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CHARACTERIZATION OF MITOCHONDRIAL DNA VARIABILITY IN A HYBRID SWARM BETWEEN SUBSPECIES OF BLUEGILL SUNFISH (*LEPOMIS MACROCHIRUS*)

JOHN C. AVISE, ELDREDGE BERMINGHAM, LOUIS G. KESSLER, AND NANCY C. SAUNDERS
Department of Molecular and Population Genetics, University of Georgia, Athens, Georgia 30602

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Genetic variation in mitochondrial DNA (mtDNA) has been studied in populations of mammals, *Drosophila,* and a few other higher animals (reviews in Avise and Lansman, 1983; Brown, 1983). Among the noteworthy features of mtDNA variability pertinent to the analysis of evolutionary process are the following: a rapid rate of evolution involving primarily base substitutions plus very small addition/deletions (Brown et al., 1979; Cann and Wilson, 1983); a remarkable stability of gene content and arrangement; apparent mtDNA sequence homogeneity (homoplasmicity) in somatic cells within an individual organism; extensive sequence polymorphism among conspecifics; and maternal transmission of mtDNA to progeny. Empirical and conceptual reservations apply to some of these generalities. For example, low frequency mtDNA variants within an individual would normally escape empirical detection, and, given the ubiquity of between-individual sequence differences, it would seem that at least some transient mtDNA heteroplasmicity (perhaps in germ cell lineages) is inevitable (see Olivo et al., 1983). Also, the consequential possibility remains that some mtDNA molecules are successfully transmitted to progeny from the male parent (paternal leakage; but see Lansman et al., 1983).

The main purpose of this study is to begin the characterization of mtDNA polymorphism in another class of vertebrates—the fishes. Our approach has been to map mtDNA restriction site variation in the bluegill sunfish (*Lepomis macrochirus*; Centrarchidae), using a large number of restriction endonucleases. We have chosen an evolutionary setting (a local subspecies hybrid swarm) which should facilitate analysis of several possible features of mtDNA variation. Distinctive mtDNA genotypes contributed by the parental subspecies to the hybrid swarm are identified. As described later, these provide a special opportunity to recognize paternally-generated mtDNA heteroplasmicity, if it does exist, both in somatic and germ cells. Study of the association between allozyme and mtDNA markers permits comparison of the evolutionary consequences of biparental versus uniparental transmission of genotypes across animal generations. We will also include a description of mtDNA sequence variation across a broader range of the species. At this macrogeographic level, is the evolutionary divergence in mtDNA, reflecting matriarchal phylogeny, concordant with the subspecies distributions defined by morphology and by allozymes encoded in the nuclear genome?

MATERIALS AND METHODS

Taxa Examined

Two subspecies of bluegill, originally described by morphologic criteria, are native to the southeastern United States (Hubbs and Allen, 1944; Miller and Winn, 1951). *Lepomis macrochirus purpurescens,* abundant in peninsular Florida and Atlantic coastal streams, differs slightly in quantitative morphologic and physiologic features from *L. m. macrochirus,* the bluegill inhabiting the Mississippi and other Gulf drainages east to the Chattahoochee River in Georgia (Hubbs and Lagler, 1958). A recent protein-electrophoretic survey revealed significant

