in the reduced set of samples (see Methods and Materials) explained, respectively, 14.9% and 9.9% of the observed variance in allele frequencies among samples. The plot of sample scores on these axes grouped samples from the Red and Arkansas river basins according to basin of occurrence rather than according to species membership (Fig. 4). Thus, *M. hyostoma* from the Red and Arkansas river basins grouped with, respectively, *M. australis* and *M. tetranema*. Correspondingly, the phylogenetic analysis indicated paraphyly for *M. hyostoma*, with the Red and Arkansas river populations appearing more closely related to the endemic species in the two basins than to populations of *M. hyostoma* from other basins (Fig. 5).

Figure 5 supports monophyly for *M. australis* and *M. tetranema*. However, except for the occurrence of one rare allele (GPI-A^5) in *M. tetranema* (frequency = 0.02-0.04 in all samples) and one rare allele (PGM-1^b) in *M. australis* (0.05 in only two samples), there were no synapomorphic alleles supporting the monophyly of these two species, and the indication of monophyly is based only on frequencies of alleles shared by other species.

Overall, there were extremely low levels of genetic divergence among the three species of

the speckled chub complex in the Arkansas and Red river basins. There were no fixed allele differences that could serve to diagnose any one of the three species. As a result, the hierarchical analysis of genetic diversity across all samples of the three species (*M. tetranema*, *M. australis*, and *M. hyostoma* from all localities sampled) indicated that only 7% of the diversity reflects differences among species, whereas 17% was attributable to differences among samples within species. The portion representing differences among species increased by more than twofold (to 15%) when we excluded Arkansas and Red river *M. hyostoma* from the analysis; the portion attributable to differences among samples within species declined to 13%. Considering just the samples of *M. tetranema* and *M. hyostoma* from within the Arkansas River Basin, only 0.4% of the diversity reflected differences between species and 1.3% was attributable to differences among samples within species. The corresponding numbers in a similar analysis of *M. australis* and *M. hyostoma* from the Red River Basin were 1.9% and 0.8%. Thus, within both basins, most of the genetic diversity (>97%) was contained within the average single sample, regardless of species.

After the Bonferroni correction for Type I error, neither *M. tetranema* nor *M. hyostoma* showed significant geographic variation in allele frequencies. Correspondingly the analysis of the distribution of genetic diversity within *M. tetranema* indicated that, on average, 98.9% occurred within a single sample and only 1.1% was attributable to differences among samples; the comparable values for *M. australis* were 99.4 and 0.6%.

To address the question of reproductive isolation between sympatric forms, we performed locus-by-locus tests of Hardy-Weinberg expectations in the combined sample of *M. hyostoma* (n = 57) and *M. australis* (n = 23) from sites 23 and 24, the only sites where *M. hyostoma* was taken together with one of the other species. The results revealed no evidence of the heterozygote deficiencies expected in combined samples of two reproductively isolated species.

In agreement with Eisenhour's (1997) morphological analysis, our results indicate that *M. hyostoma* from the Brazos River in Texas is the most divergent member of its species. The population appears basal to the clade comprising *M. hyostoma*, *M. australis*, and *M. tetranema* (Fig. 5). Also in agreement with Eisenhour's (1997) results, *M. aestivalis* and *M. marconis* appeared phylogenetically distinct from other members of the speckled chub complex (Fig. 5).

VI. DISCUSSION:

The results from allozyme variation are consistent with the hypothesis that genetic introgression explains Eisenhour's (1997) conclusion from morphology that *M. hyostoma* in the Red and Arkansas river basins converges toward the morphotype of, respectively, *M. tetranema* and *M. australis*. Whereas Eisenhour (1997) found greater morphological intergradation in *M. hyostoma* from more upstream areas of the two basins, our results indicate that genetic introgression involving genes encoding allozymes may have occurred throughout the distributions of both species in each basin. This would account for the extremely low levels of genetic divergence among the three species, and it would account for the near absence of observable genetic divergence among samples of the species pairs in both the two river basins.

There were limited opportunities to examine the question of whether *M. hyostoma* is genetically isolated from the other two species. Instances of co-occurrence in our samples occurred only between *M. hyostoma* and *M. australis* at localities 23 and 24. Combining these into a single

sample revealed no evidence of the heterozygote deficiency (Wahlund effect) expected in combined samples of two reproductively isolated species. This suggests that either reproductive isolation is very weak or absent or the two species are so similar in allele frequencies that larger sample sizes would be required to demonstrate the Wahlund effect. Extremely high levels of genetic similarity typical of those seen among samples of the same population have been reported in other instances of morphologically well-defined fish species occurring in sympatry (Humphries, 1984; Allendorf et al., 1987). Thus, we cannot discount the conclusion from morphology that the speckled chub complex in the Red and Arkansas rivers is divisible into three species. Indeed, the phylogenetic analysis of allozyme variation supported, albeit rather weakly, the monophyly of both of the endemic species, *M. tetranema* and *M. australis*, indicating that they may retain remnants of past allozyme divergence from *M. hyostoma*.

One other point bears on the question of how many species are represented by the three morphotypes in the Red and Arkansas basins. Two morphotypes once occurred sympatrically in the Cimarron River, where the *M. tetranema* morphotype was much more common and widespread than the *M. hyostoma* morphotype, which was known only from three collections from downstream areas of the maintstem (Eisenhour, in press). By the 1970s, both forms had been extirpated from the drainage, possibly because of drought (Luttrell, 1997). Subsequently, and despite heavy collecting efforts, neither species was collected from the Cimarron River drainage until 1992 when *M. hyostoma* was taken from a downstream locality. By 1993, *M. hyostoma* had spread about 200 km upstream, but the *M. tetranema* morphotype had not reappeared (Luttrell, 1997). This suggests that the morphotypes are different species and not ecophenotypes of the same population, unless the stream environment has changed to the point where one ecophenotype is not expressed.

The tendency for Red and Arkansas river populations of *M. hyostoma* to cluster with, respectively, *M. australis* and *M. tetranema*, is explainable as a result of evolution in geographic isolation followed by contact and genetic introgression. Eisenhour's (1997) phylogenetic analysis of morphology indicated a sister relationship between *M. tetranema* and *M. australis*. He suggested that they evolved from a common ancestor in a south-flowing stream in western Kansas and Oklahoma that may or may not have been part of Metcalf's (1966) Ancestral Plains Stream, which, by early Pleistocene extended southward from the Dakotas and may have emptied into the Gulf of Mexico independently of the Mississippi River (Cross et al. 1986). Divergence of *M. tetranema* and *M. australis* might have begun during Mid-Pleistocene when the headward eroding Arkansas River breached the Ozark-Ouachita Highlands area and captured a large part of the Ancestral Plains Stream, forming the upper Arkansas River Basin (Eisenhour, 1997). Contact and resultant introgressive hybridization with *M. hyostoma* presumably occurred as a result of dispersal of that species from elsewhere in the Ancestral Plains Stream system, and might have occurred either before or after the geologic event separating *M. tetranema* and *M. australis* (Eisenhour, 1997).

In conclusion, the three species of the speckled chub complex in the Red and Arkansas river basins are remarkably similar in genetic composition as indicated by allozymes. This does not, however, refute Eisenhour's (1997) taxonomy, which recognizes *M. tetranema* from the Arkansas, *M. australis* from the Red, and *M. hyostoma* in both. The morphological distinctiveness of the three forms has remained intact despite evidence of considerable genetic introgression in the past. The phylogenetic analysis of allozymes provides some support for the conclusion from morphology that the three forms are separate historical entities that exchanged genetic material after secondary contact between divergent forms.

VII. ACKNOWLEDGMENTS:

A. F. Echelle and M. Jones provided extensive help in the field and the laboratory, and R. Larson provided the sample of *M. aestivalis*, and D. Eisenhour provided most other samples from outside the Red and Arkansas river basins.

RECOMMENDATIONS (PARTS A AND B)

1. *Repatriation of* M. tetranema *in the Salt Fork of the Arkansas upstream of Great Salt Plains Reservoir.* The present, highly restricted and disjunct nature of the distribution of *M. tetranema* renders the species particularly vulnerable to extinction. The population in the South Canadian River probably should serve as the source of stock for repatriation of the species because there is morphological evidence (Eisenhour, 1997; in press) that the Ninnescah population may be more affected by genetic introgression from *M. hyostoma.*

2. *A survey of the status of the Red River endemic,* Macrhybopsis aestivalis. This species still seems common in most of its historical range, but this needs better documentation.

3. *Further genetic analyses of the question of reproductive isolation between* M. australis *and* M. hyostoma *in the Red River.* This test is not available for the *M. hyostoma-M. tetranema* pair because the remaining populations in the Arkansas River Basin are not in sympatry. Such an analysis would include both Hardy-Weinberg tests and tests of linkage disequilibrium for rapidly evolving markers such as microsatellites. Microsatellites are another form of nuclear marker that evolves much more rapidly than allozymes, thus providing more allele variation. In addition, because microsatellites do not encode proteins, they are free of balancing selection, a factor that might explain the high levels of allele-frequency similarity for allozymes among the various forms in the harsh, plains stream environment they occupy.

