GENETIC STRUCTURE OF SPOTTED BASS (*MICROPTERUS PUNCTULATUS*) IN THE RED RIVER BASIN

By

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Bachelor of Arts

University of Windsor

Windsor, Ontario

1993

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 2001

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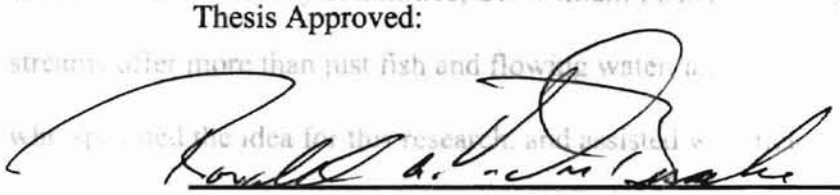
Thesis Approved:



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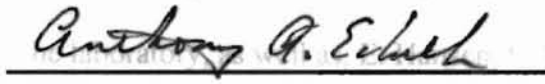

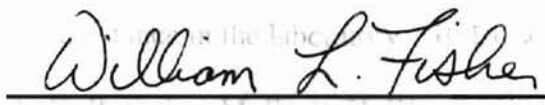

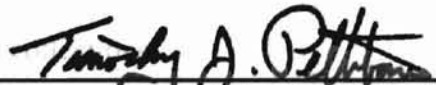

Dean of the Graduate College

Finally, to my wife Sarah, who has had such a positive influence on my life. Her strong will and belief in me has fuelled my personal confidence and helped me realize that with her by my side, we can achieve anything.

ACKNOWLEDGMENTS

I would like to thank my co-advisors Dr. Anthony Echelle and Dr. Ronald Van Den Bussche for their support and guidance throughout this research, but most of all for providing me with an opportunity to pursue post-graduate studies. Their patience and commitment to training new scientists are appreciated most. I would also like to thank the third member of my committee, Dr. William Fisher, who helped me realize that streams offer more than just fish and flowing water, and Larry Cofer, a co-investigator, who spawned the idea for this research, and assisted with field collections.

My indebted thanks go out to T. Malloy for providing microsatellite DNA primers and training in the laboratory, as well as, E. Hansen, S. Hoofer, G. Wilson, and D. Onorato for their assistance in the laboratory. B. Brown, A. F. Echelle, J. Harper, J. Long, P. Mauck, D. Rutledge, M. Scott, H. Woods, and S. Wooldridge provided assistance with field collections.

This research could not have been conducted without the support of the Oklahoma Cooperative Fish and Wildlife Research Unit, which provided field vehicles, boats and sampling equipment. This project was supported by a Federal Aid in Sport Fish research grant administered through the Oklahoma Department of Wildlife Conservation.

I am grateful to my parents and siblings for their patience during the years of "finding myself" and emotional support for the move from Canada to Oklahoma.

Finally, to my wife Sarah, who has had such a positive influence on my life. Her strong will and belief in me has fuelled my personal confidence and helped me realize that with her by my side, we can achieve anything.

This scholastic effort is dedicated to the next generation of Coughlin's

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ABSTRACT

Mitochondrial DNA sequence variation and allele frequencies for five microsatellite DNA loci was used to assess the genetic structure of spotted bass populations in the upper Red, Ouachita, and Arkansas river basins, with emphasis on those in the Red River Basin. Results for 318 spotted bass from 14 localities provide no evidence that the present spotted bass populations in western reaches of the Red River Basin carry remnants of variation that originated in a pre-glacial Ouachita River. The population in East Cache Creek, which potentially supported native populations of the nominal subspecies *M. p. wichitae*, appears to have been introduced from farther east in the Red River Basin. The pattern of overall similarity for both mtDNA and microsatellite DNA indicates that spotted bass in the Ouachita River Basin are more similar to those in the Arkansas River Basin than to those in the Red River Basin, a result that conflicts with expectations based on a previous Pleistocene model for the biogeography of the fishes of the region. These results, together with a nested clade analysis of mtDNA variation, suggest that the present pattern of genetic variation in spotted bass of the region is a result of recent events, possibly post-Pleistocene dispersal into the region. The results for microsatellite DNA showed no evidence of hybridization with smallmouth bass as a factor in the present genetic structure of spotted bass. The corresponding results for mtDNA provided no added resolution to this question because mtDNA is not divergent between the two species in the study area.

INTRODUCTION

Mitochondrial DNA (mtDNA) and microsatellite DNA variation was used to describe the genetic structure of spotted bass (*Micropterus punctulatus*) in the Red River Basin of Oklahoma, Texas, and Arkansas. The spotted bass in western portions of this basin are of particular interest from a conservation standpoint because of the presence of disjunct populations in the Wichita Mountains region of southwestern Oklahoma (Hubbs and Bailey 1940; Cofer 1995). Biogeographic considerations indicate that several other primarily eastern fishes with disjunct populations in the Wichita Mountains are relicts of pre-glacial times (Mayden and Matthews 1989, Taylor et al. 1993).

Native populations of spotted bass occur from the Great Plains east to the Appalachian Divide and from the Ohio and Wabash River drainages south to the Gulf of Mexico (MacCrimmon and Robbins 1975). Two subspecies, both described by Hubbs and Bailey (1940), are currently recognized: *M. p. punctulatus* throughout most of the native range of the species, and *M. p. henshalli*, from the Mobile Bay drainage of Mississippi, Alabama, and Georgia. In Oklahoma, native populations are primarily restricted to the eastern half of the state, but a disjunct population occurs in Cache Creek, a tributary of the Red River in Comanche County, southwestern Oklahoma (Miller and Robison 1973; MacCrimmon and Robbins 1975; Vogele 1975). This population, which represents the western-most native population of the species (Hubbs and Bailey 1940), was first described as *M. pseudaplites* (Hubbs and Ortenburger 1929) based upon collections taken from the West Cache Creek portion of the system in 1923-1928. Hubbs and Bailey (1940) subsequently classified these specimens as *M. punctulatus wichitae*. More recently however, Cofer (1995) concluded that recognition of this taxon was based

on hybrids between spotted bass and non-native smallmouth bass (*M. dolomieu*) that were introduced into West Cache Creek as early as 1907. Regardless of taxonomic status, however, the possibility remains that the historically disjunct populations in the Cache Creek drainage are genetically divergent.

Various surveys since 1930 indicated extirpation of the spotted bass from the West Cache Creek drainage, probably because of habitat changes associated with construction of a number of small reservoirs in the 1930s and replacement of the species by largemouth bass (Cook 1979, Cofer 1995, Hostettler and Cofer 1996). Extant populations of spotted bass are present in the nearby East Cache Creek drainage (Cofer 1995) where the species occurs in approximately 200 square miles of artificial reservoir habitat (lakes Lawtonka and Ellsworth) and 50 miles of stream (L. Cofer, unpubl.).

The genetic status of the East Cache Creek populations is not clear, but it is possible that they comprise either non-natives or natives genetically introgressed by non-native spotted bass stock. During 1977-1979, the Oklahoma Department of Wildlife Conservation released non-native spotted bass in Lake Ellsworth (Federal Aid Project F-36-R-3, Job 4). This involved three different stockings of fingerlings: 6000 in 1978 from Lake Texoma (Red River Basin) broodstock, 22,000 in 1978 from Holdenville Lake (Arkansas River Basin) broodstock, and several hundred fingerlings of unknown origin in 1979. Further, there is hearsay evidence that in 1972 fishermen caught a small number of spotted bass into Lake Kemp, a western Red River Basin reservoir in the Wichita River drainage of Texas, and released them alive in Lake Lawtonka (Ken Cook, pers. comm.). Finally, the Lake Kemp population itself may have been introduced (Lewis and Dalquest 1957), but the non-native status of this population is not well substantiated because it is

based only on the observation that the species was restricted to Lake Kemp during a basin-wide survey of the Wichita River from 1953 to 1955. There are no documented introductions of spotted bass in eastern portions of the Red River Basin. The species is historically common in this area and most stockings of spotted bass would have been incidental to introductions of other fishes, particularly smallmouth bass. Further, because of the prior presence of spotted bass populations, such incidental stockings are likely to have had little effect on native stocks.