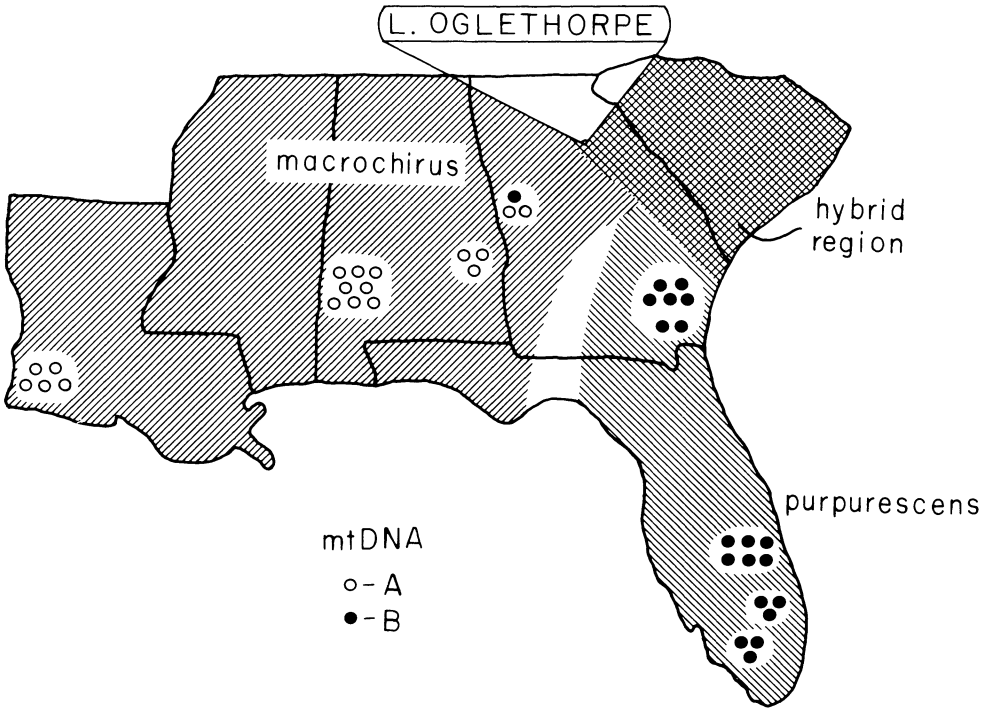

FIG. 1. Schematic map of the partial geographic distributions of *L. m. macrochirus, L. m. purpurescens,* and their hybrids, as previously defined by allozyme markers and by morphology (see Avise and Smith, 1974). Also shown are the distributions of mtDNA genome types A and B described in the present study (each circle represents one individual). The geographic area without shading has not been sampled.

allozyme differences between the subspecies, refined knowledge of their geographic distributions, and uncovered a broad zone of probable secondary contact and hybridization in Georgia and South Carolina (Avise and Smith, 1974; see also Felley and Smith, 1978). In particular, two allozyme loci exhibit essentially fixed allelic differences between the subspecies and serve as markers of hybridization: an esterase (*Es-3*) and an aspartate aminotransferase (*Got-2*) (described in Avise and Smith, 1974). In most populations from the hybrid zone, genotypes at these loci are randomly associated, suggesting that interbreeding is extensive (Avise and Smith, 1974; Avise and Felley, 1979).

The geographic distributions of the forms in the southeast, as presently understood, are indicated by shading in Figure 1. Much of the current study concerns a large sample from a subspecies hybrid population in Lake Oglethorpe, Oglethorpe Co., GA. ($N = 151$). This population was chosen because of its proximity to our laboratory, and because a preliminary survey indicated that allozyme markers for both subspecies were present in high frequency.

Smaller numbers of specimens were taken from other scattered localities as follows: L. Ogden, Palm Beach Co., FLA. ($N = 3$); Tamiami Canal, Broward Co., FLA. ($N = 3$); St. Lucie Canal, Martin Co., FLA. ($N = 6$); Saltilla River, Brantley Co., GA. ($N = 2$); Altamaha River, Long Co., GA. ($N = 5$); West Point Reservoir, Troup Co., GA. ($N = 3$); Uphappe Creek, Macon Co., ALA. ($N = 3$); Tombigbee River, Greene Co., ALA. ($N = 8$); Calcasieu River, Calcasieu Co., LA. ($N = 5$).