4. *A mitochondrial DNA survey of the speckled chub complex.* This would provide unique perspective on the hypothesis of introgression following secondary contact in the Red and Arkansas river basins. The prediction is that populations in each of the two basins will each have a divergent mtDNA lineage and that these will be absent from *M. hyostoma* outside these basins. Further, because of its maternal, essentially haploid mode of inheritance, mtDNA can show striking discordance with nuclear genes with respect to geographic/taxonomic distribution (Avise, 1994). Thus, mtDNAs in the Red and Arkansas river basins may sort more closely with morphology than do the nuclear genes assayed by allozymes. Alternatively, the haplotypes unique to the Red and Arkansas river basins might occur as a polymorphism with the lineage characteristic of *M. hyostoma* outside the basin. This would indicate that, as appears to be the case with allozymes, all three forms in the Red and Arkansas river basins are genetically introgressed.

5. *Surveys of both* M. tetranema *and* M. australis *with the above mentioned markers* (either

microsatelllites or mtDNA). Because of their more rapidly evolving nature relative to allozymes, microsatellite DNA and mtDNA would allow a more sensitive assessment geographic variation. Such information might be valuable in future management of the species; e.g., in choosing stocks for maintenance in captivity or for use in repatriation efforts.

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LITERATURE CITED (PARTS A AND B)

Allendorf, F., N. Ryman, and F. Utter. 1987. Genetics and fishery management: Past, present, and future, p. 1-20. *In*: N. Ryman and F. Utter (eds.). Population genetics and fishery management. Univ. of Washington Press, Seattle.

Avise, J. C. 1994. Molecular markers, natural history, and evolution. Chapman and Hall, NY.

Becker, G. C. 1983. Fishes of Wisconsin. Univ. of Wisconsin Press, Madison.

Berlocher, S. H., and D. L. Swofford. 1997. Searching for phylogenetic trees under the frequency parsimony criterion: an approximation using generalized parsimony. Syst. Biol. 46:211-215.

Booth, W. 1988. Reintroducing a political animal. Science 241:154-158.

Bottrell, C. E., R. H. Ingersol, and R. W. Jones. 1964. Notes on the embryology, early development, and behavior of *Hybopsis aestivalis tetranemus* (Gilbert). Trans. Am. Micros. Soc. 83:391-399.

Buth, D. G. 1983. Duplicate isozyme loci in fishes: Origins, distribution, phyletic consequences and locus nomenclature, p. 381-400. *In*: M. C. Rattazzi, J. F. Scandalios, and G. S. Whitt (eds.). Current topics in biological and medical research. Vol. 10. A. R. Liss, NY.

Conway, W. 1988. Editorial. Conserv. Biol. 2:132-134.

Cross, F. G. 1967. Handbook of fishes of Kansas. Mus. Nat. Hist., Univ. of Kansas Misc. Publ. 45:1-357.

------, R. L. Mayden, and J. D. Stewart. 1986. Fishes in the western Mississippi basin (Missouri, Arkansas, and Red rivers), p. 363-412. *In*: C. H. Hocutt and E. O. Wiley (eds.). The zoogeography of North American freshwater fishes. Wiley-Interscience, NY.

Dimmick, W. W. 1993. A molecular perspective on the phylogenetic relationships of the barbeled minnows, historically assigned to the genus *Hybopsis* (Cyprinidae: Cypriniformes). Mol. Phyl. Evol. 2:173-184.

Eisenhour, D. J. 1997. Systematics, variation, and speciation of the *Macrhybopsis aestivalis* complex (Cypriniformes: Cyprinidae) west of the Mississippi River. Unpubl. Ph.D. diss., Southern Illinois University, Carbondale.

------. In press. Systematics of *Macrhybopsis tetranema* (Cypriniformes: Cyprinidae). Copeia.

Griffith, B., J. M. Scott, J. W. Carpenter, and C. Reed. 1989. Translocation as a species conservation tool: Status and strategy. Science 245:477-480.

Hendrickson, D. A., and J. E. Brooks. 1991. Transplanting short-lived fishes in North American deserts: Review, assessment, and recommendations, p. 283-298. *In*: W. L. Minckley and J. E. Deacon (eds.). Battle against extinction: Native fish management in the American west. Univ. of Arizona Press, Tucson.

Humphries, J. M. 1984. Genetics of speciation in pupfishes from Laguna Chichancanab, Mexico, p. 129-140. *In*: A. A. Echelle and I. Kornfield (eds.), Evolution of fish species flocks. Univ. of Main Press, Orono.

Luttrell, G. R. 1997. Conservation biology of the speckled chub complex (Cyprinidae: cf. *Macrhybopsis aestivalis*) in the Arkansas River Basin. Unpubl. Ph.D. diss., Oklahoma State University, Stillwater.

------, A. A. Echelle, W. L. Fisher, and D. J. Eisenhour. Submitted. Declining status of two species of the *Macrhybopsis aestivalis* complex (Teleostei: Cyprinidae) in the Arkansas River

Basin and related effects of reservoirs as barriers to dispersal. Copeia (accepted pending revision).
Levene, H. 1949. On a matching problem arising in genetics. Ann. Math. Stat. 20:21-94.
Metcalf, A. L. 1966. Fishes of the Kansas River system in relation to zoogeography of the Great Plains. University of Kansas Publications of the Museum of Natural History 17:23-189.
Miller, R. J., and H. W. Robison. 1973. The fishes of Oklahoma. Oklahoma State University Press, Stillwater.
Murphy, R. W., J. Sites, D. G. Buth and C. H. Haufler. 1996. Proteins I: isozyme electrophoresis, p. 62-120. *In*: D. M. Hillis, C. Moritz and B. K. Mable (eds.). Molecular systematics, 2nd ed. Sinauer, Sunderland, MA.
Page, L. M., and B. M. Burr. 1991. A field guide to freshwater fishes. Houghton Mifflin, Boston.
Reed, M. J. 1996. Using statistical probability to increase confidence of inferring species extinction. Conserv. Biol. 10:1283-1285.
Starrett, W. C. 1951. Some factors affecting the abundance of minnows in the Des Moines River, Iowa. Ecology 32:13-24.
Steel, R. G. D., and J. H. Torrie. 1980. Principles and procedures of statistics: a biometrical approach. 2nd ed. McGraw-Hill, NY.
Swofford, D. L., and S. H. Berlocher. 1987. Inferring evolutionary trees from gene frequency data under the principle of maximum parsimony. Syst. Zool. 36:293-325.
------, and R. B. Selander. 1981. BIOSYS-1: a FORTRAN program for comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. 72:281-281.
Wikramanayake, E. D. 1990. Conservation of endemic rain forest fishes of Sri Lanka: Results of a translocation experiment. Conserv. Biol. 4:32-37.
Williams, J. E., and 12 co-authors. 1988. American Fisheries Society guidelines for introduction of threatened and endangered fishes. Fisheries 13:5-11.
Winston, M. R., C. M. Taylor, and J. Pigg. 1991. Upstream extirpation of four minnow species due to damming of a prairie stream. Trans. Am. Fish. Soc. 120:98-105.
Page, L. M., and B. M. Burr. 1991. A field guide to freshwater fishes. Houghton Mifflin, Boston.Reed, M. J. 1996. Using statistical probability to increase confidence of inferring species extinction. Conserv. Biol. 10:1283-1285.
Starrett, W. C. 1951. Some factors affecting the abundance of minnows in the Des Moines River, Iowa. Ecology 32:13-24.
Steel, R. G. D., and J. H. Torrie. 1980. Principles and procedures of statistics: a biometrical approach. 2nd ed. McGraw-Hill, NY.
Swofford, D. L., and S. H. Berlocher. 1987. Inferring evolutionary trees from gene frequency data under the principle of maximum parsimony. Syst. Zool. 36:293-325.
------, and R. B. Selander. 1981. BIOSYS-1: a FORTRAN program for comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. 72:281-281.
Wikramanayake, E. D. 1990. Conservation of endemic rain forest fishes of Sri Lanka: Results of a translocation experiment. Conserv. Biol. 4:32-37.
Williams, J. E., and 12 co-authors. 1988. American Fisheries Society guidelines for introduction of threatened and endangered fishes. Fisheries 13:5-11.
Winston, M. R., C. M. Taylor, and J. Pigg. 1991. Upstream extirpation of four minnow species due to damming of a prairie stream. Trans. Am. Fish. Soc. 120:98-105.

TABLE 1. Back-transformed means and standard deviations of habitat variables for the upper and lower Salt Fork of the Arkansas (= Salt Fork), Cimarron, and Medicine Lodge rivers. Means with the same letters were not significantly different (Tukey's HSD; $\alpha = 0.01$); *ns* = not significant (ANOVA; $P > 0.01$); n = number of seine-hauls.