This study addresses the following questions: 1) Are the populations in the East Cache Creek drainage and Lake Kemp native or non-native? 2) Is there evidence of genetic introgression of a native East Cache Creek stock with stocks from elsewhere (Lake Texoma and Lake Kemp)? 3) Is there any indication of introgression between spotted bass and non-native smallmouth bass? 4) Does the genetic structure of populations in the Red River Basin indicate the presence of evolutionarily significant units (ESU; Waples 1991) deserving special attention from fish managers interested in preserving genetic diversity?

Moritz (1994) recommended that ESU recognition be based on reciprocal monophyly for mtDNA, and that populations or clusters of populations representing "management units" (Waples 1991) within ESUs be recognized on the basis of allele frequency divergence at nuclear loci (microsatellite DNA in this study). ESU recognition might be warranted even if the genetic structure suggests genetic introgression by non-natives, particularly if the introgressed population is the last remaining representative of a significant portion of the evolutionary legacy of a species (Campton 1987; Allendorf and Leary 1988, Dowling and Childs 1992). Previous genetic studies of spotted bass have

dealt primarily with hybridization between the species and various congeners (Koppelman 1994; Avise et al. 1997; Pierce and Van Den Avyle 1997). In the only other study addressing population structure of spotted bass, Fuller (1998) concluded, from allozymes and randomly amplified polymorphic DNA, that two populations in Louisiana belong to the subspecies *M. p. punctulatus* and not *M. p. henshalli*.

METHODS

In 1999 and 2000, samples of 19-30 specimens each were collected from 12 sites encompassing the major streams in the Red River Basin in Arkansas, Oklahoma, and Texas, and two Oklahoma localities in the Arkansas River Basin. Localities were as follows (in parentheses, locality numbers as in Fig. 1 and Table 1): (1) Medicine Creek at state highway 115 crossing upstream of Lake Lawtonka, Comanche Co., Oklahoma; (2) East Cache Creek on Fort Sill Military Reservation, Comanche Co., Oklahoma; (3) Lake Ellsworth, Comanche Co., Oklahoma; (4) Lake Kemp near the dam, Baylor Co., Texas; (5) Washita Arm of Lake Texoma, Marshall and Bryan Counties, Oklahoma; (6). Blue River at Blue River Public Hunting and Fishing Area, Johnston Co., Oklahoma; (7) McGee Creek Reservoir, McGee and Potapo arms, Atoka Co, Oklahoma; (8) Sardis Reservoir just N of junction between state highways 2 and 43, Pushmataha Co., Oklahoma; (9) Pine Creek Reservoir 6 km W of New Ringold, McCurtain Co., Oklahoma; (10) Broken Bow Reservoir in the Hochatown State Park area, McCurtain Co., Oklahoma; (11) Greeson Reservoir near U.S. Highway 70 bridge, Pike Co., Arkansas; (12) DeGray Reservoir, Clark Co., Arkansas; (13) Lake Tenkiller, Cherokee Co., Oklahoma; (14) Skiatook Lake, Osage County, Oklahoma.

To allow assessment of genetic introgression and as an initial outgroup for the mtDNA phylogenetic analysis, smallmouth bass (*Micropterus dolomieu*) from the following three sources were included: captive stock at Byron State Fish Hatchery, Byron, Alafalfa Co., Oklahoma (derived originally from the Cumberland River Basin, Tennessee, n = 22); Baron Fork of the Illinois River 1.2 km S, 0.4 km E of Welling, Cherokee Co., Oklahoma (n = 2); Mountain Fork River near Jet, McCurtain Co., Oklahoma (n = 2). For the phylogenetic analysis, one specimen of redeye bass (*M. coosae*) from Alabama River, Walker Co., Alabama, and a largemouth bass (*M. salmoides*) from Stillwater Creek, Payne Co., Oklahoma were included.

Collections were made by seining, angling, and boat and backpack electrofishing. Captured specimens were kept alive in water or immediately put on ice until tissues were removed for analysis. At time of collection muscle and/or liver tissue was removed from spotted bass and placed in 15 ml tubes with 5 ml of lysis buffer (2M TRIS HCL pH 8.0, 0.5M EDTA, 5M NaCl, ddH_2O, 10% SDS). In the lab, 0.1 g of tissue was used for DNA extraction following Longmire et al. (1997).

An initial screening of 14 previously developed microsatellite primers (Colbourne et al. 1996; Malloy et al. 2000) was conducted on seven specimens from each of eight different collections of spotted bass. Five polymorphic loci were then selected for analysis (Table 2). In the initial screening and the subsequent survey, forward primers for the polymerase chain reaction (PCR) were end-labeled with α^{32}P dCTP and conditions for the reaction were as follows: an initial 12 min denaturation at 95 °C, followed by 10 cycles at 94 °C for 15 s, 55 °C for 60 s, 72°C for 30 s, followed by 25 cycles at 89 °C for 15 s, 55 °C for 60 s, and 72 °C for 30 s, and ending with a 30-min

elongation at 72 °C. The resulting amplicons were electrophoresed through 5% and acrylamide gels and visualized with autoradiography.

Universal primers L15926 (Kocher et al. 1989) and H16498 (Meyer et al. 1990) were used to amplify a 447-bp portion of the mitochondrial DNA control-region following protocol described in Echelle et al. (2000). All spotted bass, two specimens from each of the three smallmouth bass collections, and one specimen each of redeye and largemouth basses were sequenced in one direction (forward primer L15926) using an ABI Prism™ 377 automated sequencer (Applied Biosystems Inc., Foster City, CA). Sequences were aligned using CLUSTAL X (Thompson et al. 1997) and visually verified in MacClade 4.0 (Maddison and Maddison 2000). Haplotypes were determined by using the Search and Merge option under Redundant Taxa in MacClade 4.0. Those haplotypes differing by only a single nucleotide were sequenced in both directions as a check against PCR error.

For microsatellite DNA data, the computer program GENEPOP (Raymond and Rousset 1995; web version 3.1c at Http://wbiomed.curtin.edu.au/genepop/index. html) was used to calculate departures from Hardy-Weinberg equilibrium (HWE), and allele frequencies. Arlequin version 2.0 (Schneider et al. 2000) was used for analyses of genetic structure, including observed and expected heterozygosity, pairwise F_{ST} values, and hierarchical analysis of gene diversity based on Wright's F-statistics (Wright 1951). The hierarchical analyses partitioned the genetic diversity into proportions attributable to variation among populations in different basins, among populations within basins, and within populations. For this analysis, localities were grouped into three basins as

follows: Red River (sites 1-10 in Fig. 1), Ouachita (11 and 12), and Arkansas (13 and 14).

Two different approaches were employed to analyze the mtDNA sequence data. Arlequin was used to estimate haplotype and nucleotide diversities and pairwise Φ_{ST}-values among populations and to obtain a hierarchical analysis of molecular structure based on Φ_{ST} statistics (AMOVA; Excoffier et al. 1992). For the hierarchical analysis, localities were grouped as described for microsatellite DNA data.

Significance levels of all multiple tests were adjusted using the sequential Bonferroni correction (Rice 1989) for Type I error (tablewide $\alpha = 0.05$). To summarize patterns of overall genetic similarity among localities, separate minimum spanning trees were constructed by hand to summarize the pairwise F_{ST}-values from microsatellite DNA loci and the Φ_{ST}-values from the mtDNA sequences. In a minimum spanning tree, each collection is connected to the collection which it is most similar.