Laboratory Procedures

Fish were returned live to the laboratory, where fresh liver, heart, spleen, kidney, and muscle were used as somatic tissue sources for purification of mtDNA by differential centrifugation and banding in CsCl gradients (Lansman et al., 1981). Some fish were sexually ripe at time of capture, and from these mtDNA was also purified from sperm or eggs in separate workups. Closed-circular mtDNA was then digested with each of the following restriction endonucleases (with recognition sequences): 1) *Ava*I (CPyCGPuG); 2) *Bam*HI (GGATCC); 3) *Bcl*I (TGATCA); 4) *Bgl*I (GCCn_5GGC); 5) *Bgl*II (AGATCT); 6) *Bst*EII (GGTNACC); 7) *Cla*I (ATCGAT); 8) *Hind*III (AAGCTT); 9) *Pst*I (CTGCAG); 10) *Pvu*II (CAGCTG); 11) *Sac*I (GAGCTC); 12) *Xba*I (TCTAGA); 13) *Hinc*II (GTPyPuAC); 14) *Kpn*I (GGTACC); and 15) *Nde*I (CATATG). The latter two enzymes apparently produced no cuts in bluegill mtDNA, and are not considered further. Digested fragments were "end-labeled" with α-^{32}P-dCTP using the large fragment of DNA polymerase I as described by Brown (1980). After separation by molecular weight on agarose gels, the labeled fragments were detected by autoradiography (Lansman et al., 1981). In general, the clarity of the mtDNA digestion profiles in bluegill was excellent (Fig. 2).

Maps of all restriction sites (with the exception of *Hinc*II) were constructed using conventional "double-digestion" procedures (Lawn et al., 1978; Maniatis et al., 1982). *Bam*HI, *Bgl*II, *Cla*I, and *Sac*I proved to be most useful for the mapping experiments, because each enzyme produced one or two cuts in most bluegill mtDNAs and the sites were widely spaced about the genome. Maps were considered complete when cleavage sites for a new enzyme could be assigned to locations internally consistent with those for the above enzymes, although additional double-digests with other endonucleases were often conducted for further verification. The approximately 16,200 base-pair mtDNA of *Lepomis macrochirus* was assigned a total map length of 100 units, with position zero arbitrarily chosen as one of the two *Sac*I sites consistently recognized in all individuals. The accuracy of site determination inevitably varies from site to site but at worst is approximately ±250 base pairs (1.5 map units). Molecular weight markers were provided by a mixture of a *Hind*III digest of bacteriophage λ, and a *Pvu*II/*Hinc*II digest of pBR322. Estimates of nucleotide sequence divergence (*p*) between restriction maps were calculated by the cleavage site method of Nei and Li (1979).

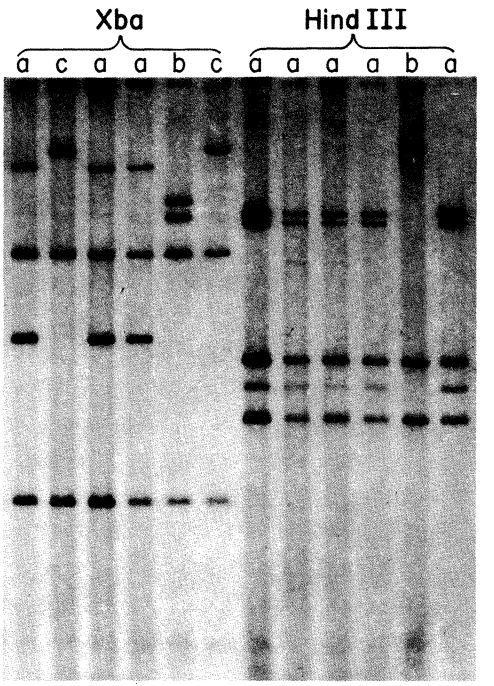

FIG. 2. *Xba*I and *Hind*III digestion profiles used as markers of the A and B mtDNA genomes observed in bluegill. The faint bands in lanes 8 and 9 (from the left) have molecular weights consistent with the interpretation that they represent incomplete digests.

Genotypes at the allozyme loci *Es-3* and *Got-2* were scored in all fish from horizontal starch gels, according to published procedure (Avise and Smith, 1974).