	Chubs absent				Chubs present					
	Medicine Lodge River ($n = 30$)		Upper Salt Fork ($n = 30$)		Lower Salt Fork ($n = 20$)		Cimarron River ($n = 70$)			
Variables	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	*F*	*P*
Mean Depth (cm)	15.24[a]	5.24	14.96[a]	12.25	29.31[b]	14.47	35.12[b]	20.33	34.71	<0.001
Velocity (cm/s)	30.83	4.78	24.08	7.76	22.81	9.22	27.23	11.73	1.56	ns
Compaction (cm)	3.56[c]	0.49	3.25[c]	0.68	2.25	1.13	4.22[c]	2.42	34.73	<0.001
Fines (%)	0.00	0.00	9.67[d]	6.69	12.75[d]	10.94	6.64[d]	11.12	8.21	<0.001
Small-sand (%)	21.83[e]	10.38	28.77[e]	13.24	32.25[ef]	36.22	48.14[f]	27.74	16.64	<0.001
Coarse-sand (%)	52.00	20.07	32.23[g]	12.34	30.75[g]	31.80	23.79[g]	19.70	11.73	<0.001
Pea-sized gravel(%)	20.50	14.88	25.00	16.97	19.25	26.37	19.57	22.82	2.12	ns
Pebble\cobble (%)	5.67[h]	8.98	4.33[h]	6.26	0.50[i]	2.24	1.29[i]	3.37	8.94	<0.001
Boulder (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.57	4.78	1.19	ns
Bedrock (%)	0.00	0.00	0.00	0.00	4.50	14.68	0.00	0.00	3.12	ns

Table 2. Protein designations, presumptive loci, tissues and buffer systems used to assay genetic variation in the speckled chub complex. Locus abbreviations follow Buth (1983); protein names and numbers follow IUBMBNC (1992).

Protein (EC number)	Locus	Tissue	Analytical system [1]
Adenylate Kinase (EC 2.7.4.3)	AK	Muscle	TC-III
Creatine Kinase (EC 2.7.3.2)	CK-1	Eye-Brain	TC-III
	CK-2	Muscle	TC-III
Glyceraldehyde-3-phosphate	GAPDH-1	Eye-Brain	TC-III
dehydrogenase (EC 1.2.1.12)	GAPDH-2	Muscle	TC-III
Glucose-6-phosphate isomerase	GPI-A	Muscle	TC-6
(EC 5.3.1.9)	GPI-B	Muscle	LiOH.TC-6
Isocitrate dehydorginase	m-IDH-A	Eye-Brain	TC-8
(EC 1.1.1.42)	s-IDH-A	Eye-Brain	TC-8
L-Lactate dehydrogenase	LDH-A	Eye-Brain	T-EDTA
(EC 1.1.1.27)	LDH-B	Eye-Brain	T-EDTA
Malate dehydrogenase (EC 1.1.1.37)	s-MDH-A	Muscle	TC-8
	s-MDH-B	Muscle	TC-8
	m-MDH-A	Muscle	TC-8
Malate dehydrogenase (EC 1.1.1.40)	m-MDHP-A	Eye-Brain	TC-8
Manose-6-phosphate isomerase	MPI-A	Muscle	T-EDTA
(EC 5.3.1.8)			
Peptidase-A (EC 3.4.-.-)	PEP-A	Muscle	TC-8
Peptidase-B (EC 3.4.-.-)	PEP-B	Eye-Brain	T-EDTA
Phosphogluconate	PGD-A	Muscle	TC-III
dehydrogenase (EC 1.1.1.44)			
Phosphoglucomutase (EC 2.7.5.1)	PGM-A	Muscle	TC-8

[1] Analytical systems are as follows: TC-III: Stock solution 0.75 M Tris-Hydromethlaminomethane (= "Tris"), 0.25 M citric acid, pH 7.0; anodal electrode buffer:1 volume stock, 6 volumes water; cathodal electrode buffer solution: 1 volume stock, 4 volumes water; gel buffer: 1 volume stock, 19 volumes water. TC-6: Electrode buffer and stock solutions: 0.223 M Tris, 0.86 M citric acid, pH 6.0: gel buffer: 1 volume stock, 28 volumes water. LiOH: Stock solution A: 0.19 M boric acid,0.03 lithium hydroxide, pH8.1. Stock solution B: 0.05 M Tris, 0.008 M citric acid, pH 8.4. Electrode solution: undiluted stock solution A: gel buffer: 1 volume stock solution A, 9 volumes stock solution B, pH 8.3. TC-8: Electrode buffer and stock solution: 0.69 M Tris, 0.16 M citric acid, pH 8.0; gel buffer : 1 volume stock, 28 volumes water. TEDTA: Stock solution: 0.90 M Tris, 0.50 M boric acid, 0.1 M disodium EDTA, pH 8.6; electrode solution: 1 volume stock, 6.9 volumes water; gel buffer: 1 volume stock, 24 volumes water. All pH adjustments made with 10 N NaOH.

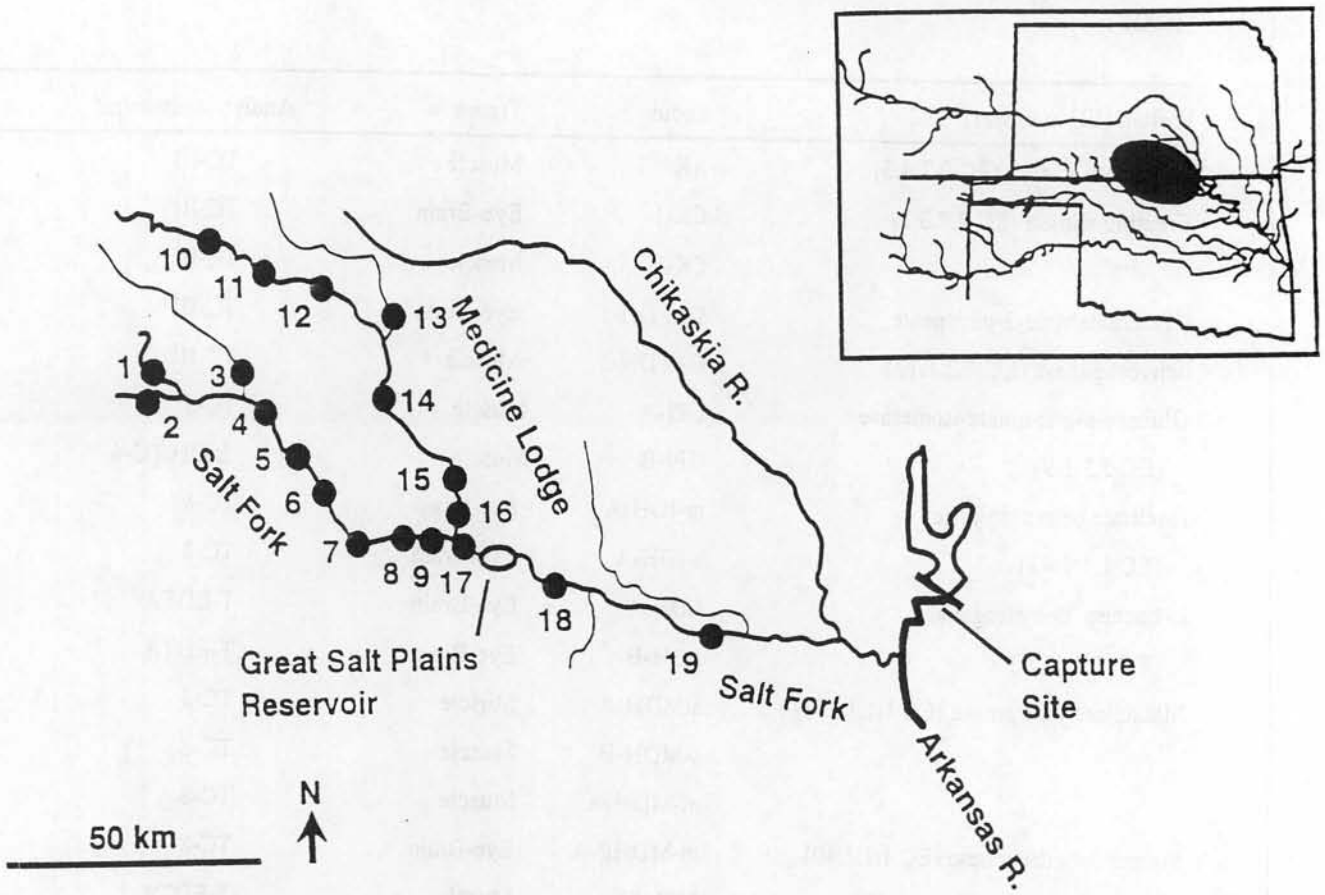

Figure 1. Map showing speckled chub capture site and release sites (7 and 12) and other study sites in the area. Habitat measurements were made at sites 7, 8, 9, 11, 12, 14, 18, and 19. Post-release seine-collections were made at sites 1-17 from 1994 through 1997. Specific site locality data are given in Appendix D. Inset shows approximate geographic location of study area.