For phylogenetic analysis of relationships among haplotypes, PAUP* (version 4.062, Swofford 1998) was used in heuristic, maximum-parsimony searches with TBR branch swapping, equal-weighting of characters, and 20 random addition replicates. This analysis included all haplotypes detected in spotted bass and three haplotypes detected in smallmouth bass; redeye bass and largemouth bass were the designated outgroups.

The second approach, nested clade analysis, uses objective statistical tests to separate population structure from population history (Templeton et al. 1992, 1995). The computer program TCS (Clement et al. 2000) was used to estimate, with the algorithm presented by Templeton et al. (1992), an unrooted network of relationships that grouped haplotypes (zero-step clades), into one-step clades; (member clades differ by one

substitution), two step clades (member clades differ by two substitutions), and so on, until the next level of nesting encompasses the whole tree (Templeton et al. 1992). The program GeoDis (version 2.0; Posada et al. 2000) incorporates spatial information (pairwise river-kilometer distances among collection sites; Table 3) to calculate for each haplotype or higher level clade, its average distance from its geographical center (clade distance = D_c), and its average distance from the geographical center of the next higher-level clade (nesting clade) to which it belonged (nested clade distance = D_n; Templeton et al. 1992). Following an inference key (latest edition at: http://bioag.byu.edu/zoology/crandall_lab/geodis.htm), the GeoDis output was used to identify which historic events (e.g., range expansion, long distance, dispersal, allopatric fragmentation, or isolation by distance) best explain the geographic structure of mtDNA variation.

RESULTS

Microsatellite DNA Variability

The five loci selected for this study were polymorphic in all samples except for monomorphy of *Mdo5* and *Mdo12* in one sample each (Table 4). Number of alleles ranged from 3 (*Mdo5* and *Mdo12*) to 11 (*Lma21*). None of the alleles detected in smallmouth bass were found in spotted bass. Tests for conformity to HWE revealed no significant deviations (initial α for each population = 0.01; calculated by dividing 0.05 by the number of loci).

All pairwise F_{ST}-values among samples from the Red River Basin were statistically significant except the one comparing the sample from Lake Ellsworth of the

East Cache Creek system with the Sardis Reservoir sample. Other instances of non-significance included the comparison of the two samples from reservoirs (Greeson and DeGray) of the Ouachita River Basin, and, somewhat surprisingly, comparisons of those two samples with the one from Lake Tenkiller of the Arkansas River Basin (Table 5).

The minimum spanning tree based on the matrix of F_{ST}-values shows that two of the spotted bass collections from three closely spaced locations in the East Cache Creek system (sites 1, 2, and 3 in Figure 1) were more similar to collections from locations farther east than they were to each other (Figure 2). The Medicine Creek collection (site 1) from that system was most similar to the one from Lake Texoma, whereas the Lake Ellsworth collection (site 3) was most similar to the one from Sardis Reservoir. In addition, the three collections from the East Cache Creek system were not notably divergent from other populations of spotted bass.

The collection from Lake Kemp was the most divergent spotted bass collection in the survey. All other collections overlapped each other in allele composition at all five microsatellite loci, whereas, for *Mdo3*, the Lake Kemp collection overlapped only with the Lake Ellsworth and Lake Texoma collections (Table 4). In addition, the Lake Kemp collection had, at moderate frequencies (0.24 and 0.57), two alleles, *Lma21*-G and *Mdo11*-E, that were absent in all other spotted bass examined.

Mitochondrial DNA Variability

The amplified d-loop segment comprised 447 base pairs of which 39 were variable across all taxa and only 7 of which were variable within spotted bass. Eight mtDNA haplotypes were detected in spotted bass (Table 6), all of which are deposited in

GenBank. Excluding Blue River, all populations contained two or more haplotypes. Haplotype diversity ranged from 0.000 to 0.540 (mean = 0.437; Table 7). Nucleotide diversity ranged from 0.000 to 0.003 (mean = 0.0017; Table 7), indicating low levels of intrapopulational sequence divergence.

Two haplotypes (A and B) represented 92% of all spotted bass analyzed. Haplotype A occurred in all collections except the two from the Ouachita River system (Greeson and DeGray reservoirs), and haplotype B occurred in all except two collections from the Red River basin (Lake Kemp and Blue River). Among the remaining haplotypes detected in spotted bass, C, was shared between collections from the Ouachita and Arkansas river basins, occurring in, respectively, DeGray and Tenkiller reservoirs, G occurred in Kemp and Sardis reservoirs of the Red River basin, and the remaining haplotypes were detected only in single individuals.

The smallmouth bass samples included three different haplotypes, one of which occurred in a spotted bass from Lake Tenkiller (Table 6). The remaining seven spotted bass haplotypes were no more divergent from smallmouth bass haplotypes (1 to 4 substitutions; uncorrected p = 0.004-0.010) than they were from each other (also 1-4 substitutions). Correspondingly, the maximum parsimony analysis of relationships gave no resolution. There were 259 equally parsimonious trees (tree length = 43; consistency index = 0.88; retention index = 0.74), and the strict consensus included one large polytomy comprising all haplotypes detected in spotted and smallmouth bass. The haplotypes of the spotted/smallmouth bass clade differed from those of the outgroup taxa, redeye and largemouth bass, at, respectively, 12 to 14 (uncorrected p = 0.027-0.031) and 27 to 29 (p = 0.061-0.065) base-pair positions.

The Φ_{ST}-values ranged from near zero for a number of pairwise combinations of collections to 0.98 between the Blue River and Greeson Reservoir collections (Table 5). The Blue River population was significantly divergent from all others except the population in Lake Kemp. The latter was significantly divergent from all populations except for a group comprising four of the five other western-most populations in the Red River Basin: the one in Blue River, the Lake Texoma population, and two of the three East Cache Creek populations (sites 2 and 3, Figure 1). The two collections from the Ouachita River Basin (Greeson and DeGray reservoirs) differed significantly from all collections from the Red River Basin. The two samples from the Arkansas River Basin differed significantly from all of the six western-most populations in the Red River Basin, but a number of between-basin (Arkansas vs Red and Ouachita) combinations were not significantly divergent (Table 5).

The minimum spanning tree for the matrix of Φ_{ST}-values reflects some aspects of the comparable tree based on F_{ST}-values for microsatellites (Figure 2). In both trees, the East Cache Creek populations are weakly divergent from eastern populations in the Red River Basin, the Lake Kemp population is one of the most divergent populations, and the two populations from the Ouachita River system (DeGray and Greeson reservoirs) cluster most closely with those from the Arkansas River Basin (Tenkiller and Baron Fork).

Hierarchical Analysis of Genetic Diversity

For both forms of DNA data, the majority of the genetic diversity (72.6% for microsatellite DNA; 60.3% for mtDNA) was attributable to variation within populations. The proportion attributable to variation among samples within basins was 16.9% for

microsatellites and 12.6% for mtDNA, both of which were highly significant ($P <$ 0.0001). The corresponding values for proportion attributable to variation among populations in different basins was 10.3% and 29.9% ($P = 0.009$ and 0.011).