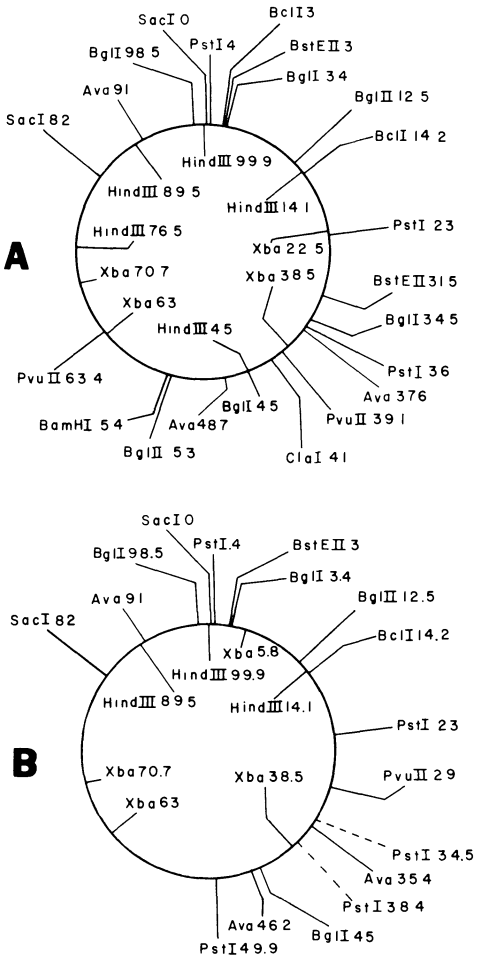

FIG. 3. Map positions of restriction sites recognized by 12 endonucleases in the A and B mtDNA genomes of bluegill. In B, the *Pst*I site indicated by a dashed line was mapped only to one of the two alternative positions shown.

RESULTS

Two highly distinctive mtDNA genotypes (henceforth labeled "A" and "B") were observed in fish collected from Lake Oglethorpe. Map positions for the total of 37 sites recognized by 12 endonucleases are shown along the circular mtDNA genome in Figure 3. Genotypes A and B exhibit 31 and 23 sites, respectively, and differ from each other by 20 counted mutation steps. Estimated nucleotide sequence divergence is $p = .085$. All observed genetic differences between A and B can reasonably be attributed to gains or losses of particular restriction sites (perhaps through base substitution), without additions, deletions, or rearrangements affecting more than about 50–250 base pairs (based on uncertainties in fragment size determination).

In addition to the large number of mapped site changes, the genomes differ qualitatively in the more complex (but unmapped) digestion profiles produced by *Hinc*II, where at least seven of a total of 15 detected fragments distinguish A and B. Overall, there can be no doubt that the A and B mtDNA genomes are highly divergent.

The genome maps were constructed from mtDNAs isolated from six individual fish from Lake Oglethorpe. The entire battery of endonucleases was also used to assay two fish from Greene Co., ALA. (representing *L. m. macrochirus*) and two fish from Martin Co., FLA. (representing *L. m. purpurescens*). One Alabama fish has an mtDNA digestion profile identical to that for the genome A map in Figure 1, and the other is only two site-gains (*Bcl*I and *Ava*I) removed from A ($p = .004$). Both Florida fish differ from genome B (Fig. 1) by two site-gains (*Bst*EII, *Pvu*II and *Bst*EII, *Bam*HI, respectively; $p = .007$). The high similarity of the general A or B genome types across a broad geographic range contrasts strikingly with the large differences between those genotypes within a single geographic locale such as Lake Oglethorpe.

Two restriction enzymes (*Xba*I and *Hind*III) were subsequently employed as markers of the A and B genomes to screen an additional 179 bluegill from Lake Oglethorpe and other locations in the southeast (Figs. 2 and 3). For the entire study, digestion profiles for *Xba*I and *Hind*III were perfectly concordant. In other words, any individual exhibiting the *Xba*I "a" digestion pattern (or a commonly-observed variant of it labeled "c" and lacking the *Xba*I site at map position 22.5 [Fig. 4]) also exhibited the *Hind*III "a" pattern characteristic of the A ge-

nome; and conversely, any individual exhibiting *Xba*I "b" also showed the *Hind*III "b" profile characteristic of the B genome. Such complete disequilibrium between sites is expected for a strictly unisexually transmitted, non-recombining genome. The possibility of gross misclassification of a mtDNA genotype for an individual fish (i.e., through simultaneous convergent mutations) is negligible in this case, because the A and B genomes usually differ by two recognition sites for each of the marker enzymes employed.

