Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 5066–5069, May 1994
Evolution

Cytonuclear genetics of experimental fish hybrid zones inside Biosphere 2

(mosquitofish/introgression/allozymes/mitochondrial DNA)

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Contributed by John C. Avise, February 24, 1994

ABSTRACT **Two species of mosquitofish (family Poeciliidae) known to hybridize in nature were introduced into freshwater habitats inside Biosphere 2, and their population genetics were monitored after 2 years. Within four to six generations, nuclear and cytoplasmic markers characteristic of *Gambusia holbrooki* had risen greatly in frequency, although some *Gambusia affinis* alleles and haplotypes were retained primarily in recombinant genotypes, indicative of introgressive hybridization. The temporal cytonuclear dynamics proved similar to population genetic changes observed in replicated experimental hybrid populations outside of Biosphere 2, thus indicating strong directional selection favoring *G. holbrooki* genotypes across the range of environments monitored. When interpreted in the context of species-specific population demographies observed previously, results suggest that the extremely rapid evolution in these zones of secondary contact is attributable primarily to species differences in life-history traits.**

Hybrid zones provide excellent settings to study microevolutionary processes for at least two reasons (1–5). First, numerous genetic markers that distinguish the participating taxa normally can be uncovered, thus facilitating studies of natural selection, gene flow, and mate choice in the hybrid region. Second, ecological interactions between the parental species, and genetic interactions between the genomes they contribute to hybrids, might both tend to be magnified in contact zones, thereby making their effects easier to document. However, most prior research on secondary hybrid zones has involved static descriptions of contact regions in nature, where pertinent variables (such as migration of parental types into the region and sizes and ages of the admixed founding populations) remained uncontrolled (4, 5). Here we employ a series of controlled experimental populations to monitor temporal cytonuclear changes in artificial contact zones between two mosquitofish species that are known to hybridize over a broad area in the southeastern United States (6–8).

The current set of experiments was conducted inside Biosphere 2, a futuristic glass and steel "greenhouse" near Tucson, Arizona, that was designed as a self-sustaining mesocosm and as a prototype for the scientific study of closed ecological systems (9). Covering about 3.5 acres, Biosphere 2 is almost completely sealed off from atmospheric or other material exchange with the outside world ("Biosphere 1") and contains about 4000 species of introduced plants and animals distributed among a variety of habitats ranging from desert to tropical rain forest and coral reef. The availability of two freshwater systems inside Biosphere 2 (a stream and a marsh) provided a unique opportunity for the experimental study of fish contact zones in reasonably complex but closed habitats. Genetic results for the *Gambusia* populations inside Biosphere 2 will be compared to those previously observed in experimental populations in ecologically simpler pool and pond environments in South Carolina (10).

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MATERIALS AND METHODS

In September of 1991, immediately prior to the first prolonged (2 year) closure of Biosphere 2, approximately equal numbers of *Gambusia affinis* and *G. holbrooki* were introduced into each of two separate and distinctive bodies of water within the facility: "savannah stream" (total $n \approx 70$) and "freshwater marsh" ($n \approx 150$). The founders were obtained from allopatric natural populations that previously had been characterized genetically (6): *G. affinis* from Lake Arthur, Louisiana, and *G. holbrooki* from the Savannah River Site near Aiken, South Carolina. Immediately following the reopening of Biosphere 2 in September of 1993, 112 presumed descendents of these *Gambusia* founders were collected. These fish were genetically typed for proteins encoded by five autosomally inherited nuclear (allozyme) loci and for two maternally inherited mitochondrial DNA (mtDNA) markers, all of which were known to be species diagnostic (6, 11, 12).

Similar experiments over a 2-year period (1989–1990) had been conducted on experimental *Gambusia* populations in artificial pools and ponds at the Savannah River Site (10, 13). These experimental populations were initiated with founders from the same source populations as above. In the "pool" experiments, three males and three virgin females of each species ($n = 12$ founders) were introduced into each of two small pools 2.4 m in diameter and 30 cm deep. In the "pond" experiments, 60 adult males and 60 adult females of each species ($n = 240$ founders) were introduced into each of two ponds about 12 m long, 7 m wide, and 1.5 m deep. Escape cover and plankton food sources were added at monthly intervals, but both sets of habitats remained "uncomplicated" (small in size, structurally simple, and lacking in predators and competing species). At the end of the second breeding season, cytonuclear genotypes were determined for all individuals in the pool populations (total $n = 171$) and for 100 juveniles sampled at random from each pond population.