Nested Clade Analysis

The nested clade analysis resulted in three one-step clades (1-1, 1-2, and 1-3) grouped into a single two-step clade (2-1; Figure. 3). An ambiguous loop involving haploypes A, B, F, and G was resolved according to nesting rules in Templeton and Sing (1993). Based on the assumption that more widespread haplotypes constitute older lineages (Templeton et al. 1995), clade 1-1 was designated as interior to clades 1-2 and 1-3

There was significant geographic and genetic structure at the 1-step and entire cladogram levels (Table 9). Clade 1-1 had significantly large D_c and small D_n values (Figure 4). The inference chain for this clade, which occurred in 12 of the 14 populations sampled and all three of the major basins, indicated restricted gene flow as a result of long distance dispersal over areas not occupied by the species (Figure 4). The same biological inference applied to the geography of the entire network (clade 2-1), which had significantly large clade and nested-clade distances. Clade 1-3, which occurred in all collections except those from the Ouachita River Basin, had a significantly large D_c value and a nonsignificant D_n value, and the inference chain indicated restricted gene flow due to isolation by distance

DISCUSSION

Results of this study are compatible with the hypothesis that present populations of spotted bass in the East Cache Creek drainage are non-natives introduced from populations farther east in the Red River Basin. For both mtDNA and microsatellite DNA, these populations exhibit no unique genetic markers and they do not cluster as a separate group from other populations on the basis of marker frequencies. For mtDNA, haplotype frequencies in the East Cache Creek populations are not divergent from those in several eastern populations, and for microsatellite DNA alleles they are more similar to various eastern populations than they are to each other. The latter is unusual for native populations in such close proximity with no natural barriers to gene flow. In the other two such instances among the collections (Pine Creek and Broken Bow reservoirs and DeGray and Greeson reservoirs), which were from sites separated by greater distances than the East Cache Creek sites (about 150-275 river-km vs <90), the highest similarities were with the collection from the nearest location. The rather heterogeneous nature of the East Cache Creek populations may reflect the earlier mentioned stockings of Lakes Ellsworth and Lawtonka with non-native spotted bass after establishment of dams serving as barriers to gene flow.

Other possibilities for the East Cache Creek populations are that they are native populations that have been genetically introgressed by introduced non-native spotted bass stocks, or that they are native populations that have been subjected to genetic drift subsequent to population fragmentation, for example, as a result of reservoir construction. My results cannot eliminate these hypotheses, but if either possibility is true, then the native populations were originally weakly divergent from populations elsewhere in the

Red River Basin. There is no evidence of genetic markers restricted to the East Cache Creek area.

The Lake Kemp population was divergent from all other samples on the basis of both mtDNA and microsatellite DNA. There was no evidence of a fixed genetic difference between this population and all others, but it did exhibit some unique microsatellite alleles, two of which occurred at moderate frequencies (0.24 and 0.56). As previously mentioned, this population may have been introduced (Lewis and Dalquest 1957), but this is not well documented. Insight into the native/non-native status of this population would require a more extensive survey of the geographic range of spotted bass.

These results provide little evidence of genetic introgression of spotted bass by smallmouth bass. Hybdrization between the two species can be common, particularly in areas where one has been introduced into the native range of the other (Koppelman 1994; Avise et al. 1997; Pierce and Van Den Avyle 1997). Nonetheless, this survey revealed no sharing of microsatellite DNA alleles between the two species. The mitochondrial DNA is so similar between the collections of spotted and smallmouth bass that the results are somewhat uninformative regarding hybridization, a situation that has also been observed in populations of the two species in Missouri (J. Koppelman, pers. comm.). The occurrence in a single spotted bass from Lake Tenkiller of an mtDNA haplotype that otherwise occurred only in smallmouth bass from a nearby location (both specimens from Baron Fork River) might reflect contemporary hybridization. However, without further study of phylogenetic relationships among haplotypes in the two species, contemporary hybridization cannot be separated from shared ancestral mtDNA lineages or from lineage

sharing as a result of ancient hybridization and replacement of the mtDNA of one of the two species by that of the other.

A notable result of this survey was the observation from both mtDNA and microsatellite DNA that populations in the Ouachita River Basin (DeGray and Greeson reservoirs) were more similar to those in the Arkansas River Basin than to those in the Red River Basin. This pattern is in conflict with results of a phylogenetic analysis of genetic variation in smallmouth bass (Stark and Echelle 1998) and with expectations based on the hypothesis (Mayden 1988; Taylor et al. 1993) that the localities examined in the Ouachita River Basin and most of those in the Red River Basin were part of a pre-glacial Ouachita River. This result, together with biological inferences from the nested clade analysis, which indicate restricted gene flow based on either isolation by distance or occasional long dispersal across largely uninhabited area, suggests that the present pattern of genetic variation in the study region is largely explained by relatively recent events, possibly post-Pleistocene dispersal into the upper Red, Arkansas, and Ouachita river basins.

LITERATURE CITED

Allendorf, F. W., and R. F. Leary. 1988. Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. Conservation Biology 2:170-184.

Avise, J. C., P. C. Pierce, M. J. Van Den Avyle, M. H. Smith, W. S. Nelson, and M. A. Asmussen. 1997. Cytonuclear introgressive swamping and species turnover of bass after an introduction. Journal of Heredity 88:14-20.

Campton, D. E. 1987. Natural hybridization and introgression in fishes. Pp. 161-192 *in* Population genetics and fishery management. N. Ryman and F. Utter (eds.). University of Washington Press, Seattle.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9:1657-1659.

Cofer, L. M. 1995. Invalidation of the Wichita spotted bass, *Micropterus punctulatus wichitae*, subspecies theory. Copeia 1995:487-490.

Cook, K. D. 1979. Fish population study of West Cache Creek with emphasis on search for the Wichita spotted bass, *Micropterus punctulatus wichitae*. Proceedings of the Oklahoma Academy of Science 59:1-3.

Colbourne, J. K., B. D. Neff, J. M. Wright, and M. R. Gross. 1996. DNA fingerprinting of bluegill sunfish (*Lepomis macrochirus*) using (GT)n microsatellites and its potential for assessment of mating success. Canadian Journal of Fisheries and Aquatic Sciences 53:342-349.

Dowling, T. E., and M. R. Childs. 1992. Impact of hybridization on a threatened trout of the southwestern United States. Conservation Biology 6:35-364.

Echelle, A. A., R. A. Van Den Bussche, T. P. Malloy, Jr., M. L. Haynie, and C. O. Mickley. 2000. Mitochondrial DNA variation in pupfishes assigned to the species *Cyprinodon macularius* (Atherinomorpha: Cyprinodontidae): taxonomic implications and conservation genetics. Copeia 2000:353-364.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479-491.

Fuller, B. 1998. Molecular systematics of selected members of the black basses, genus *Micropterus*, with concentration on the spotted bass (*M. punctulatus*) species complex. PhD. dissertation, Louisiana State University, Baton Rouge, LA.

Hostettler, L., and L. Cofer. 1996. Potential for spotted bass in West Cache Creek-Wichita Mountains Wildlife Refuge. Oklahoma Department of Wildlife Conservation, Oklahoma City, OK.

Hubbs, C. L., and A. J. Ortenburger. 1929. Fishes collected in Oklahoma and Arkansas in 1927. Publications of the University of Oklahoma Biological Survey 1:45-112.

Hubbs, C. L., and R. M. Bailey. 1940. A revision of the black basses (*Micropterus and Huro*) with descriptions of four new forms. Miscellaneous Publications of the Museum of Zoology, University of Michigan 48:19-22.

Kocher, T. D., W. K. Thomas, A. Meyer, S. V. Edwards, S. Paabo, F. X. Villablanca, and A. C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of the National Academy of Science 86:6196-6200.

Koppelman, J. B. 1994. Hybridization between small mouth bass, *Micropterus dolomieu*, and spotted bass, *M. punctulatus*, in the Missouri River System, Missouri. Copeia 1994:204-210.

Lewis, L. D., and W. W. Dalquest. 1957. A fisheries survey of the Big Wichita River system and its impoundments. Texas game and Fish Commission, Austin, Texas, 64 pp.