Geographic distributions of the A-type and B-type mtDNA genomes are shown in Figure 1. The distributions coincide almost perfectly with the previously-recognized subspecies ranges—A is associated with *L. m. macrochirus* and B with *L. m. purpurescens.* The only observed exception involved a single fish from the Chattahoochee drainage in west Georgia, which by allozyme and geographic criteria should have been *L. m. macrochirus,* but nonetheless exhibited the B mtDNA. Since this drainage represents the extreme eastern edge of *L. m. macrochirus* and is adjacent to other drainages occupied by the eastern subspecies and subspecies hybrids, this fish may reflect the effects of past introgression from *L. m. purpurescens.* Our sample sizes outside the hybrid zone are too small to permit accurate assessments of levels of introgression.

Protein electromorph and mtDNA genotype frequencies in the 151 bluegill collected from Lake Oglethorpe are presented in Table 1. The proportions of various genetic characters originally derived from *L. m. macrochirus* and *L. m. purpurescens* are all near .50 and are statistically homogeneous. For both *Got-2* and *Es-3,* genotype frequencies are in accord with Hardy-Weinberg expectations for a random-mating population (Table 2). However, considered jointly, there is a significant departure from expected frequencies attributable largely to an excess of the double-heterozygote class and deficits of the *aa/bb* and *bb/aa* homozygotes

TABLE 1. Frequencies of protein electromorphs and mtDNA genotypes in the Lake Oglethorpe population of bluegill. Genetic characters denoted by "a" or A were originally contributed by *L. m. macrochirus,* and those denoted by "b" or B were ultimately derived from *L. m. purpurescens.*[1]

Genetic character	Freq. ± 2 SD	Derived from . . .
Got-2 a	.473 ± .057	*L. m. macrochirus*
Es-3 a	.477 ± .057	*L. m. macrochirus*
mtDNA A	.530 ± .057	*L. m. macrochirus*
Got-2 b	.527 ± .057	*L. m. purpurescens*
Es-3 b	.523 ± .057	*L. m. purpurescens*
mtDNA B	.470 ± .057	*L. m. purpurescens*

[1] No frequencies in the table are significantly different at the .05 level (Sokal and Rohlf, 1969 p. 608).

(Table 2). Double-heterozygote excesses are not invariably observed in other bluegill populations in the hybrid zone (Avise and Smith, 1974).

Associations of the mtDNA and allozyme genotypes in the Lake Oglethorpe population are shown in Table 3. Both mtDNA genotypes were observed in high frequency with all *Got-2* and *Es-3* allozyme genotypes considered separately, and with seven of the nine *Got-2/Es-3* dilocus genotypes. For *Got-2,* we cannot reject the null hypothesis that frequency of mtDNA genotype is independent of nuclear genotype; for *Es-3,* and for *Got-2/Es-3,* associations with mtDNA genotypes were mildly significant, attributable largely to excesses of the mtDNA A genome with *Es-3* "ab," and with *Got-2* "*ab*"/*Es-3* "*ab,*" respectively. Interestingly, the A and B mtDNA genomes in Lake Oglethrope were randomly associated with the double-homozygote allozyme genotypes (*aa/aa* and *bb/bb*) characteristic of the "pure" parental subspecies elsewhere (Table 3).

For each individual in the study, the somatic-cell mtDNA genome could be unambiguously identified as the A or B type by the marker endonucleases employed. In other words, there was no evidence for individual heteroplasmicity involving A and B mtDNAs. Under our assay conditions, a rare molecule would have been detected if it comprised more

TABLE 2. Observed and expected numbers of allozyme genotypes in the Lake Oglethorpe population of bluegill.[1]

Loci	Genotype	Number of individuals: Observed	(Expected: H-W equilibrium)	χ^2	P
Considered singly					
Got-2	*aa*	28	(33.8)		
	ab	87	(75.3)	3.65	$.1 > P > .05$
	bb	36	(41.9)		
Es-3	*aa*	30	(34.3)		
	ab	84	(75.3)	1.99	$.5 > P > .1$
	bb	37	(41.3)		
Considered jointly					
Got-2/Es-3	*aa/aa*	10	(7.7)		
	aa/ab	16	(16.9)		
	aa/bb	2	(9.3)		
	ab/aa	17	(17.1)		
	ab/ab	54	(37.6)	26.3	$P < .005$
	ab/bb	16	(20.6)		
	bb/aa	3	(9.2)		
	bb/ab	14	(20.9)		
	bb/bb	19	(11.5)		

