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Geographic Distributions of Eastern and Western Mosquitofishes (Poeciliidae: *Gambusia*): Delineation of Ranges Using Fin Ray Counts

GEOGRAPHIC DISTRIBUTIONS OF EASTERN AND WESTERN MOSQUITOFISHES (POECILIIDAE: *GAMBUSIA*): DELINEATION OF RANGES USING FIN RAY COUNTS

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ABSTRACT

Dorsal and anal fin ray counts were made on preserved specimens from 104 sites throughout the southeastern United States to delineate the ranges of the eastern (*Gambusia holbrooki*) and western (*G. affinis*) mosquitofishes. These meristic characters differentiate the species quite well, although not absolutely. Hybrid zones were detected in tributaries to the Mobile River and Gulf of Mexico in the vicinity of Mobile, Alabama and in the mid- and upper-Chattahoochee River drainage. Eastern and western mosquitofishes do not mix randomly, even in populations where hybrids occur. This implies behavioral and/or ecological differentiation between the species. We propose a biogeographic model for the current distributions of *G. affinis* and *G. holbrooki*.

INTRODUCTION

The mosquitofishes, formerly considered to be a single species, *Gambusia affinis* (Poeciliidae), range widely over the southeastern United States. The fact that they are differentiated into eastern and western forms has been known for years (e.g. Hubbs, 1955); however, the taxonomic significance of the differentiation has been subject to different opinions (see Wooten et al., 1988, for a summary). In recent years, various studies have provided genetic data indicating that the western mosquitofish, *G. affinis* (Baird and Girard) and eastern mosquitofish, *G. holbrooki* (Girard) are distinct species (Wooten et al., 1988; Scribner and Avise, 1993).

The distributions of the eastern and western mosquitofishes often have been discussed in general terms, but have not been accurately delimited. For example, Rivas (1963), who considered the two forms to be subspecies, stated that the range of *G. affinis* extended from southern Illinois and Indiana into Alabama, through Louisiana and southern Texas to the Rio Grande. The range of *G. holbrooki* extended from the Delaware River system of New Jersey and Pennsylvania southward and westward along the Atlantic and Gulf drainages of Georgia, Florida and Alabama.

The existence of areas of hybridization also has been long recognized but the precise locations of these populations have not been described. Krumholz (1948) indicated that hybrids occur "between Bay St. Louis, Mississippi and Apalachicola, Florida." Hubbs (1955) mentioned "intergrades, obviously produced through hybridization near the Gulf of Mexico in Mississippi and Alabama." In another paper in which he discussed the long-term stability of intergrade populations, Hubbs (1961) did not specifically identify their location. Hubbs and Lagler (1964: p. 97) referred to *G. affinis* "intergrading in Alabama with *G. holbrooki*." Black and Howell (1979: p. 509) stated that *G. a. affinis* "intergrades in south Alabama and northwest Florida with the subspecies *G. a. holbrooki*." Studies of geographic trends in genetic variation by Wooten et al. (1988) and Scribner and Avise (1993) did not include enough sites in enough drainages to permit accurate resolution of species' boundaries.

Accurate characterization of the distributions of the eastern and western mosquitofishes, as well as regions of hybridization, would be useful (1) in identifying hybrid zones, where further studies may concentrate on the extent of reproductive and genetic isolation between the species, and (2) in providing information that may contribute to our understanding of the biogeography and evolution of southeastern fishes in general. Whereas previous genetic studies have provided extremely useful information about variation between and within the species, time and cost constraints have necessarily limited the number of sites per study and the sample sizes which could be accommodated. In contrast, this study utilized a relatively "quick and dirty" method - based on readily observable meristic characters - to differentiate the two species. Our goal was to keep the means of identification simple so that we could include many more sites, and larger sample sizes per site, than had been possible in the genetic studies. This would allow us to provide more detailed information on the distributions of the two species of mosquitofishes, and their areas of hybridization, than has been available.

MATERIALS AND METHODS

Meristic characters

This investigation utilized dorsal and anal fin ray counts to distinguish between *G. affinis* and *G. holbrooki* and their hybrids. These characters discriminate the eastern and western mosquitofishes quite well: *G. affinis* typically has 6 dorsal and 9 anal rays (following the methods of Hubbs and Lagler, 1964); *G. holbrooki* typically has 7 dorsal and 10 anal rays. For both the dorsal and anal fins, Hubbs and Lagler (1964) considered the (apparent) last two rays to be a single branched ray and accordingly suggested that they be counted as one. These species also differ in characteristics of the gonopodium (D'Ancona, 1939; Rosen and Bailey, 1963). We did not routinely use these characters because (1) gonopodia are present only in males, and (2) the dorsal and anal fin ray counts were sufficient to serve our needs.