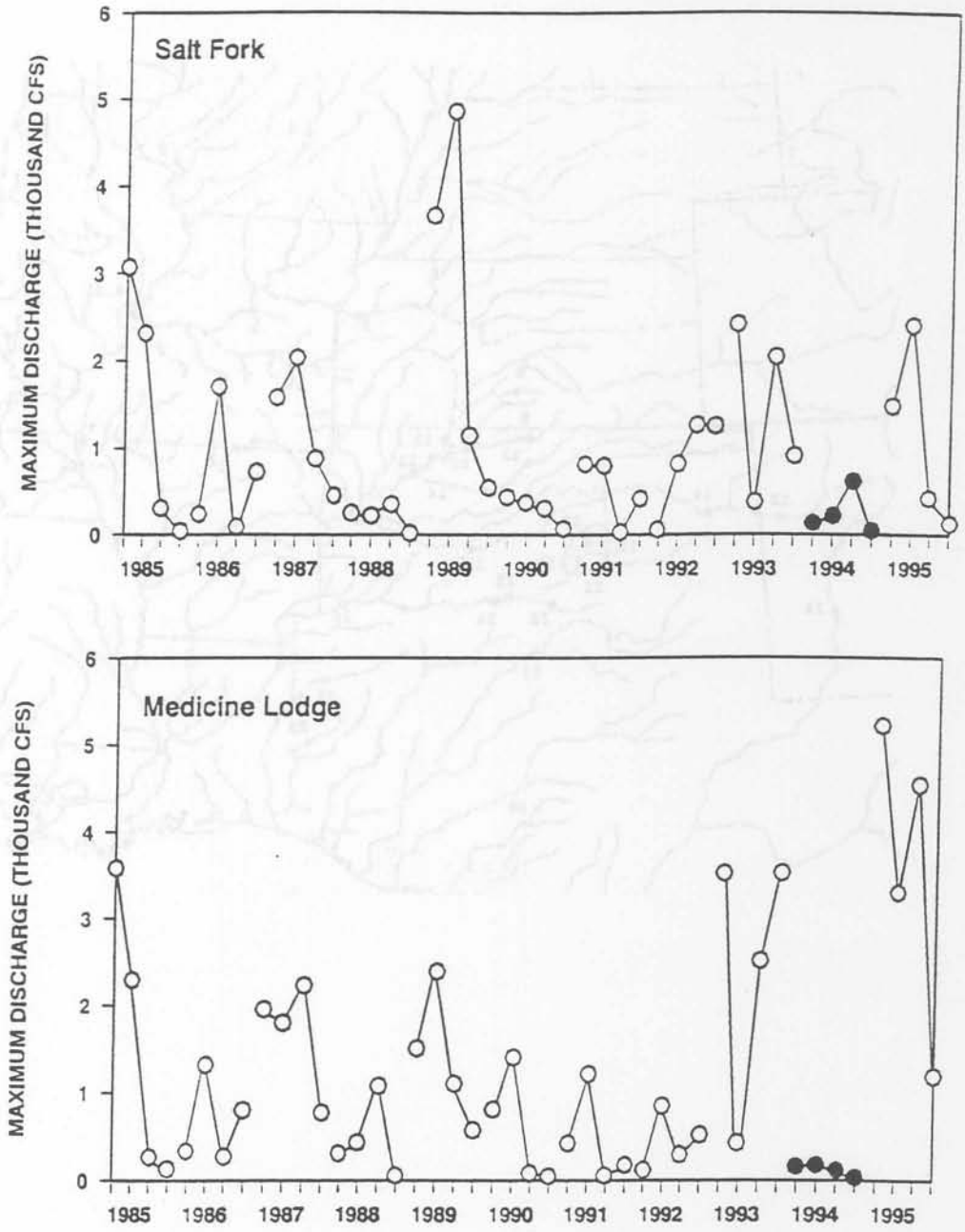

Figure 2. Maximum daily discharges for the Salt Fork of the Arkansas River near Alva, Oklahoma (upper pane) and the Medicine Lodge River near Kiowa, Kansas (lower pane) in May-August of 1985-1995. Closed circles indicate flows during 1994.

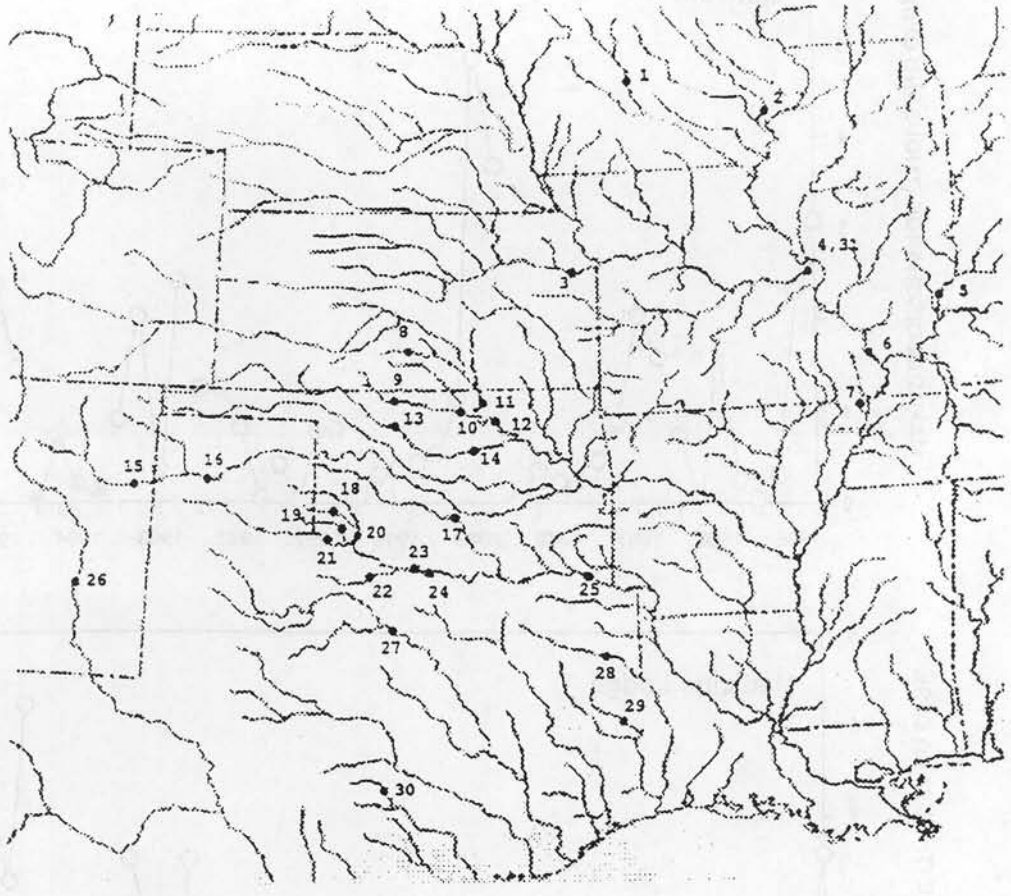

Figure 3. Sample sites for collections used in the genetic analysis of the speckled chub complex. Collection numbers correspond with those in Appendix A.