Longmire, J. L., M. Maltbie, and R. J. Baker. 1997. Use of "Lysis Buffer" in DNA isolation and its implication for museum collections. Occasional Papers of the Museum of Texas Tech University 163:1-3.

MacCrimmon, H. R., and W. H. Robbins. 1975. Distribution of the black basses in North America. Pp. 56-66 *in* Black Bass Biology and Management. H. Clepper (ed.). Sport Fishing Institute, Washington, DC.

Maddison, W. P., and D. R. Maddison. 2000. MacClade, version 4.0: Analysis of phylogeny and character evolution. Sinauer, Sunderland, MA.

Malloy, T. P., Jr., R. A. Van Den Bussche, W. D. Coughlin, and A. A. Echelle. 2000. Isolation and characterization of microsatellite loci in smallmouth bass, *Micropterus dolomieu*. (Teleostei: Centrarchidae), and cross-species amplification in spotted bass, *M. punctulatus*. Molecular Ecology 9:1946-1948.

Mayden, R. L. 1988. Vicariance biogeography, parsimony, and evolution in North American freshwater fishes. Systematic Zoology 37:329-355.

Mayden, R. L., and W. J. Matthews. 1989. Zoogeographic implications of *Luxilus cardinalis* (Cyprinidae) and *Etheostoma radiosum* (Percidae) in the Washita River system, Oklahoma. The Southwestern Naturalist 34:415-416.

Meyer, A., T. D. Kocher, P. Basasibwaki, and A. C. Wilson. 1990. Monophyletic origin of Lake Victoria fishes suggested by mitochondrial DNA sequences. Nature 347:550-553.

Miller, R. J., and H. W. Robison. 1973. The fishes of Oklahoma. Oklahoma State University Press, Stillwater.

Moritz, C. 1994. Defining 'evolutionary significant units' for conservation. Trends in Ecology and Evolution 9:373-375.

Pierce, P. C., and M. J. Van Den Avyle. 1997. Hybridization between introduced spotted bass and smallmouth bass in reservoirs. Transactions of the American Fisheries Society 126:939-947.

Posada, D., K. A. Crandall, and A. R. Templeton. 2000. GeoDis: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. Molecular Ecology 9:487-488.

Raymond, M., and F. Rousset. 1995. GENEPOP (Version 1.2): a population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248-249.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin ver. 2.000: A software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Stark, W. J., and A. A. Echelle. 1998. Genetic structure and systematics of smallmouth bass, with emphasis on Interior Highlands populations. Transactions of the American Fisheries Society 127:393-416.

Swofford, D. L. 1998. PAUP*: Phylogenetic analysis using parsimony (*and other methods). Version 4, Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

Taylor, C. M., M. Pyron, and M. R. Winston. 1993. Zoogeographic implications for the first record of *Crystallaria asprella* (Percidae) from the Kiamichi River drainage, and for the occurrence of *Notropis boops* (Cyprinidae) and *Luxilus*

chrysocephalus (Cyprinidae) in the Wichita Mountains, Oklahoma. The Southwestern Naturalist. 38:302-303.

Templeton, A. R. 1998. Nested clade analysis of phylogeographic data: testing hypotheses about gene flow and population history. Molecular Ecology 7:381-397.

Templeton, A. R., K. A. Crandall, and C. F. Sing. 1992. A cladistic analysis of phenotypic associations and haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132:619-633.

Templeton, A. R., E. Routman, and C. A. Phillips. 1995. Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. Genetics 140:767-782.

Templeton, A. R., and C. F. Sing. 1993. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. IV. Nested analyses with cladogram uncertainty and recombination. Genetics 134:659-669.

Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. 1997. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 24:4876-4882.

Turner, T. F., J. C. Trexler, J. L. Harris, and J. L. Haynes. 2000. Nested cladistic analysis indicates population fragmentation shapes genetic diversity in a freshwater mussel. Genetics 154:777-785.

Vogele, L. E. 1975. The spotted bass. Pp. 34-45 in Black Bass Biology and Management. H. Clepper (ed.). Sport Fishing Institute, Washington, DC.Wenburg, J. K., P. Bentzen, and C. J. Foote. 1998. Microsatellite analysis of genetic population structure in an endangered salmonid: the coastal cutthroat trout (*Oncorhynchus clarki clarki*). Molecular Ecology 7:733-749.

Waples, R. S. 1991. Pacific salmon, *Oncorhynchus* spp., and the definition of "species" under the Endangered Species Act. U.S. National Marine Fisheries Service Marine Fisheries Review 53:11-22.

Wright, S. 1951. The genetical structure of populations. Annals of Eugenics 15:232-354.

Table 1. Spotted bass sampling locations. Locality numbers correspond with those in Figure 1.

State	Basin	Drainage	Stream/Reservoir
1. Oklahoma	Red River	Cache Creek	Medicine Creek
2. Oklahoma	Red River	Cache Creek	East Cache Creek
3. Oklahoma	Red River	Cache Creek	Lake Ellsworth
4. Texas	Red River	Wichita River	Lake Kemp
5. Oklahoma	Red River	Red River	Lake Texoma
6. Oklahoma	Red River	Blue River	Blue River
7. Oklahoma	Red River	Muddy Boggy River	McGee Creek Reservoir
8. Oklahoma	Red River	Kiamichi River	Sardis Reservoir
9. Oklahoma	Red River	Little River	Broken Bow Reservoir
10. Oklahoma	Red River	Little River	Pine Creek Reservoir
11. Arkansas	Ouachita	Ouachita River	DeGray Reservoir
12. Arkansas	Ouachita	Ouachita River	Greeson Reservoir
13. Oklahoma	Arkansas River	Illinois River	Lake Tenkiller
14. Oklahoma	Arkansas River	Verdigris River	Skiatook Reservoir

Table 2. Primer sequences for the five microsatellite loci used in this study. Forward primers are denoted by F and reverse primers by R.

Locus	Primer Sequence	Reference	Source Species
Lma21	F: CAGCTCAATAGTTCTGTCAGG	Colbourne *et al.* (1996)	*Lepomis macrochirus*
	R: ACTACTGCTGAAGATATTGTAG		
Mdo3	F: AGGTGCTTTGCGCTACAAGT	Malloy *et al.* (2000)	*Micropterus dolomieu*
	R: CTGCATGGCTGTTATGTTGG		
Mdo5	F: CAGGTTCCCTCTCACCTTCA	Malloy *et al.* (2000)	*M. dolomieu*
	R: ATGGTCTCACCAGGGACAAA		
Mdo11	F: TTGTGGAGAGGGGCATAAAC	Malloy *et al.* (2000)	*M. dolomieu*
	R: GCATCCTCCCACGTTACCTA		
Mdo12	F: CACCCTCCCTCTCTTCCTCT	Malloy *et al.* (2000)	*M. dolomieu*
	R: CCATCAACACACGGAGACAC		

Table 3. Pairwise sample site distances based upon river kilometers. Locality numbers correspond to those in Table 1 and Figure 1.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1	--												
2	60	--											
3	85	25	--										
4	400	340	365	--									
5	660	600	625	690	--								
6	950	890	915	980	290	--							
7	970	910	935	1000	310	340	--						
8	1190	1130	1155	1220	530	560	420	--					
9	1555	1495	1520	1585	895	925	785	835	--				
10	1505	1445	1470	1535	845	875	735	785	150	--			
11	2538	2478	2503	2568	1878	1908	1768	1818	1503	1453	--		
12	2463	2403	2428	2493	1803	1833	1693	1743	1428	1378	275	--	
13	3069	3009	3034	3099	2409	2439	2299	2349	2034	1984	1955	1880	--
14	3334	3274	3299	3364	2674	2704	2564	2614	2299	2249	2220	2145	325

Table 4. Microsatellite allele frequencies and measures of variability for 14 populations of spotted bass. Locality numbers correspond with those in Figure 1. Numbers in parentheses = number of individuals. Measures of variability: a = number of alleles, H_o = observed heterozygosity; H_e = expected heterozygosity.