Protein-electrophoretic assays were conducted on all specimens ($n = 483$ total). The species-diagnostic proteins examined were adenosine deaminase (E.C. 3.5.4.4) (Fig. 1), aspartate aminotransferase 1 (E.C. 2.6.1.1), malate dehydrogenase 1 (E.C. 1.1.1.37), peptidase A (E.C. 3.4.11.-) (leucylalanine as substrate), and aconitate hydratase 1 (E.C. 4.2.1.3). The allozyme assays involved conventional starch-gel electrophoretic procedures, using as tissue source cellular debris taken from the initial low-speed centrifugations employed to isolate mtDNA (see below).

Abbreviation: mtDNA, mitochondrial DNA.

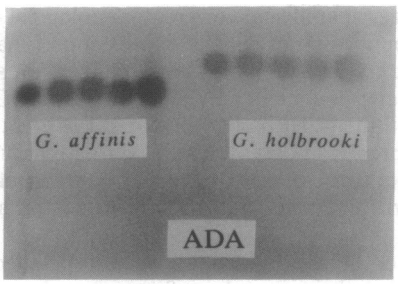

FIG. 1. Example of an electrophoretic gel for a species-diagnostic allozyme locus (adenosine deaminase; ADA).

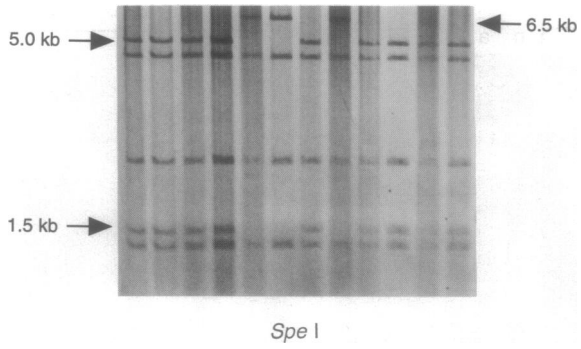

FIG. 2. Example of an autoradiograph for a species-diagnostic mtDNA restriction fragment-length polymorphism produced by *Spe* I. Lanes 5, 6, and 8 from the left are digestion profiles characteristic of *G. holbrooki*; the remainder are characteristic of *G. affinis*.

mtDNA was extracted from each fish using a rapid isolation alkaline lysis procedure (14). The mtDNA was resuspended in 60 μl of TE and dialyzed to remove excess salt (15). For all specimens exhibiting hybrid multilocus nuclear genotypes, restriction assays (*Hin*dIII, all experiments; *Spe* I, Biosphere 2 experiments only) were employed to reveal species-specific mtDNA digestion profiles (e.g., Fig. 2). Restriction fragments were end-labeled with ^{35}S radionuclides, separated on 1.0% agarose gels, and visualized by autoradiography (16, 17).

RESULTS

Similar temporal changes were observed in the genetic architectures of the stream and marsh populations of *Gambusia* fishes inside Biosphere 2 (Fig. 3). The two species clearly had hybridized, as evidenced by the presence of various recombinant genotypic classes. For example, about 5.1% of individuals in the marsh sample were most likely F_1 hybrids (heterozygous at all five diagnostic allozyme loci), 10.3% were the probable progeny of backcross matings to *G. holbrooki* (homozygous for *G. holbrooki* alleles at some loci, heterozygous at others), and 2.1% were F_2 or later-generation hybrids (alternately homozygous for *G. holbrooki* and *G. affinis* alleles at different loci). Furthermore, both populations showed pronounced and nearly identical increases in the frequency of *G. holbrooki* nuclear alleles and mitochondrial haplotypes at the expense of those from *G. affinis* by the end of year two (Fig. 3).

One unanticipated finding in the Biosphere 2 studies was the presence (in 30% frequency) of mtDNA genotypes other than those introduced by our founders (Fig. 4). Further inspection against prior geographic studies from natural *Gambusia* populations (6) indicated that these mtDNA genotypes probably stem from pure *G. holbrooki* in southern Florida that apparently had been introduced when the biomes