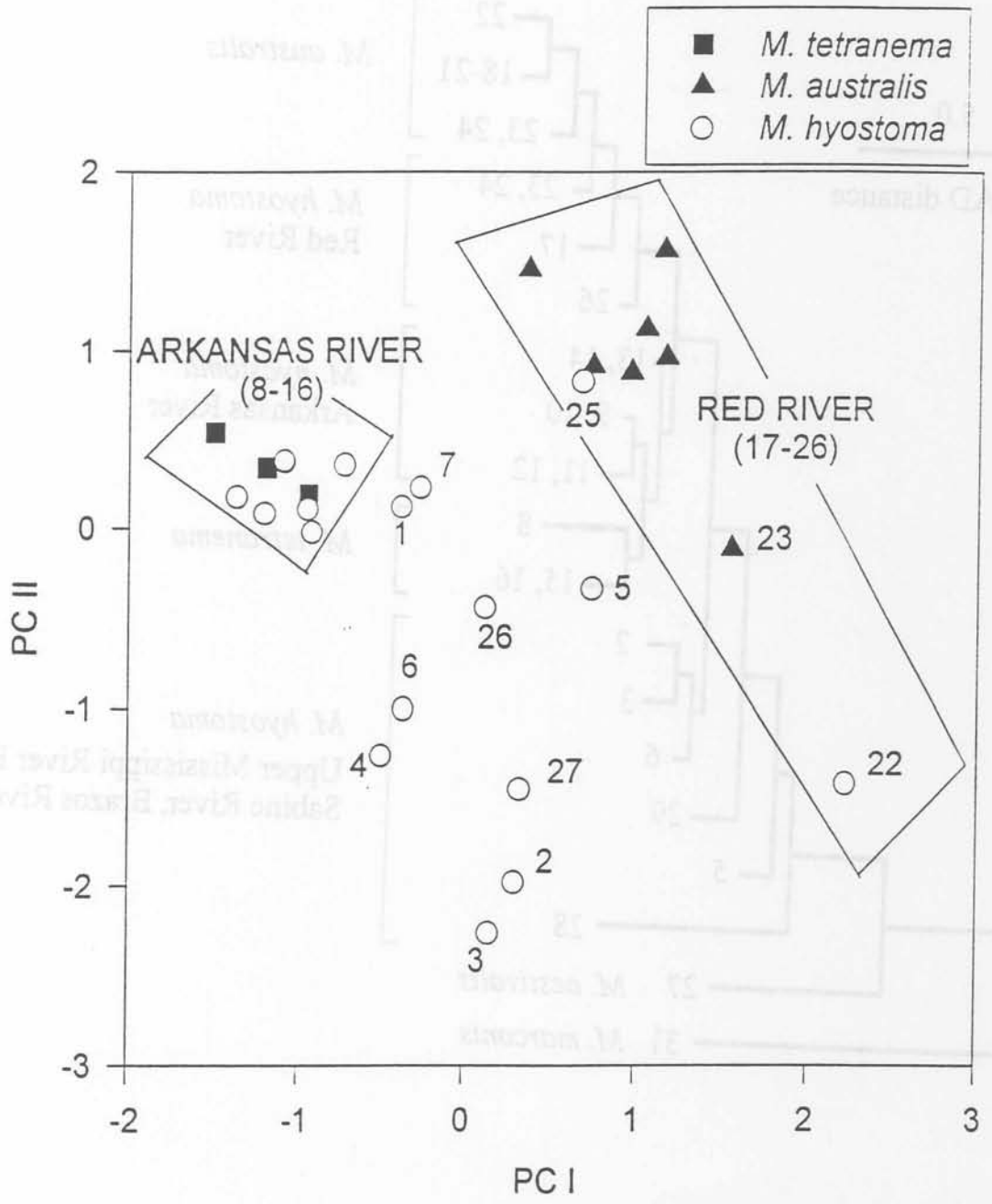

Figure 4. Plot of sample scores on the first two principle components from the PCA of arcsine-transformed allele frequencies in speckled chubs. Sample numbers correspond with those in Figure 3 and Appendix A.

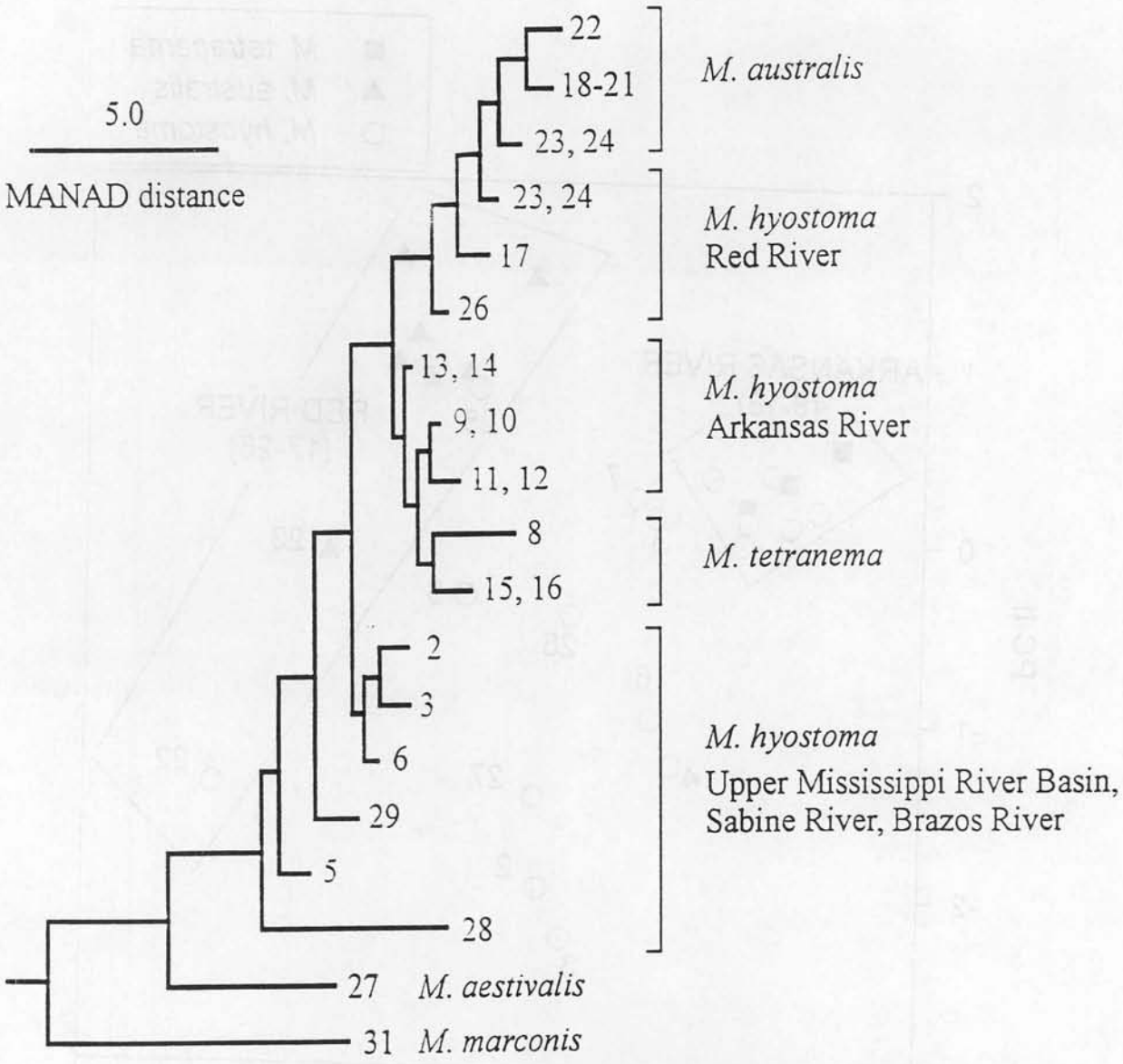

Figure 5. The shortest FREQPARS tree for members of the speckled chub complex. Numbers correspond with sample numbers in Figure 3 and Appendix A. The outgroup was *M. gelida*.

Appendix A

Locality data and dates collections were made for samples used in the genetic analysis. Sample numbers correspond to site numbers in Figure 3.

Sample	State	County, Locality, and Collection Date
1	IA	Boone Co.,Des Moines River at lowhead dam, 0.5 miles west of Fraser. 30 June 1996.
2	IA	Muscatine Co., Cedar River at Iowa state highway 22 bridge. 30 July 1996.
3	KS	Douglas Co., Kansas River in Lawrence at lowhead dam. 6 October 1995.
4	MO	St. Charles Co., Missouri River at upstream end of Cora Island. 26 May 1997.
5	IL	White Co., Wabash River at downstream end of Mink Island, 5 mi. N of Maunie. 30 Oct. 1995.
6	MO	Scott Co., Mississippi River at Gray's Point, 3 km N of Thebes. 7 June 1997.
7	MO	Mew Madrid Co., Ditch #290 at highway "B" crossing 3 mi. S of Tallapoosa. 10 June 1995.
8	KS	Kingman Co., Ninnescah River at Kingman city park. 26 October 1995.
9	OK	Grant Co., Salt Fork of the Arkansas River N of Salt Fork at highway 74 bridge. 16 Oct. 1995.
10	OK	Kay Co., Salt Fork of the Arkansas River at the mouth of the Chikaskia River.
11	OK	Osage Co., Arkansas River below Kaw Dam. 12 May 1998.
12	OK	Osage Co., Arkansas River below highway 20 bridge, at Ralston. 15 October1997.
13	OK	Major Co., Cimarron River 6.4 km W and 3.2 km S of Ames. 17 October 1995.
14	OK	Logan Co., Cimarron River at highway 77 bridge N of Guthrie.
15	NM	Quay Co., South Canadian River 5.3 mi. E of Logan. 1 September 1996.
16	TX	Oldham Co., South Canadian River at highway 385 bridge S of Boy's Ranch.1-2 September 1996.
17	OK	Garvin Co., Washita River at highway 29 bridge. 27 June 1998.
18	OK	Greer Co., Elm Fork of the Red River at highway 34 bridge. 14 July 1997.
19	OK	Greer Co., Salt Fork of the Red River at highway 34 bridge. 14 July 1997.
20	OK	Jackson Co., North Fork of the Red River at highway 62 bridge. 2 August1997.
21	OK	Jackson Co., Prairie Dog Town Fork of the Red River at highway 6 bridge, SW of El Dorado. 2 August 1997.
22	TX	Knox Co., South Fork of the Wichita River, 4 mi. N of vera. 15 June 1998.
23	OK	Jefferson Co., Red River at TX 79 crossing, 7 mi. NE of Byers. 29 June 1996.
24	OK	Jefferson Co., Red River at highway 81 bridge, S of Terrell. 27 June 1998.
25	OK	McCurtain Co., Red River at US 259 bridge. 30 June 1996.
26	NM	Chaves Co., Pecos River at Sallie Ranch. T11s, R25E, S36. 28 October 1997.
27	TX	Young Co., Brazos River at TX 7 bridge, 8 mi. S of Graham. 29 June 1996
28	TX	Panola Co., Sabine River at Watt Shoals on unnamed road opposite county road 291, 10 mi NE Carthage, 26 June 1996.
29	TX	Nacogdoches Co., Angelina River at TX 7 bridge. 26 June 1996.
30	TX	Caldwell Co., San Marcos River at US highway 90 bridge, 2.0 mi. SW of Luling. 27 June 1996.
31	MO	St. Charles Co., Missouri River at upstream end of Cora Island RM 4.5. 26 May 1997.

Appendix B. Table of allele frequencies. polymorphism (*P*). and heterozygosities (*H*). Population numbers correspond to those in Figure 3. N = sample size for each locus.