	Locality number													
Locus/ allele	1 (19)	2 (24)	3 (22)	4 (23)	5 (25)	6 (19)	7 (24)	8 (20)	9 (19)	10 (24)	11 (21)	12 (18)	13 (30)	14 (24)
Lma21														
A	0.026	--	--	--	--	--	0.042	0.150	0.053	0.080	--	--	--	--
B	--	0.063	0.188	--	--	--	0.021	0.025	0.211	0.280	0.262	0.237	0.433	0.625
C	--	--	0.083	0.500	--	--	0.021	0.025	0.132	0.300	0.262	0.342	0.100	--
D	--	--	--	--	--	--	--	0.050	--	--	--	--	0.017	0.042
E	0.947	0.938	0.688	0.044	0.860	0.643	0.917	0.725	0.605	0.320	0.452	0.368	0.450	0.333
F	0.026	--	0.042	--	0.140	0.357	--	0.025	--	0.020	--	--	--	--
G	--	--	--	0.239	--	--	--	--	--	--	--	--	--	--
H	--	--	--	0.022	--	--	--	--	--	--	--	--	--	--
I	--	--	--	0.109	--	--	--	--	--	--	0.024	0.053	--	--
J	--	--	--	0.022	--	--	--	--	--	--	--	--	--	--
K	--	--	--	0.065	--	--	--	--	--	--	--	--	--	--
Mdo3														
A	--	--	--	--	--	0.136	0.042	0.175	0.053	--	0.050	0.125	--	--
B	0.947	0.804	0.375	--	0.440	0.432	0.438	0.425	0.553	0.640	0.200	0.325	0.317	0.250
C	0.053	0.196	0.271	--	0.220	0.432	0.521	0.400	0.395	0.360	0.750	0.550	0.683	0.750
D	--	--	--	0.783	0.080	--	--	--	--	--	--	--	--	--
E	--	--	0.354	0.217	0.260	--	--	--	--	--	--	--	--	--

Table 4 *continued*

Locus allele	Locality Number 1 (19)	2 (24)	3 (22)	4 (23)	5 (25)	6 (19)	7 (24)	8 (20)	9 (19)	10 (24)	11 (21)	12 (18)	13 (30)	14 (24)
Mdo5														
A	0.974	0.750	0.566	1.000	0.854	0.925	0.729	0.425	0.263	0.520	0.167	0.290	0.217	0.396
B	0.026	--	--	--	--	--	--	0.025	--	--	--	--	--	--
C	--	0.250	0.438	--	0.146	0.075	0.271	0.550	0.737	0.480	0.833	0.711	0.783	0.604
Mdo11														
A	--	--	--	--	--	--	--	--	--	--	--	--	0.117	0.063
B	0.500	0.438	0.479	0.435	0.500	0.886	0.604	0.525	0.842	0.700	0.405	0.395	0.417	0.188
C	0.500	0.563	0.521	--	0.500	0.114	0.396	0.475	0.158	0.300	0.452	0.474	0.467	0.750
D	--	--	--	--	--	--	--	--	--	--	0.119	0.132	--	--
E	--	--	--	0.565	--	--	--	--	--	--	--	--	--	--
F	--	--	--	--	--	--	--	--	--	--	0.024	--	--	--
Mdo12														
A	0.632	0.188	0.229	--	0.583	--	--	0.125	0.361	0.300	--	--	--	0.063
B	0.263	0.729	0.604	0.457	0.354	0.750	1.000	0.650	0.639	0.660	0.952	0.895	0.950	0.771
C	0.105	0.083	0.167	0.544	0.063	0.250	--	0.225	--	0.040	0.048	0.105	0.050	0.167
Variability														
a	3	2	3	3	3	2	2	3	3	3	3	3	3	3
H_o	0.29	0.39	0.62	0.49	0.42	0.38	0.39	0.56	0.51	0.53	0.45	0.49	0.41	0.50
H_e	0.40	0.39	0.55	0.54	0.51	0.33	0.44	0.56	0.50	0.55	0.45	0.56	0.45	0.48

Table 5. Pairwise mtDNA Φ_{ST} values (above diagonal) and microsatellite F_{ST} values (below diagonal) estimates for 14 populations of spotted bass. Bold & underlined values signify statistical significance with the sequential Bonferroni correction.

Locality	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1		-0.032	0.089	**0.408**	0.058	**0.611**	-0.049	-0.048	-0.029	-0.043	**0.325**	**0.278**	**0.179**	**0.169**
2	**0.140**		0.019	0.299	-0.001	**0.490**	-0.029	-0.009	0.033	-0.038	**0.398**	**0.352**	**0.258**	**0.260**
3	**0.210**	**0.074**		0.130	-0.042	**0.314**	0.088	0.137	**0.207**	0.049	**0.592**	**0.524**	**0.429**	**0.465**
4	**0.528**	**0.470**	**0.351**		0.159	0.250	**0.393**	**0.452**	**0.534**	**0.332**	**0.842**	**0.768**	**0.670**	**0.740**
5	**0.085**	**0.097**	**0.061**	**0.393**		**0.339**	0.058	0.102	0.167	0.024	**0.542**	**0.484**	**0.393**	**0.417**
6	**0.314**	**0.183**	**0.146**	**0.379**	**0.182**		**0.579**	**0.653**	**0.722**	**0.515**	**0.976**	**0.898**	**0.787**	**0.881**
7	**0.341**	**0.088**	**0.104**	**0.480**	**0.188**	**0.122**		-0.041	-0.020	-0.038	**0.313**	**0.279**	**0.186**	0.172
8	**0.261**	**0.085**	0.031	**0.395**	**0.127**	**0.136**	**0.080**		-0.043	-0.027	**0.277**	**0.242**	0.148	0.125
9	**0.338**	**0.197**	**0.102**	**0.454**	**0.202**	**0.202**	**0.191**	**0.064**		0.003	**0.213**	**0.193**	0.101	0.062
10	**0.260**	**0.143**	**0.073**	**0.365**	**0.164**	**0.150**	**0.158**	**0.072**	**0.037**		**0.338**	**0.302**	**0.214**	0.206
11	**0.505**	**0.298**	**0.155**	**0.482**	**0.332**	**0.305**	**0.220**	**0.109**	**0.139**	**0.143**		0.116	**0.098**	0.024
12	**0.407**	**0.215**	**0.098**	**0.396**	**0.256**	**0.226**	**0.170**	**0.062**	**0.102**	**0.074**	0.003		-0.016	0.098
13	**0.477**	**0.270**	**0.146**	**0.492**	**0.324**	**0.295**	**0.205**	**0.108**	**0.131**	**0.130**	0.006	0.024		0.056
14	**0.465**	**0.282**	**0.150**	**0.482**	**0.307**	**0.329**	**0.261**	**0.148**	**0.230**	**0.174**	**0.093**	**0.078**	**0.055**	

Table 6. Distribution of mtDNA haplotypes. Locality numbers correspond to those in Figure 1. Values represent number of individuals.

Species/ Sample site	Haplotype										
	A	B	C	D	E	F	G	H	I	J	K
Spotted Bass											
1	7	12									
2	11	13									
3	14	8									
4	16						7				
5	15	10									
6	19										
7	9	15									
8	6	13					1				
9	5	14									
10	9	14						1			
11		20		1							
12		13	4	1							
13	2	20	7			1					
14	2	21			1						
Smallmouth bass											
Mountain Fork									2		
River											
Byron										1	1
Hatchery											
Baron Fork						2					
River											

Table 7. mtDNA diversity indexes for 14 populations of spotted bass in the Red and Arkansas River basins. Locality numbers correspond with those in Figure 1.