A fish was classed as having a "hybrid" ray count if it had a dorsal ray count characteristic of one species and an anal ray count characteristic of the other. This method of characterization was not expected to identify all hybrid individuals, but the presence of significant numbers of individuals with hybrid ray counts was considered indicative of a population where hybridization was occurring. Often, when mature males were identified as hybrids on the basis of dorsal and anal ray counts, the hybrid nature of these individ-

uals was supported by their gonopodium, which was intermediate in characteristics that distinguish the two species. Dorsal and anal fin ray counts were made on 20 specimens or more (males, females and juveniles) from most sites although some collections (mostly from the Mobile, AL area) consisted of fewer specimens.

Collection sites

Fishes used for this study were obtained from a total of 104 sites throughout the southeastern United States. Sources of the preserved fishes include 52 collections made from 1 June - 15 July 1983 as part of an electrophoretic study of biochemical variation (Wooten, et al., 1988). Other preserved specimens were provided on loan from museum collections: University of Alabama Ichthyological Collection (29 sites shown in this paper), Mississippi State University Ichthyological Collection (eight sites), and Clemson University Vertebrate Collection (12 sites). Collections at three sites were personally made by the authors. Dates of collection ranged from 1951 to 1991. Because a hybrid zone has long been recognized to exist in the Mobile, Alabama area, numerous sites from that region were included. In fact, fin rays were counted on fish from more sites in the Mobile vicinity than could be shown in Fig. 1. Results for all sites included in this study are available in tabular form from the authors.

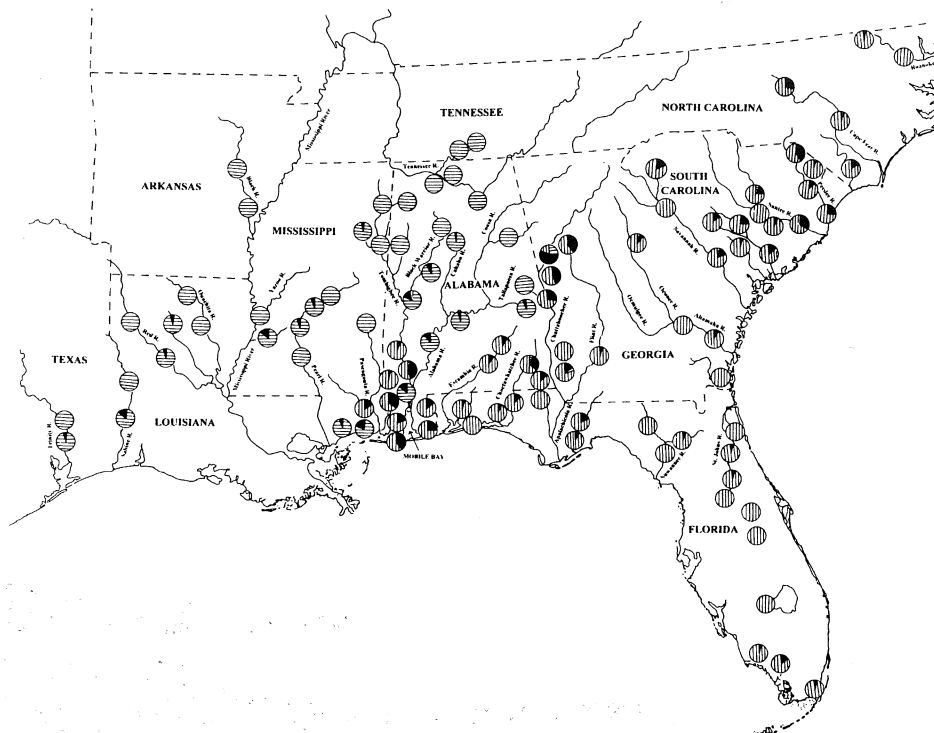


Figure 1. Composition of mosquitofish populations from throughout the southeastern United States. The proportion of *Gambusia affinis* in each population is indicated by the circle sector with horizontal striping, the proportion of *G. holbrooki* by vertical striping, and the proportion of hybrid fin ray phenotypes by black shading.