	Population								
Locus	1	2	3	4	5	6	7	8	9
AK-1									
(N)	12	21	18	20	53	19	20	21	25
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	1.000	.976	1.000	.975	1.000	.974	1.000	1.000	.980
C	.000	.024	.000	.025	.000	.026	.000	.000	.020
CBP-1									
(N)	12	21	18	20	53	19	20	21	25
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CK-1									
(N)	12	21	18	20	53	19	20	21	25
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	1.000	1.000	1.000	1.000	.991	1.000	1.000	.952	1.000
C	.000	.000	.000	.000	.009	.000	.000	.048	.000
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
CK-2									
(N)	12	21	18	20	53	19	20	21	25
A	.000	.024	.000	.000	.009	.000	.000	.000	.000
B	1.000	.976	1.000	1.000	.991	1.000	1.000	1.000	1.000
C	.000	.000	.000	.000	.000	.000	.000	.000	.000
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
GAP-1									
(N)	12	21	18	20	53	19	20	21	25
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GAP-2									
(N)	12	21	18	20	53	19	20	21	25
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GPI-1									
(N)	12	21	18	20	53	19	20	21	25
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.048	.056	.075	.047	.053	.000	.000	.020
C	.000	.000	.000	.025	.000	.000	.000	.000	.000
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
E	1.000	.857	.917	.850	.953	.921	.975	.952	.960
F	.000	.000	.000	.000	.000	.000	.000	.000	.000
G	.000	.000	.000	.000	.000	.000	.000	.048	.000
H	.000	.095	.028	.025	.000	.026	.025	.000	.000
I	.000	.000	.000	.025	.000	.000	.000	.000	.020

	Population								
Locus	1	2	3	4	5	6	7	8	9
GPI-2									
(N)	12	21	18	20	53	19	20	21	26
A	.000	.000	.000	.000	.009	.000	.000	.095	.212
B	.000	.000	.000	.000	.000	.000	.000	.000	.019
C	.042	.119	.250	.125	.047	.132	.025	.024	.019
D	.792	.738	.667	.875	.840	.684	.825	.786	.712
E	.125	.143	.083	.000	.075	.158	.125	.048	.038
F	.000	.000	.000	.000	.000	.000	.000	.048	.000
G	.042	.000	.000	.000	.028	.026	.025	.000	.000
IDH-1									
(N)	12	21	18	20	53	19	20	21	25
A	.000	.000	.028	.000	.028	.000	.000	.024	.000
B	1.000	.976	.972	1.000	.972	1.000	1.000	.976	.980
C	.000	.000	.000	.000	.000	.000	.000	.000	.000
D	.000	.024	.000	.000	.000	.000	.000	.000	.000
E	.000	.000	.000	.000	.000	.000	.000	.000	.020
IDH-2									
(N)	12	21	18	20	53	19	20	21	25
A	.000	.000	.028	.000	.000	.000	.000	.000	.000
B	1.000	1.000	.972	1.000	1.000	1.000	1.000	1.000	1.000
LDH-1									
(N)	12	21	18	20	53	19	20	21	25
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.000	.000	.000	.000	.000
C	1.000	1.000	1.000	1.000	.981	1.000	1.000	1.000	1.000
D	.000	.000	.000	.000	.019	.000	.000	.000	.000
LDH-2									
(N)	12	21	18	20	53	19	20	21	25
A	.000	.024	.056	.025	.000	.026	.000	.000	.000
B	.000	.000	.000	.000	.000	.000	.000	.000	.000
C	.583	.643	.611	.700	.877	.711	.575	.143	.180
D	.417	.333	.333	.275	.123	.263	.425	.857	.820
E	.000	.000	.000	.000	.000	.000	.000	.000	.000
MDH-1									
(N)	12	21	18	20	52	19	20	21	25
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	1.000	.976	1.000	1.000	.846	1.000	.975	1.000	1.000
C	.000	.024	.000	.000	.154	.000	.000	.000	.000
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
E	.000	.000	.000	.000	.000	.000	.025	.000	.000
MDH-2									
(N)	12	21	18	20	53	19	20	21	25
A	.000	.000	.028	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.000	.000	.000	.000	.000
C	1.000	1.000	.972	1.000	1.000	1.000	1.000	1.000	1.000
D	.000	.000	.000	.000	.000	.000	.000	.000	.000

	Population								
Locus	1	2	3	4	5	6	7	8	9
MDH-3									
(N)	12	21	18	20	53	19	20	21	25
A	.000	.214	.028	.025	.000	.026	.000	.000	.000
B	1.000	.786	.972	.875	1.000	.921	1.000	.952	1.000
C	.000	.000	.000	.000	.000	.000	.000	.024	.000
D	.000	.000	.000	.075	.000	.053	.000	.024	.000
E	.000	.000	.000	.025	.000	.000	.000	.000	.000
F	.000	.000	.000	.000	.000	.000	.000	.000	.000
ME-1									
(N)	12	21	18	20	53	19	20	21	25
A	.000	.000	.000	.000	.009	.026	.000	.000	.000
B	1.000	1.000	.972	1.000	.991	.974	1.000	1.000	1.000
C	.000	.000	.028	.000	.000	.000	.000	.000	.000
MPI-1									
(N)	12	21	18	12	53	19	20	21	25
A	.000	.000	.000	.000	.009	.026	.000	.048	.000
B	1.000	1.000	1.000	1.000	.981	.974	1.000	.952	1.000
C	.000	.000	.000	.000	.009	.000	.000	.000	.000
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
E	.000	.000	.000	.000	.000	.000	.000	.000	.000
PEP-1									
(N)	11	21	18	20	53	19	20	21	25
A	.000	.119	.000	.025	.028	.000	.000	.000	.000
B	.636	.429	.306	.250	.726	.395	.525	.119	.200
C	.364	.452	.694	.725	.245	.579	.475	.738	.720
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
E	.000	.000	.000	.000	.000	.026	.000	.143	.060
F	.000	.000	.000	.000	.000	.000	.000	.000	.020
PEP-2									
(N)	12	21	18	20	53	19	20	21	25
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	1.000	1.000	1.000	1.000	.981	1.000	1.000	1.000	1.000
C	.000	.000	.000	.000	.019	.000	.000	.000	.000
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
PGM-1									
(N)	12	21	18	20	53	19	20	21	25
A	.000	.000	.000	.000	.009	.000	.000	.000	.020
B	.000	.000	.000	.000	.000	.000	.000	.000	.000
C	1.000	1.000	1.000	1.000	.972	1.000	.950	.881	.960
D	.000	.000	.000	.000	.019	.000	.050	.119	.020
E	.000	.000	.000	.000	.000	.000	.000	.000	.000

Locus	Population 1	2	3	4	5	6	7	8	9
PGDH									
(N)	12	21	18	20	49	19	20	21	25
A	.000	.000	.000	.000	.020	.000	.000	.000	.000
B	.000	.000	.000	.000	.061	.000	.000	.000	.000
C	.000	.000	.000	.000	.020	.000	.000	.000	.000
D	1.000	1.000	1.000	.950	.857	1.000	.975	.857	.940
E	.000	.000	.000	.025	.010	.000	.025	.000	.040
F	.000	.000	.000	.000	.000	.000	.000	.000	.000
G	.000	.000	.000	.025	.031	.000	.000	.119	.000
H	.000	.000	.000	.000	.000	.000	.000	.024	.020
P	.143		.429 .381	.429	.333	.667	.381	.333	.476
H	.065	.111	.091	.083	.087	.092	.075	.093	.074

	Population								
Locus	10	11	12	13	14	15	16	17	18
AK-1									
(N)	25	10	19	25	25	25	25	20	21
A	.020	.000	.000	.020	.020	.020	.040	.000	.000
B	.960	1.000	1.000	.960	.980	.980	.960	1.000	1.000
C	.020	.000	.000	.020	.000	.000	.000	.000	.000
CBP-1									
(N)	25	10	19	25	25	25	25	20	21
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CK-1									
(N)	24	10	19	25	25	25	24	20	21
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	1.000	.850	1.000	1.000	1.000	1.000	1.000	.975	1.000
C	.000	.100	.000	.000	.000	.000	.000	.025	.000
D	.000	.050	.000	.000	.000	.000	.000	.000	.000
CK-2									
(N)	25	10	19	25	25	25	25	20	21
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
C	.000	.000	.000	.000	.000	.000	.000	.000	.000
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
GAP-1									
(N)	25	10	19	25	25	25	25	20	21
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GAP-2									
(N)	25	10	19	25	25	25	25	20	21
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GPI-1									
(N)	25	10	19	25	25	25	25	20	21
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.040	.000	.053	.000	.100	.020	.000	.100	.024
C	.000	.000	.000	.000	.000	.000	.000	.000	.000
D	.000	.000	.000	.000	.000	.000	.000	.000	.024
E	.900	1.000	.895	.940	.900	.960	.940	.875	.952
F	.000	.000	.000	.000	.000	.000	.000	.000	.000
G	.020	.000	.000	.040	.000	.000	.020	.000	.000
H	.000	.000	.000	.000	.000	.000	.000	.025	.000
I	.040	.000	.053	.020	.000	.020	.040	.000	.000
GPI-2									
(N)	25	10	19	25	25	25	25	20	21
A	.180	.100	.184	.100	.060	.000	.000	.000	.024
B	.000	.000	.000	.000	.000	.000	.000	.000	.000
C	.000	.050	.000	.040	.000	.020	.000	.050	.071
D	.820	.850	.737	.840	.900	.880	1.000	.775	.738
E	.000	.000	.026	.020	.040	.020	.000	.075	.071
F	.000	.000	.000	.000	.000	.080	.000	.000	.000
G	.000	.000	.053	.000	.000	.000	.000	.100	.095