Population	Haplotype diversity (h)	Nucleotide diversity (π)
1. Medicine Creek	0.4912 +/- 0.0683	0.0022 +/- 0.0017
2. East Cache Creek	0.5181 +/- 0.0344	0.0023 +/- 0.0018
3. Lake Ellsworth	0.4848 +/- 0.0637	0.0022 +/- 0.0017
4. Lake Kemp	0.4427 +/- 0.0797	0.0010 +/- 0.0010
5. Lake Texoma	0.5000 +/- 0.0480	0.0022 +/- 0.0017
6. Blue River	0.0000 +/- 0.0000	0.0000 +/- 0.0000
7. McGee Creek Reservoir	0.4891 +/- 0.0569	0.0022 +/- 0.0017
8. Sardis Reservoir	0.5105 +/- 0.0907	0.0021 +/- 0.0017
9. Pine Creek Reservoir	0.4094 +/- 0.1002	0.0018 +/- 0.0015
10. Broken Bow Reservoir	0.5399 +/- 0.0619	0.0025 +/- 0.0019
11. Greeson Reservoir	0.0952 +/- 0.0843	0.0002 +/- 0.0004
12. DeGray Reservoir	0.4510 +/- 0.1174	0.0011 +/- 0.0011
13. Lake Tenkiller	0.5126 +/- 0.0874	0.0017 +/- 0.0014
14. Skiatook Reservoir	0.2355 +/- 0.1093	0.0009 +/- 0.0010

Table 8. Hierarchical distribution of genetic diversity for microsatellite loci and mtDNA. For this analysis populations were grouped into one or the other of three river basins: Red, Ouachita, and Arkansas (see text). Asterisks indicate statistical significance as follows: * <0.01, ** <0.0001.

	Percentage of variation	
Source of variation	Microsatellites	mtDNA
Among basins	10.3*	29.9*
Among populations within basins	16.9**	12.6**
Within populations	72.8**	57.5**

Table 9. Nested contingency analysis of geographical associations based upon 1000 resamples. Clades are the same as in Figure 3 and include only those with a probability value less than 0.05, indicating significant geographical structure. Clades with no genetic or geographic structure are not provided.

Clade	X^2 Statistic	Probability
1-1	52.5	0.014
1-3	50.3	0.029
Entire Clade	124.7	<0.001

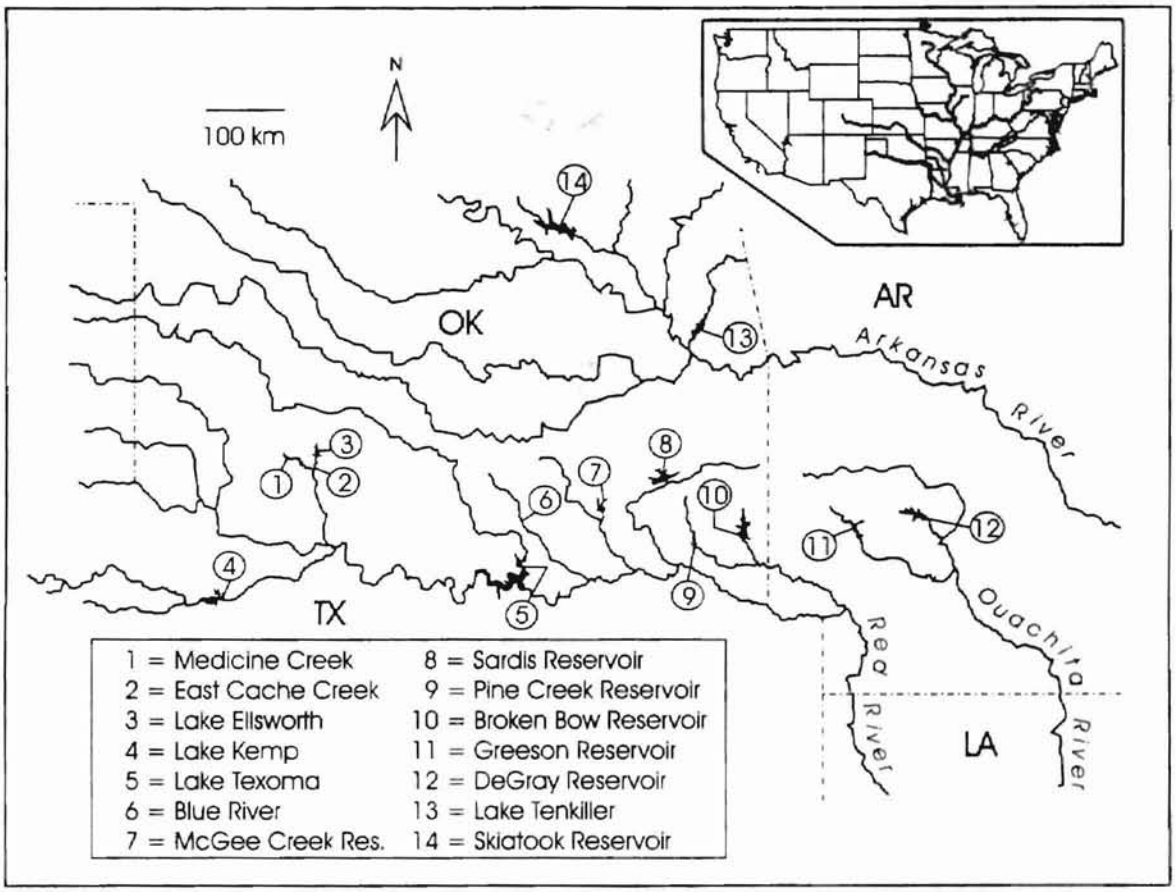

Figure 1. Map of sampling locations.