[1] At *Es-3*, two alleles ("*96*" and "*92*") are characteristic of *L. m. macrochirus* in the southeast (Avise and Smith, 1974 and this study). For simplicity in this and the other tables, these alleles were artificially pooled into the *Es-3* "a" allelic class. Actual frequencies of *Es-3* "*96*" and "*92*" in Lake Oglethorpe were .41 and .06, respectively.

than about 5% of the total mtDNA population. Furthermore, the mtDNA genotype of germ cells (eggs or sperm) invariably agreed with the somatic-cell designation (Table 4). The significance of these observations is discussed below.

DISCUSSION

Descriptive surveys of natural populations cannot answer all questions about the nature of mtDNA variability. Detailed molecular studies (e.g., genome sequencing—Anderson et al., 1982) and controlled laboratory experiments (e.g., manipulated crosses for study of transmission genetics—Lansman et al., 1981) will also play a very important role. However, any final conclusions about mtDNA evolution must be sensitive to and compatible with the unique kinds of information obtainable only from population-level analysis. Our characterization of mtDNA variability in a hybrid swarm provides several observations particularly relevant to a developing understanding of the evolutionary dynamics of mtDNA.

Transmission Genetics and Tests for Somatic Cell Heteroplasmicity

In a population survey involving restriction digests, individual heteroplasmicity would be evidenced by the appearance on gels of additional bands whose summed molecular weights exceed known mtDNA genome size. As discussed by Avise and Lansman (1983), such supernumerary bands are commonly observed. However, most authors conservatively interpret the extra bands as results of incomplete digests—i.e., the molecular heterogeneity is thought to be an artifact of incomplete cleavage of some fraction of mtDNA molecules under the particular assay conditions. For mtDNAs which differ by a single site, incomplete digests would normally produce digestion profiles not readily distinguishable from true mtDNA sequence heterogeneity. For example, suppose an individual truly possessed a mixed population of mtDNAs differing by one site (such as the *Xba*I maps "a" and "c" [or "b" and "c"] of Fig. 4). The

TABLE 3. Association of mtDNA genotypes with allozyme genotypes for the Lake Oglethorpe population of bluegill.

Loci	Genotype	Observed (and expected) numbers of mtDNA genotypes[1] A	B	G^2	P
Considered singly					
Got-2	*aa*	16 (14.8)	12 (13.2)		
	ab	51 (45.8)	36 (41.2)	5.45	$.1 > P > .05$
	bb	13 (19.0)	23 (17.0)		
Es-3	*aa*	12 (15.7)	18 (14.3)		
	ab	52 (43.9)	32 (40.1)	6.16	$.05 > P > .01$
	bb	16 (19.4)	21 (17.6)		
Considered jointly					
Got-2/Es-3	*aa/aa*	6 (5.3)	4 (4.7)		
	aa/ab	6 (9.0)	11 (8.0)		
	aa/bb	0 (1.6)	3 (1.4)		
	ab/aa	10 (8.5)	6 (7.5)		
	ab/ab	38 (28.6)	16 (25.4)	14.76[3]	$.05 > P > .01$
	ab/bb	4 (7.4)	10 (6.6)		
	bb/aa	0 (1.1)	2 (.9)		
	bb/ab	7 (8.5)	9 (7.5)		
	bb/bb	9 (10.1)	10 (8.9)		

[1] From observed frequencies of mtDNA A and B genomes in the Lake Oglethorpe population at large.
[2] R × C test of independence using the *G*-test (Sokal and Rohlf, 1969).
[3] Classes with expected numbers less than 6 were pooled.

additive fragment pattern on the gel would include only fragments also expected in an incomplete digest of the more complex map.