RESULTS

Fig. 1 illustrates the fin ray counts obtained for mosquito-fish populations from throughout the southeastern United States. In the figure, each collection site is represented by a circle. Within each circle, the fraction of the fish with both dorsal and anal counts typical of *G. affinis* is shown by a sector with horizontal stripes. The fraction of the fish with both dorsal and anal counts typical of *G. holbrooki* is shown by a sector with vertical stripes. The fraction of the fish with a dorsal count typical of one species and an anal count typical of the other (e.g. a "hybrid" combination) is shown by a shaded sector.

The long-recognized hybrid zone in the vicinity of Mobile, Alabama can be readily discerned in Fig. 1 by the presence of numerous individuals with hybrid ray counts in those populations. A second hybrid zone is also apparent in the Chattahoochee River drainage from the point where the river meets the Alabama-Georgia state line northeast toward the headwaters. This zone was recently described by Lydeard, et al. (1991).

Many of the populations in the rivers draining into the Atlantic north of the Altamaha also contained substantial numbers of individuals with "hybrid" ray counts. These do not represent the products of recent hybridization; *G. affinis* has not been collected in most of these drainages. For possible explanations of this phenomenon, see the Discussion.

Other sites throughout the southern and southeastern U.S. infrequently contain individuals with "hybrid" ray counts. We consider these to represent variation within species because the populations are remote from any areas of known contact between species.

DISCUSSION

Dorsal and anal ray counts differentiate the eastern and western mosquitofishes well, although not absolutely as some variability occurs within species. Thus, not all fish with "hybrid" ray counts represent the products of interspecific hybridization (at least not recent hybridization). Nevertheless, these characters are consistent enough to be useful in identifying the ranges of *G. affinis* and *G. holbrooki* and areas where hybridization is occurring. A hybrid zone is identifiable as a cluster of populations, all with significant numbers of individuals with hybrid ray counts.

Genetics

Analysis of the fin ray counts in hybrid zones provides information on the genetic basis of these traits. It appears that the genetic bases of fin ray counts are more complex than previously thought. Hubbs (1955) observed that F_1 hybrids between *G. affinis* and *G. holbrooki* always had 7 dorsal and 10 anal rays, indicating that *G. holbrooki* is "completely dominant in this regard." Although he made no mention of

observing typical Mendelian ratios in backcross or F_2 progeny, Hubbs concluded that the dorsal and anal fin ray differences were due to single genes. One might expect that, if the genes for *G. holbrooki* ray counts are dominant, the result would be an abundance of *G. holbrooki* phenotypes in hybrid zone populations. However, this is not seen.

There are two common "hybrid" combinations of dorsal and anal ray counts (6,10 or 7,9). In populations where *G. holbrooki* was the predominate species, a highly significant majority of hybrid counts fell into the 6,10 category (Table 1). In the relatively few populations where *G. affinis* predominated, a significant majority fell into the 7,9 category (Table 1). In hybrid populations, the anal ray count tends to be that of the parental species that predominates, but the dorsal ray count tends to be that of the species that is rare or absent. A simple single-gene Mendelian model will not explain this situation. Additionally, this unbalanced distribution of ray counts indicates that random two-way introgression of genes is not occurring within hybrid populations. This is consistent with results obtained in a recent molecular genetic study (Scribner and Avise, 1993).

One *G. affinis* population included in this study had a notable tendency toward a reduced number of dorsal rays. Half (10/20) of the fish from a population in the Elk River in Lincoln County, Tennessee (UAIC 1981) had 5 dorsal rays instead of the normal 6. Because this variation is away from the *G. holbrooki* condition, this variation would not produce pseudo-hybrid phenotypes. Nearby populations from the same drainage did not show this trend. It thus appears that the population has experienced rather unique alteration in frequency of the genes influencing dorsal ray count. At present, it is not possible to determine whether this has been due to local selective forces or whether random genetic drift in a somewhat isolated deme is responsible for the variation in this character.

Table 1. Total numbers of fish with each of the two possible hybrid phenotypes. Collection sites were divided into two groups according to the species that was numerically dominant at the site. Chi-square values were calculated based on the hypothesis of equal numbers of hybrid types in each species. Contingency table analysis supports the hypothesis that the distribution of fin ray counts differs significantly between species ($\chi^2 = 40.9$, $p < 0.001$).