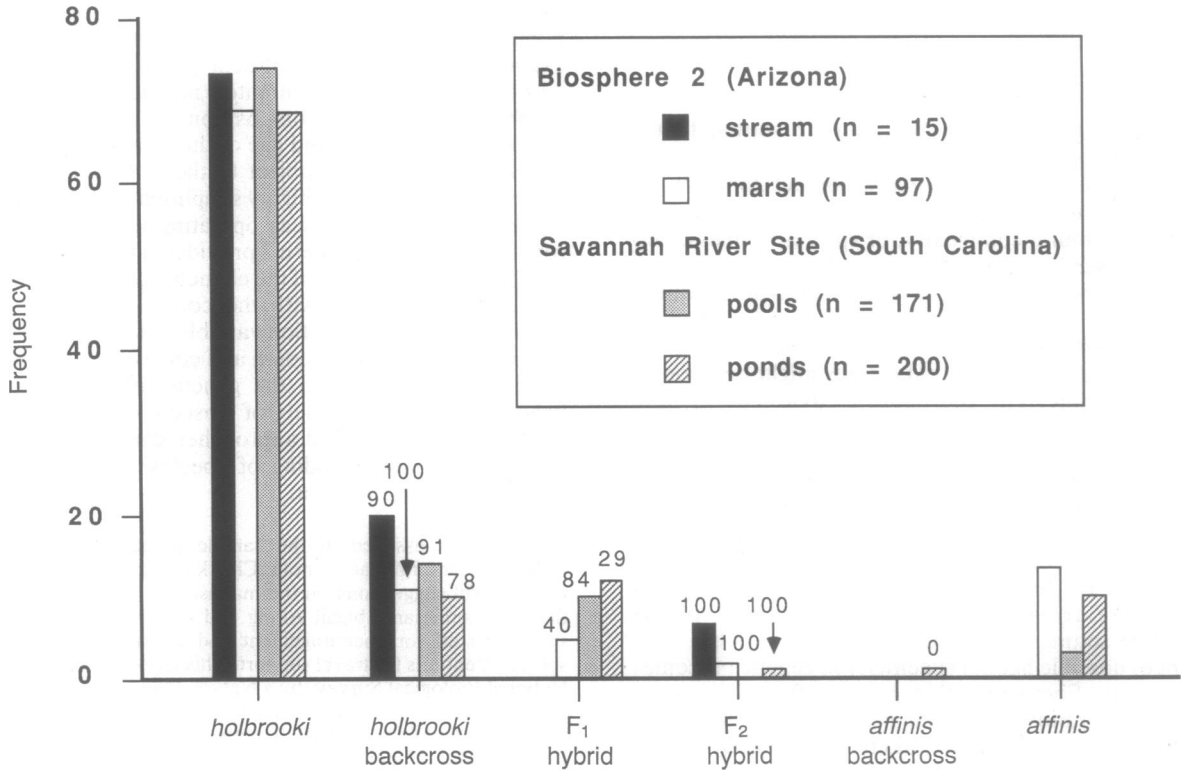

FIG. 3. Histograms showing frequencies of individuals classified as pure parentals, probable F_1's, backcrosses, and other recombinants in each of four experimental studies of *Gambusia* hybridization, after two breeding seasons (approximately four to six generations). These classifications were based on five species-diagnostic allozyme loci. Also shown by numerical values above the histogram bars are percent frequencies of *G. holbrooki* mtDNA. In the pools and ponds, sample sizes cover both replicates, and frequencies of genotypic classes represent mean values. Sample size from the Biosphere 2 stream was small because few *Gambusia* fishes could be found there after the 2 years.

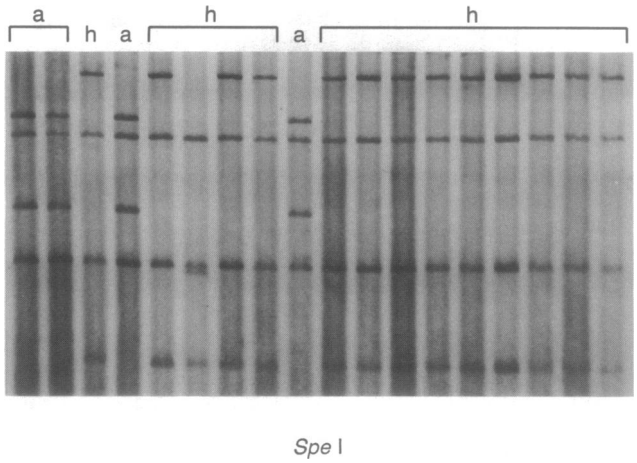

FIG. 4. *Spe* I digestion profiles for the mtDNAs of *G. affinis* (a) and *G. holbrooki* (h). The banding patterns in lanes 3, 5, 7, 8, and 10–18 may trace to maternal lineages of *G. holbrooki* from Florida (see text).

originally were installed in 1990. The exact number (and reproductive fate) of inadvertently introduced males and females from Florida remains unknown; this adds an unwanted complication to interpretations of the observed temporal genetic changes.

In any event, the genetic outcomes in the Biosphere 2 populations were nearly identical to those observed in the experimental populations at the Savannah River Site (Fig. 3). As in the marsh and stream habitats of the Biosphere facility, interspecific hybridizations clearly had occurred in the South Carolina pools and ponds, and there were rapid and parallel increases in the frequencies of *G. holbrooki* nuclear alleles and mitochondrial haplotypes at the expense of those from *G. affinis*.