	Population								
Locus	10	11	12	13	14	15	16	17	18
ME-1									
(N)	24	10	19	25	25	25	25	20	21
A	.000	.050	.000	.000	.020	.020	.000	.000	.000
B	1.000	.950	1.000	1.000	.980	.980	1.000	1.000	1.000
C	.000	.000	.000	.000	.000	.000	.000	.000	.000
MPI-1									
(N)	23	10	19	22	25	25	24	20	21
A	.000	.100	.026	.091	.000	.120	.063	.000	.000
B	1.000	.900	.974	.909	1.000	.880	.938	.850	.929
C	.000	.000	.000	.000	.000	.000	.000	.150	.071
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
E	.000	.000	.000	.000	.000	.000	.000	.000	.000
PEP-1									
(N)	25	10	19	25	25	25	25	20	21
A	.000	.050	.000	.000	.000	.000	.000	.000	.024
B	.220	.150	.184	.220	.220	.040	.020	.675	.690
C	.700	.700	.605	.740	.760	.780	.800	.300	.286
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
E	.080	.050	.211	.040	.000	.140	.140	.025	.000
F	.000	.050	.000	.000	.020	.040	.040	.000	.000
PEP-2									
(N)	25	10	19	25	25	25	25	20	21
A	.020	.000	.026	.000	.000	.000	.020	.000	.000
B	.980	.950	.974	1.000	1.000	1.000	.980	.875	.952
C	.000	.050	.000	.000	.000	.000	.000	.125	.048
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
PGM-1									
(N)	25	10	19	25	25	25	25	18	21
A	.000	.000	.000	.020	.000	.100	.060	.083	.000
B	.000	.000	.000	.000	.000	.000	.000	.000	.024
C	.960	1.000	1.000	.980	.920	.820	.880	.722	.452
D	.040	.000	.000	.000	.080	.080	.060	.194	.524
E	.000	.000	.000	.000	.000	.000	.000	.000	.000
PGDH									
(N)	23	10	19	24	25	25	25	20	21
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.000	.000	.000	.025	.000
C	.000	.000	.000	.021	.000	.000	.000	.000	.024
D	.978	1.000	.895	.958	.920	1.000	1.000	.900	.976
E	.022	.000	.053	.000	.040	.000	.000	.025	.000
F	.000	.000	.000	.000	.000	.000	.000	.000	.000
G	.000	.000	.053	.021	.040	.000	.000	.050	.000
H	.000	.000	.000	.000	.000	.000	.000	.000	.000
P	.476	.333	.429	.429	.476	.429	.381	.476	.571
H	.074	.078	.079	.076	.075	.072	.048	.115	.112

	Population								
Locus	10	11	12	13	14	15	16	17	18
IDH-1									
(N)	25	10	19	25	25	25	25	20	21
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.980	1.000	.974	1.000	1.000	1.000	1.000	1.000	1.000
C	.000	.000	.000	.000	.000	.000	.000	.000	.000
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
E	.020	.000	.026	.000	.000	.000	.000	.000	.000
IDH-2									
(N)	23	10	19	24	25	25	25	20	21
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LDH-1									
(N)	25	10	19	25	25	25	25	20	21
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.000	.000	.000	.000	.000
C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.950	.905
D	.000	.000	.000	.000	.000	.000	.000	.050	.095
LDH-2									
(N)	25	10	19	25	25	25	25	20	21
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.000	.000	.000	.000	.000
C	.140	.100	.026	.200	.200	.100	.020	.025	.095
D	.860	.900	.974	.800	.780	.900	.980	.975	.905
E	.000	.000	.000	.000	.020	.000	.000	.000	.000
MDH-1									
(N)	25	10	19	25	25	25	25	20	21
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	1.000	1.000	1.000	1.000	.980	1.000	1.000	1.000	.976
C	.000	.000	.000	.000	.020	.000	.000	.000	.024
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
E	.000	.000	.000	.000	.000	.000	.000	.000	.000
MDH-2									
(N)	25	10	19	25	25	25	25	20	21
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.040	.000	.020	.000	.000	.024
C	1.000	1.000	1.000	.960	1.000	.980	1.000	1.000	.976
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
MDH-3									
(N)	25	10	19	25	25	25	25	20	21
A	.040	.000	.000	.000	.020	.000	.020	.000	.024
B	.960	1.000	.974	1.000	.980	1.000	.980	1.000	.952
C	.000	.000	.000	.000	.000	.000	.000	.000	.000
D	.000	.000	.026	.000	.000	.000	.000	.000	.024
E	.000	.000	.000	.000	.000	.000	.000	.000	.000
F	.000	.000	.000	.000	.000	.000	.000	.000	.000

Locus	Population 19	20	21	22	23	24	25	26	27
AK-1									
(N)	10	20	20	20	57	24	20	25	17
A	.000	.000	.000	.000	.000	.000	.000	.000	.059
B	1.000	1.000	1.000	1.000	.982	.958	1.000	.980	.941
C	.000	.000	.000	.000	.018	.042	.000	.020	.000
CBP-1									
(N)	10	20	20	20	57	25	20	25	17
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CK-1									
(N)	10	20	20	20	57	25	20	25	17
A	.000	.000	.000	.000	.000	.000	.000	.000	.029
B	1.000	1.000	1.000	1.000	.965	.980	.950	1.000	.941
C	.000	.000	.000	.000	.035	.020	.050	.000	.029
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
CK-2									
(N)	10	20	20	20	57	25	20	25	17
A	.000	.000	.000	.000	.009	.000	.000	.000	.000
B	1.000	1.000	1.000	1.000	.991	1.000	1.000	.000	1.000
C	.000	.000	.000	.000	.000	.000	.000	1.000	.000
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
GAP-1									
(N)	10	20	20	20	57	9	20	20	17
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GAP-2									
(N)	10	20	20	20	57	9	20	20	17
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GPI-1									
(N)	10	20	20	20	57	25	20	25	17
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.075	.150	.025	.088	.040	.050	.000	.912
C	.000	.000	.000	.000	.009	.020	.000	.000	.000
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
E	1.000	.875	.800	.950	.842	.860	.850	1.000	.088
F	.000	.000	.000	.000	.000	.000	.000	.000	.000
G	.000	.000	.000	.000	.009	.020	.050	.000	.000
H	.000	.025	.025	.025	.053	.040	.050	.000	.000
I	.000	.025	.025	.000	.000	.020	.000	.000	.000
GPI-2									
(N)	10	20	20	20	57	25	20	25	17
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.000	.000	.000	.000	.000
C	.050	.000	.075	.025	.132	.020	.050	.080	.059
D	.900	.925	.750	.875	.754	.880	.850	.740	.882
E	.050	.050	.050	.000	.053	.080	.025	.140	.059
F	.000	.000	.000	.000	.000	.000	.000	.000	.000
G	.000	.025	.125	.100	.061	.020	.075	.040	.000

	Population								
Locus	19	20	21	22	23	24	25	26	27
IDH-1									
(N)	10	20	20	20	57	25	20	25	17
A	.000	.050	.000	.000	.009	.020	.000	.000	.000
B	1.000	.925	1.000	1.000	.982	.960	1.000	.900	1.000
C	.000	.025	.000	.000	.009	.000	.000	.000	.000
D	.000	.000	.000	.000	.000	.020	.000	.100	.000
E	.000	.000	.000	.000	.000	.000	.000	.000	.000
IDH-2									
(N)	10	20	20	20	57	25	20	25	17
A	.000	.000	.000	.000	.009	.000	.000	.000	.000
B	1.000	1.000	1.000	1.000	.991	1.000	1.000	1.000	1.000
LDH-1									
(N)	10	20	20	20	57	25	20	25	17
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.018	.000	.000	.000	.000
C	.750	.850	.750	.750	.912	.920	.925	1.000	.000
D	.250	.150	.250	.250	.070	.080	.075	.000	1.000
LDH-2									
(N)	10	20	20	20	57	25	20	25	17
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.000	.000	.000	.000	.000
C	.100	.025	.050	.025	.105	.080	.075	1.000	.941
D	.900	.975	.950	.975	.895	.920	.925	.000	.059
E	.000	.000	.000	.000	.000	.000	.000	.000	.000
MDH-1									
(N)	10	20	20	20	57	25	20	25	17
A	.000	.025	.000	.000	.000	.020	.000	.000	.000
B	1.000	.975	1.000	.975	.991	.960	1.000	1.000	1.000
C	.000	.000	.000	.000	.009	.020	.000	.000	.000
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
E	.000	.000	.000	.025	.000	.000	.000	.000	.000
MDH-2									
(N)	10	20	20	20	57	25	20	25	17
A	.000	.000	.000	.000	.018	.000	.000	.000	.000
B	.000	.000	.000	.000	.026	.000	.000	.000	.000
C	1.000	1.000	1.000	1.000	.947	.980	1.000	1.000	1.000
D	.000	.000	.000	.000	.009	.020	.000	.000	.000
MDH-3									
(N)	10	20	20	20	57	25	20	25	17
A	.000	.000	.000	.000	.000	.020	.000	.000	.000
B	1.000	1.000	1.000	1.000	.991	.960	1.000	1.000	.971
C	.000	.000	.000	.000	.000	.000	.000	.000	.000
D	.000	.000	.000	.000	.009	.020	.000	.000	.000
E	.000	.000	.000	.000	.000	.000	.000	.000	.000
F	.000	.000	.000	.000	.000	.000	.000	.000	.029