Numerically Dominant Species	Fin Ray Counts (dorsal, anal)		χ^2	Probability
	6,10	7,9		
<i>G. holbrooki</i>	190	48	83.5	< <0.001
<i>G. affinis</i>	10	25	5.6	0.018

Geographic Patterns

Geographic patterns of variation in meristic characters (this study) correlate well with patterns of biochemical variation observed in mosquitofishes in other studies. Wooten et al. (1988) surveyed allozyme-encoding genetic variation in mosquitofish populations throughout the southeastern United States. They observed a major disjunction in the Mobile Bay, Alabama area. To the north and west, the populations were primarily *G. affinis*, whereas to the east, along the Gulf coast, the populations were primarily *G. holbrooki*. Scribner and Avise (1993) surveyed mitochondrial DNA and allozyme genetic markers in populations throughout the southeastern U.S. They found a pronounced genetic discontinuity along a line extending from southeastern Mississippi to northeastern Georgia demarking the area where the distributions of *G. affinis* and *G. holbrooki* abut.

Wooten, et al. (1988) detected a zone of hybridization in the Mobile area that has been recognized for years (e.g. Hubbs, 1955). The zone is also reflected morphologically by an abundance of hybrid ray counts in populations in the Mobile area. The geographic extent of the hybrid zone is well delineated in Fig. 1.

Wooten et al. (1988) distinguished a secondary genetic subdivision along the Atlantic coastal region of Georgia between the Savannah and Altamaha rivers in a region inhabited by *G. holbrooki*. One form was found in North and South Carolina and Georgia, the other from the Altamaha river drainage in Georgia, west to the Mobile Bay region. Scribner and Avise (1993) also detected a genetic subdivision among *G. holbrooki* populations which distinguished many (but not all) populations from the Gulf coastal plain from those to the east in peninsular Florida, Georgia, and the Carolinas.

Our data support the idea of geographic genetic subdivision in *G. holbrooki*. Populations north of the Altamaha show much more variation in fin ray counts than do populations to the south (Fig. 1). In some of the Atlantic coast populations, as many as one third of the individuals had "hybrid" ray counts. These sites do not appear to represent a hybrid zone since *G. affinis* has not been found in the region. The extent and taxonomic significance of the genetic differentiation in *G. holbrooki* remains uncertain. In fact, not all studies reveal this subdivision. For example, Hernandez (1988) did not find genetic evidence indicative of two forms of Atlantic coast *G. holbrooki*.

Other zones of contact and hybridization between *G. affinis* and *G. holbrooki* have been recently identified. Lydeard et al. (1991) used both allozyme and fin ray count data to demonstrate the presence of *G. affinis* in the Savannah and Chattahoochee drainages, previously thought to be entirely within the range of *G. holbrooki*. Based on comparisons between fin ray counts and allozyme genotypes, they concluded that intergradation in hybrid zones has apparently gone beyond the F₁ stage.

The present study confirms and extends the findings of Lydeard et al. (1991). In the Chattahoochee (Fig. 1), a zone of hybridization is evident in the collections from the middle regions of the river in the vicinity of Auburn, AL. Although

this study includes few collections from the headwaters of the Savannah, one of them shows about 20% of the individuals having hybrid ray counts.

Systematics

Rivas (1963) considered the eastern and western mosquitofishes to be subspecific, based primarily on the presence of areas of intergradation. However, data from subsequent studies have indicated that the two forms are distinct species. Both Black and Howell (1979) and Reznick (1981) identified a degree of reproductive isolation between the eastern and western mosquitofishes. Wooten et al. (1988) identified "substantial genetic differentiation between the two forms...indicative of differentiation at the species level." Their genetic survey also indicated that, although hybrid zones exist, the two forms of mosquitofish do not appear to be merging as a result of introgression of genes. Overall, Wooten et al. (1988) found little evidence of significant gene flow between *G. affinis* and *G. holbrooki*. Based on the previous reports of reproductive isolation and on their own observations of abrupt shifts in gene frequencies between the forms, Wooten et al. (1988) recommended restoration of D'Ancona's (1939) taxonomic designations for southern U.S. mosquitofishes as separate species: *G. holbrooki* and *G. affinis*.

Scribner and Avise (1993) used both nuclear and mitochondrial genetic data in their study. They also found a pronounced genetic discontinuity between *G. holbrooki* and *G. affinis*. Although they found evidence of hybridization, they saw pronounced gametic disequilibria among nuclear genotypes in hybrids, indicating that randomization of genotypes under hybridization was not occurring.