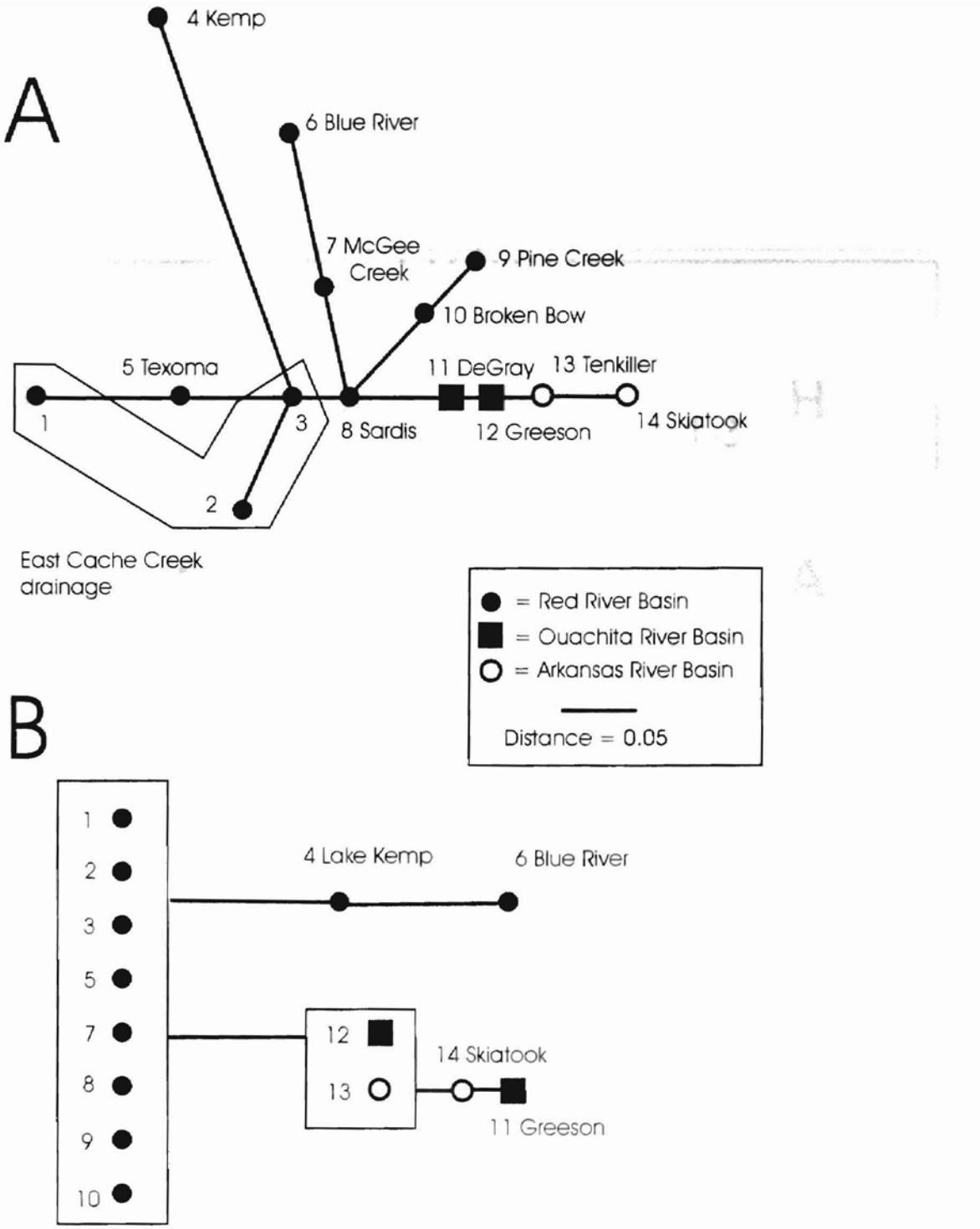

Figure 2. Minimum spanning trees based on (A) pairwise F_{ST} values from five microsatellite DNA loci and (B) pairwise Φ_{ST} values for mtDNA. Locality numbers correspond to those in Figure 1; names indicated are for the corresponding streams or reservoirs. In B, sites within the same box were not divergent; negative Φ_{ST} values were treated as zero (negative values are artifacts of computation; range of Φ_{ST} is 0.0 to 1.0).

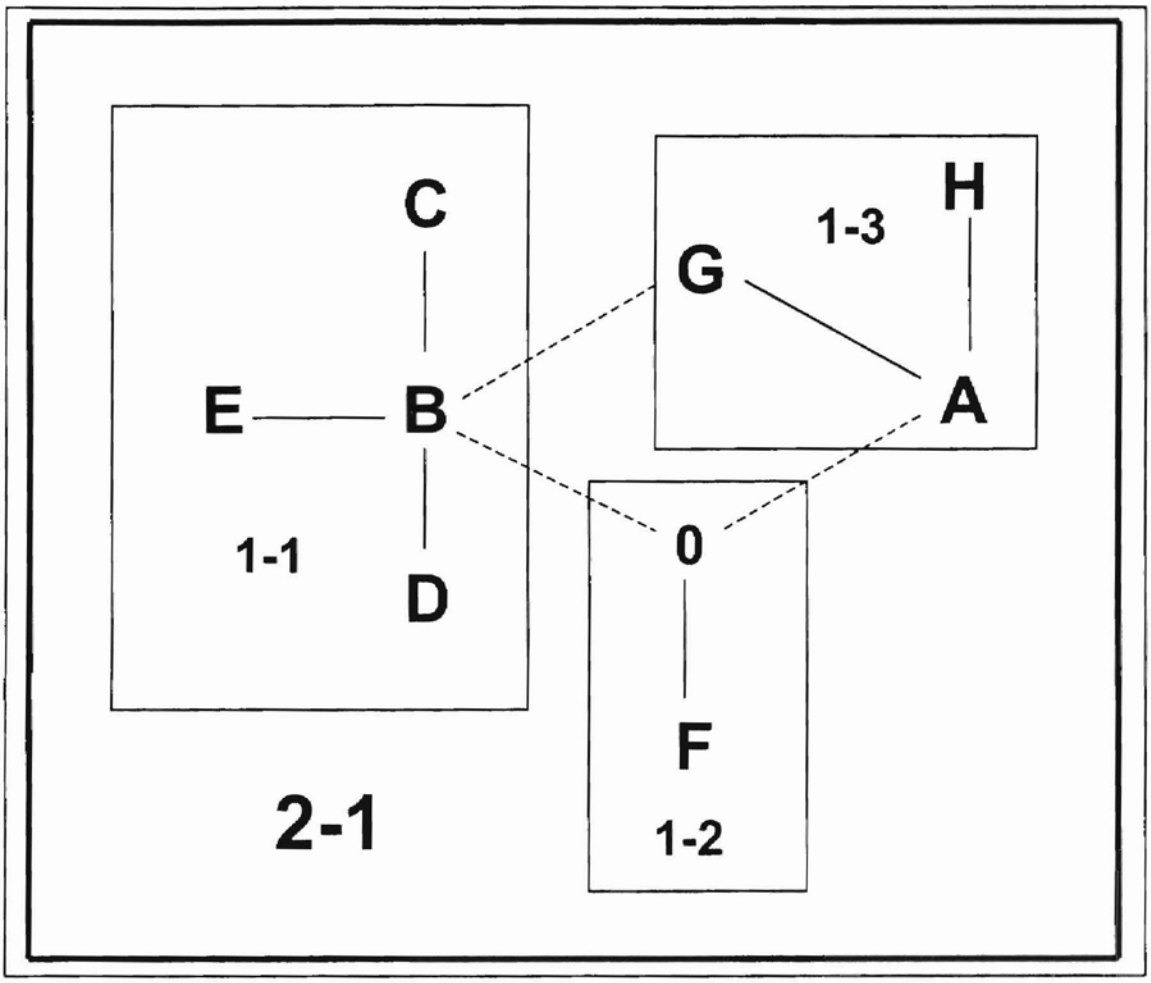

Figure 3. Estimated cladogram based upon parsimony at the 95% level among eight haplotypes found in 14 spotted bass populations. Solid branches between haplotypes represent single mutations while dashed lines identify an ambiguous connection. The zero indicates a missing haplotype. Thin-lined boxes surround 1-step clades (1-1, 1-2, 1-3), and the thick-lined box surrounds the entire clade (2-1).

Haplotypes			1-Step Clades		
Clades	D_c	D_n	Clades	D_c	D_n
B	1652^S	1659^S			
C	1268^S	1709			
D	275	1583			
E	0	1967			
I-T	616^L	-51^S			
1-2-3-5-6-7-8 Yes: RGFLDD			1-1	1675^L	1635^L
F	-	-	1-2	0	2078
A	871	868			
G	488^S	799			
H	0	1081			
I-T	437^L	37			
1-2-3-4 No: RGFIBD			1-3	873^S	1311^S
			I-T	809^L	318^L
			1-2-3-5-6-7-8 Yes: RGFLDD		

Figure 4. Results of the nested clade analysis showing geographical distance for eight haplotypes found in spotted bass. Enclosed boxes represent clades that were nested together based upon one mutational step differences. The opening of each box leads to the next higher level nesting clade. Superscript 'L' indicates a significantly large distance ($P < 0.05$), and superscript 'S' indicates a significantly small distance. Shaded areas represent interior clades. 'I-T' refers to the average distances between interior minus tip clades within a nesting clade. Nested clades with a sequence of numbers in the bottom of their boxes represent the inference chain followed; RGFLDD = restricted gene flow with some long distance dispersal over intermediate areas not occupied by the species; RGFIBD = restricted gene flow with isolation by distance.

VITA

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