	Population								
Locus	19	20	21	22	23	24	25	26	27
ME-1									
(N)	10	20	20	20	57	25	20	20	17
A	.000	.000	.000	.000	.009	.020	.025	.000	.000
B	1.000	1.000	1.000	1.000	.974	.980	.975	1.000	1.000
C	.000	.000	.000	.000	.018	.000	.000	.000	.000
MPI-1									
(N)	7	20	20	16	57	16	20	25	17
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.857	.850	.825	.906	.877	.906	.775	1.000	1.000
C	.143	.150	.150	.094	.123	.094	.225	.000	.000
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
E	.000	.000	.025	.000	.000	.000	.000	.000	.000
PEP-1									
(N)	10	20	20	20	57	25	20	25	17
A	.000	.050	.025	.025	.035	.060	.025	.000	.059
B	.650	.675	.625	.700	.579	.560	.425	1.000	.824
C	.350	.275	.350	.275	.386	.380	.525	.000	.118
D	.000	.000	.000	.000	.000	.000	.025	.000	.000
E	.000	.000	.000	.000	.000	.000	.000	.000	.000
F	.000	.000	.000	.000	.000	.000	.000	.000	.000
PEP-2									
(N)	10	20	20	20	57	25	20	25	17
A	.000	.000	.000	.000	.000	.000	.000	.020	.000
B	1.000	1.000	1.000	.975	.904	.960	.975	.980	.882
C	.000	.000	.000	.025	.079	.040	.025	.000	.118
D	.000	.000	.000	.000	.018	.000	.000	.000	.000
PGM-1									
(N)	10	20	20	20	57	25	20	25	17
A	.000	.000	.000	.050	.018	.000	.000	1.000	.000
B	.000	.000	.000	.050	.000	.000	.000	.000	.000
C	.300	.300	.450	.425	.623	.460	.825	.000	.882
D	.700	.700	.550	.450	.351	.520	.175	.000	.118
E	.000	.000	.000	.025	.009	.020	.000	.000	.000
PGDH									
(N)	10	20	20	20	57	25	20	25	17
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.025	.000	.009	.000	.000	.000	.000
C	.100	.000	.000	.000	.000	.000	.000	.000	.206
D	.850	.925	.925	.975	.974	.960	1.000	.000	.676
E	.050	.000	.000	.000	.000	.000	.000	.020	.000
F	.000	.000	.025	.000	.000	.000	.000	.000	.000
G	.000	.075	.025	.025	.018	.040	.000	.980	.000
H	.000	.000	.000	.000	.000	.000	.000	.000	.118
P	.333	.476	.381	.476	.857	.762	.476	.238	.476
H	.107	.105	.129	.103	.137	.125	.107	.035	.097

	Population			
Locus	28	29	30	31
AK-1				
(N)	20	15	34	20
A	.000	.000	.000	.000
B	1.000	1.000	1.000	1.000
C	.000	.000	.000	.000
CBP-1				
(N)	20	15	34	20
A	1.000	1.000	1.000	1.000
CK-1				
(N)	20	15	34	20
A	.000	.000	.000	.000
B	1.000	1.000	.971	1.000
C	.000	.000	.000	.000
D	.000	.000	.029	.000
CK-2				
(N)	20	15	34	20
A	.000	.000	.000	.000
B	1.000	1.000	.000	.000
C	.000	.000	1.000	.975
D	.000	.000	.000	.025
GAP-1				
(N)	20	15	34	20
A	1.000	1.000	1.000	1.000
GAP-2				
(N)	20	15	34	20
A	1.000	1.000	1.000	1.000
GPI-1				
(N)	20	15	34	20
A	.025	.000	.000	.525
B	.025	.033	.000	.000
C	.000	.000	.000	.000
D	.000	.000	.000	.000
E	.825	.800	.088	.475
F	.000	.033	.000	.000
G	.000	.000	.000	.000
H	.125	.133	.912	.000
I	.000	.000	.000	.000
GPI-2				
(N)	20	15	33	20
A	.000	.000	.000	.000
B	.000	.000	.000	.000
C	.050	.133	.818	1.000
D	.950	.867	.182	.000
E	.000	.000	.000	.000
F	.000	.000	.000	.000
G	.000	.000	.000	.000

	Population			
Locus	28	29	30	31
IDH-1				
(N)	20	15	34	20
A	.025	.000	.000	.500
B	.950	1.000	.000	.500
C	.025	.000	1.000	.000
D	.000	.000	.000	.000
E	.000	.000	.000	.000
IDH-2				
(N)	20	15	34	20
A	.000	.000	.000	1.000
B	1.000	1.000	1.000	.000
LDH-1				
(N)	20	15	34	20
A	.000	.000	.000	.000
B	.000	.000	.000	.000
C	1.000	1.000	1.000	1.000
D	.000	.000	.000	.000
LDH-2				
(N)	20	15	34	20
A	.000	.000	1.000	.000
B	.000	.000	.000	1.000
C	1.000	1.000	.000	.000
D	.000	.000	.000	.000
E	.000	.000	.000	.000
MDH-1				
(N)	20	15	34	20
A	.000	.000	.000	.000
B	1.000	1.000	1.000	.000
C	.000	.000	.000	.000
D	.000	.000	.000	1.000
E	.000	.000	.000	.000
MDH-2				
(N)	19	15	34	20
A	.026	.000	.000	.000
B	.000	.000	.000	.000
C	.974	1.000	1.000	1.000
D	.000	.000	.000	.000
MDH-3				
(N)	20	15	34	20
A	.025	.000	.000	.000
B	.975	1.000	1.000	.000
C	.000	.000	.000	.000
D	.000	.000	.000	1.000
E	.000	.000	.000	.000
F	.000	.000	.000	.000

	Population			
Locus	28	29	30	31
ME-1				
(N)	20	15	34	20
A	.000	.000	.000	.000
B	1.000	1.000	1.000	1.000
C	.000	.000	.000	.000
MPI-1				
(N)	20	15	30	10
A	.075	.200	.000	.050
B	.925	.800	.000	.500
C	.000	.000	.000	.000
D	.000	.000	.000	.450
E	.000	.000	1.000	.000
PEP-1				
(N)	20	15	33	20
A	.000	.067	.000	.000
B	.250	.400	.924	.000
C	.725	.500	.076	.375
D	.000	.000	.000	.000
E	.025	.033	.000	.625
F	.000	.000	.000	.000
PEP-2				
(N)	20	15	33	20
A	.000	.000	.000	.000
B	.950	.967	.000	1.000
C	.025	.033	.000	.000
D	.025	.000	1.000	.000
PGM-1				
(N)	20	15	34	20
A	.025	.033	.000	.525
B	.000	.000	.000	.000
C	.925	.900	1.000	.475
D	.050	.067	.000	.000
E	.000	.000	.000	.000
PGDH				
(N)	19	15	33	20
A	.000	.000	.000	.000
B	.105	.133	.470	.000
C	.000	.000	.000	.000
D	.789	.667	.530	1.000
E	.000	.000	.000	.000
F	.026	.000	.000	.000
G	.079	.200	.000	.000
H	.000	.000	.000	.000
P	.476	.333	.238	.286
H	.085	.109	.056	.126

Appendix C. Key to the three species of the speckled chub complex in the Arkansas and Red River drainages (from Eisenhour, 1997).

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1. (A) Two prominent pairs of barbels present, posterior barbels usually > orbit length, anterior barbels usually > 50% of orbit length; pectoral fin ray tuberculation of nuptial males usually biserial on primary branches, with 3-4 rows of tubercles at midsection of pectoral rays; lips fleshy and greatly expanded posteriorly; eyes round..... 2

   (B) One or two pairs of barbels present, posterior barbels usually < orbit length, anterior barbels absent or < 50% of orbit length; pectoral fin ray tuberculation of nuptial males uniserial, with 1-2 rows of tubercles at midsection of pectoral rays; lips not as fleshy and not as greatly expanded posteriorly; eyes oval..... *M. hyostoma*

2. (A) Anal fin rays modally 7; pectoral fins of adult males extending beyond pelvic bases; vertebrae 34-36..... *M. australis.*

   (B) Anal fin rays modally 8; pectoral fins of adult males just reaching pelvic bases; vertebrae 36-39..... *M. tetranema*