DISCUSSION

One rationale for adding the Biosphere 2 setting to the experimental design was that distinctive cytonuclear dynamics might well characterize *Gambusia* contact zones in different environmental regimes. For example, in habitats of greater size, structural complexity, or species richness, perhaps species-specific differences in habitat choice or mate selection are allowed fuller expression, such that mosquitofish would display less propensity to compete or to hybridize. Furthermore, patterns of survival and reproduction of the parental species or various hybrid classes might be strongly environment-specific. The Biosphere 2 environments were undoubtedly more complex than the experimental pools and ponds in South Carolina in several regards: they were larger in size (the marsh covered 400 square meters), contained far more physical and vegetative structural diversity, and included several other introduced fish species that might have served as potential competitors and predators. Nonetheless, most aspects of the temporal population genetic changes in *Gambusia* were remarkably consistent across all of the environmental settings monitored.

One hint of possible habitat specificity in genetic outcomes was uncovered. First-generation hybrids from the larger enclosures in both Biosphere 2 (the marsh) and the Savannah River Site (ponds) were more likely to carry *G. affinis* mtDNA than were F_1 hybrids from the small pool environments (Fig. 3). Experimental studies of mating preference (K.T.S., unpublished data) indicate that when offered a choice, female *G. holbrooki* tend to mate assortatively (with homospecific partners), whereas *G. affinis* females mate randomly with males of both species. Perhaps in the situations of larger spatial scale or greater ecological complexity, enhanced opportunities for the expression of mating preferences by *G. holbrooki* females were realized, thus accounting for the lower frequency of F_1 individuals with *G. holbrooki* mtDNA. On the other hand, all F_2 and the great majority of backcross progeny possessed *G. holbrooki* mtDNA (Fig. 3), an outcome consistent with earlier reports of decreased fecundity in F_1 females with *G. affinis* maternal parentage (18, 19).

Most prior studies addressing genotype-specific fitness differences in hybrid zones have focused on the fecundity and viability of hybrids relative to parental genotypes. However, species-specific demographic factors (e.g., gestation length, offspring birth size, growth rates, and size and age at sexual maturity) could in principle also dramatically influence genetic outcomes in contact regions (20, 21). Such life-history differences have been reported for mosquitofish (18) and shown to confer upon *G. holbrooki* (relative to *G. affinis*) a higher population carrying capacity, higher recruitment rate, lower overwinter mortality, and diminished susceptibility to cannibalism (a major source of juvenile mortality in *Gambusia*). Thus, life-history differences between these species probably contribute importantly to the dramatic and consistent temporal shifts toward *G. holbrooki* that were observed in Biosphere 2, and in the other experimental pool and pond populations. These demographic features may also contribute to certain aspects of the cytonuclear patterns observed in the natural hybrid zone between *G. affinis* and *G. holbrooki* across the southeastern United States (6), including the strong genetic disequilibria (associations among species-specific genotypes at different loci) in the hybrid region and the apparent introgression of *G. holbrooki* mtDNA into *G. affinis* populations.

It is difficult to disentangle the multitudinous evolutionary forces that can shape the genetic features of hybrid zones in nature. The experiments conducted on hybrid *Gambusia* populations in the pools and ponds in South Carolina allowed explicit control of important demographic variables that are notoriously difficult to monitor in natural settings (e.g., numbers and genetic composition of founders, levels of exogenous gene flow, and age of the hybrid population), but the findings remained subject to the criticism that the processes revealed in these highly simplified systems may not be representative of processes operating in nature. The environments within Biosphere 2 provided a useful compromise, combining at least some of the elements of habitat complexity found in Biosphere 1 with the considerable experimental control of critical population variables that is possible only in a closed system. The strength and consistency of directional selection documented in the genetic assays of *Gambusia* fishes across a wide variety of closed environments demonstrate how experimental approaches can contribute significantly to an understanding of species' contact zones and hybridization phenomena.

Ray Fields assisted in the sample preparations and mtDNA isolations. Drs. Michael Arnold, Chuck Lydeard, and Mike Wooten made useful suggestions on the manuscript. We especially wish to thank Biospherian Abigail Alling and other members of the Biosphere 2 staff for encouragement and assistance and Space Biospheres Ventures for travel support. This work was supported by the National Biological Survey, by a National Science Foundation grant to J.C.A., and by the Savannah River Ecology Laboratory under Contract DE-AC09-765R00819 between the U.S. Department of Energy and the University of Georgia's Institute of Ecology.

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