Genetic markers provided by the highly distinct A and B mtDNA genomes in the bluegill hybrid swarm do, however, offer an improved test of the possibility of paternally-derived heteroplasmicity. Consider again Figure 4. The *Xba*I maps "a" and "b" differ by two independent site changes. A mixture of "a" and "b" molecules in a single *Xba*I digest would produce an additive gel pattern with six bands, including those of length 8.4, 5.7, and 5.3 kilobases. Because of the particular positions of the sites involved, the pair of bands representing the 5.7 and 5.3 kilobase fragments would not be produced in an incomplete digest of *Xba*I "a"; and the 8.4 kilobase fragment would not be expected in an incomplete digest of *Xba*I "b" (refer to the maps in Fig. 4). Since we did not observe mixtures of A and B genome types in somatic tissue from individual fish, in tests using genetic markers that could distinguish mtDNA mixtures from incomplete digests, we conclude that the transmission of mtDNA is uniparental at least at the 95% level in these fishes.

MtDNA heteroplasmicity could in principle arise from at least two sources: new mutations within a female lineage, or genetic variants introduced through paternal leakage across female genealogies. The two sources should be clearly distinguished, because they entail different evolutionary consequences. Paternal leakage would provide a gene-flow bridge between female lines otherwise completely isolated from one another with respect to mtDNA and, when frequent, would force a reassessment of the use of mtDNA in interpretation of matriarchal phylogeny. The present work demonstrates that no individual bluegill exhibits heteroplasmicity at a 5% or greater level that can be explained by paternal mtDNA transmission. If results of this study can be generalized to other groups where true heteroplasmicity may have been observed (e.g., Coote et al., 1979; Brown et al., 1983), the heteroplasmicity

TABLE 4. Agreement between somatic and germ cell mtDNA genotype in 26 sexually ripe bluegill collected from Lake Oglethorpe.

Somatic cell mtDNA	mtDNA genome			
	Eggs		Sperm	
	A	B	A	B
A	12	0	4	0
B	0	6	0	4

is perhaps more likely attributable to mutationally-derived transient polymorphism *within* a female lineage.

Germ Cell mtDNA

Under some genetic models (Chapman et al., 1982; Hauswirth and Laipis, 1982*a*; Birky et al., 1983), mtDNA polymorphism might be maintained in germ-cell lineages but only rarely manifested in somatic cells of any individual. Few studies have explicitly examined mtDNA in germ-line tissues. However, Brown (1983) reports polymorphism in mtDNA isolated from batches of unfertilized eggs from individual sea urchins. Hauswirth and Laipis (1982*b*) and Olivo et al. (1983) cite indirect observational support for germ-line (but not somatic cell) mtDNA polymorphism in a herd of cattle.

In this study, the agreement between an individual's somatic cell mtDNA genotype and its mature germ-cell genotype was perfect (Fig. 2, Table 4). Furthermore, we found no evidence (at the 5% detection level) for A + B genome heteroplasmicity in the germ line. As with somatic cells, if germ-line polymorphism had commonly arisen through paternal leakage in the hybrid swarm, we should have detected it with the distinctive mtDNA markers available.

mtDNA and Allozyme Genotypes in the Subspecies Hybrid Swarm

As is true of most reservoirs in the southern United States, the current Lake Oglethorpe population of bluegill probably includes descendents of fish native to the stream at the time of impoundment (in 1970), plus periodic additions

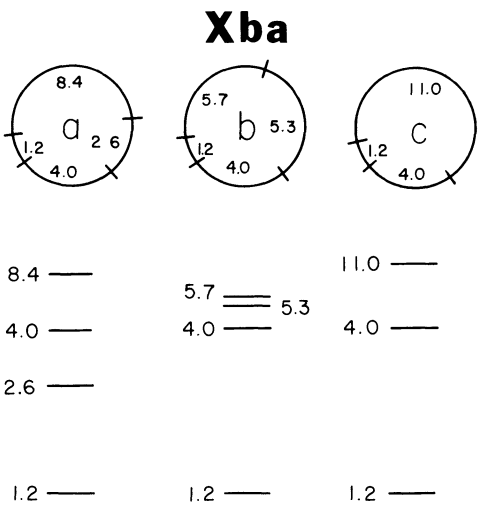

FIG. 4. Map positions and digestion profiles for the *Xba*I "a," "b," and "c" genotypes observed in bluegill. Marker sites provided by "a" and "b" were particularly useful in testing the possibility of paternally-generated individual heteroplasmicity (see text). Among the sample of 80 bluegill in Lake Oglethorpe exhibiting the A mtDNA genome, 47 and 33, respectively carried the *Xba*I "a" and "c" patterns.

from private hatchery stocks. For our purposes, the exact origin of the population is not critical. Wherever the subspecies crosses took place (e.g., even if in hatcheries), the genotype frequencies are now close to expectations for a panmictic population. The extensive genetic interchange between the bluegill subspecies should have provided many opportunities to observe paternally-generated mtDNA heteroplasmicity if it did exist.