Results of the present study support the conclusion that the eastern and western mosquitofishes are separate species. Behaviorally or ecologically they appear to avoid each other. Even in areas of sympatry where hybridization has occurred, we found a strong degree of segregation. At none of our sample sites did eastern and western mosquitofishes occur in frequencies typical of haphazardly mixed groups, as would be expected of conspecifics. At each site, even if hybrids were present, one species overwhelmingly predominated and the other was rare or (usually) absent. It was also clear that random factors alone did not determine which species would predominate in a sympatric population. Of 39 sites in the Mobile, AL area that contained fish with hybrid ray counts, *G. holbrooki* was the numerically dominant species in 33 of them. This is significantly different from a 50:50 ratio ($p < 0.001$, G test) and is consistent with the idea that *G. holbrooki* is a superior competitor to *G. affinis* and is presently expanding its range by displacing *G. affinis* (see below).

Biogeographic Scenarios

Swift et al. (1986) studied the geographic distribution limits of numerous fish species in the southeastern U.S. The major features of distributional limits summarized in that report were highly concordant with those of Bermingham and Avise (1986) who investigated geographic patterns of mtDNA

phylogenies. Both studies identified three major geographic boundary areas in the southeastern United States: the Apalachicola River, the Alabama/Tombigbee and north Florida.

Based on genetic differentiation between four species of *Lepomis*, Bermingham and Avise (1986) proposed a biogeographic scenario in which major genetic differentiation occurred in the Pliocene interglacial, when sea level was 50-80 m higher than at present. Under such conditions, they considered it likely that many of the major southern drainages, with headwaters above the physiographic Fall Line, were well isolated. The Ocala highlands region in north-central peninsular Florida offered another potential refuge area for freshwater fish. The major genetic effects of the high sea-level stand were hypothesized to be the extinction of populations in smaller Coastal Plain rivers and the opportunity for significant genetic divergence between populations in the headwaters and/or Florida refuge areas. Bermingham and Avise (1986) considered the present day distributions and boundaries as reflecting dispersal subsequent to the receding of sea level after the Pliocene high sea-level stand.

For mosquitofish, both Wooten et al. (1988) and Scribner and Avise (1993) advocated an evolutionary scenario that involves two major phylogenetic events: (1) divergence of *G. affinis* and *G. holbrooki*, perhaps as a result of isolation on opposite sides of the Appalachians during seawater elevations of the Pliocene or late Miocene, and (2) later derivation of a somewhat distinct Atlantic coastal form of *G. holbrooki*, probably from a Florida population.

We propose the following biogeographic model for the current distribution of *G. affinis* and *G. holbrooki* which is consistent with the data and conclusions of Wooten et al. (1988) and Scribner and Avise (1993). Prior to the Pliocene interglacial, *G. affinis* was widely distributed throughout the southeastern U.S. During the Pliocene high sea-level stand, *G. holbrooki* evolved in a Florida refuge area from *G. affinis* stock. Thus, *G. affinis* genetic markers can still be found in *G. holbrooki* populations (Scribner and Avise, 1993). After sea level receded, *G. holbrooki*, which appears to be a better competitor than *G. affinis* (Scribner, 1993; Scribner and Avise 1994a, 1994b), expanded its range and displaced *G. affinis*. The present distribution reflects the invasion of *G. affinis* territory by *G. holbrooki* as it dispersed from Florida northward along the Atlantic coast and westward along the Gulf coast. Because *G. holbrooki* tends to displace *G. affinis*, the only locations where both species occur are at the front edges of the current line of advance of *G. holbrooki*. Such sites include the Mobile, AL area and the Chattahoochee River near Auburn, AL.

Although populations of pure *G. affinis* apparently no longer exist in the coastal Atlantic drainages, the presence of significant numbers of fish with hybrid ray counts is consistent with the idea of the persistence of at least some *G. affinis* genes in populations of *G. holbrooki* that have since replaced them. If this model is correct, it might be possible to find relict populations of *G. affinis* in the headwaters of the streams east of the Mobile drainage. In fact, this has been done.

Lydeard et al. (1991) found *G. affinis* in the middle Chattahoochee and upper Savannah. The present study also found fish with pure *G. affinis* ray counts in the upper Chattahoochee. Lydeard et al. (1991) speculated that these *G. affinis* came either (1) from the Mobile or Tennessee River drainages via stream capture, or (2) were transplanted by humans. We think it is also possible that they represent surviving isolates from a time when *G. affinis* was distributed widely throughout streams in the southeastern U.S. If this hypothesis is correct, further collecting in the upper reaches of other southeast Atlantic drainages may also identify populations of *G. affinis* that could not have gotten there by stream capture from drainages where they presently occur.

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