The frequencies of the A and B mtDNAs in the Lake Oglethorpe population presumably reflect the relative numbers of surviving matriarchal lineages originally introduced by *L. m. macrochirus* and *L. m. purpurescens* females. Since the mtDNA and allozyme frequencies are similar (Table 1), the female ancestries appear representative of the biparental ancestries registered by allele frequencies in the nuclear genome. Nonetheless, mild non-random association of mtDNA and allozyme genotypes

was observed (Table 3). The reasons for this departure are not known, but to the list of candidate explanations could be added possible mtDNA-allozyme interactions influencing fitness.

Geographic Distribution of mtDNA Genotypes

The geographic distributions of mtDNA genotypes in bluegill appear highly concordant with the subspecies distributions as previously defined by allozymes and morphology (Fig. 1). Such concordance between independent elements of the genome might be expected if *L. m. macrochirus* and *L. m. purpurescens* had evolved in complete isolation at some time in the past. Avise and Smith (1974) present one possible evolutionary scenario to account for the current subspecies ranges. Perhaps ancestors of *L. m. purpurescens* were isolated on Florida islands during Pleistocene glacial minima and, for a while, evolved in isolation from mainland stock. The present zone of hybridization would then represent a region of secondary contact following post-Pleistocene range expansions. This and related scenarios for the southeastern fish fauna as a whole will be explored in greater depth elsewhere (Bermingham and Avise, unpubl.).

The finding of concordance between mtDNA and allozymes in bluegill is significant because it is atypical for the few such studies published to date. Ferris et al. (1983) observed a mtDNA genome normally characteristic of *Mus domesticus* in populations that were morphologically and allozymically *Mus musculus*. Similarly, Powell (1983) found that *Drosophila pseudoobscura,* where sympatric with *D. persimilis* (but not where allopatric), often exhibited an mtDNA indistinguishable from that of *D. persimilis*. In these cases, results were attributed to past hybridization between the forms involved, followed by substantial introgression of mtDNA but not of the nuclear genome. One can imagine a similar situation arising in bluegill. Suppose a few females of *L. m. macrochirus* migrated or were transplanted into the range of *L. m. purpurescens* (or vice versa). After even a few backcross generations, most nuclear gene evidence for the introduction would be lost, but those non-recombining mtDNA genomes which survived would bear continued testimony to the hybridization event. In this study, the single fish from the Chattahoochee drainage carrying the B mtDNA genome may represent an example of this phenomenon.

Results of this and previous studies clearly demonstrate that nuclear and cytoplasmic genomes have distinct transmission genetics that can importantly influence their evolutionary dynamics. In the future, it will be important to study more case histories in order to reach a concensus on how frequently the geographic distributions of nuclear and mtDNA markers will prove concordant. It will also be important to consider whether processes other than secondary hybridization (such as demographic influences during phylogeny) might potentially account for instances where concordance between mtDNA and nuclear genotype appears to be lacking.

SUMMARY

We begin a characterization of the evolutionary dynamics of mitochondrial DNA (mtDNA) in fishes by examining restriction site variability in 189 bluegill sunfish (*Lepomis macrochirus*). A total of 15 endonucleases was employed to map 37 restriction sites in mtDNA from selected individuals. Genome size was approximately 16.2 kilobases. All differences between genotypes could be accounted for by gains or losses of individual sites, without additions, deletions, or rearrangements affecting more than about 50–250 base-pairs.

Two highly distinct mtDNA genomes, differing by 20 assayed mutation steps and an estimated 8.5% sequence divergence, were discovered. Both genomes were observed in high frequency in a sample of 151 bluegill from a north-Georgia population, which on the basis

of allozyme genotype appears to represent a freely-interbreeding hybrid swarm between two bluegill subspecies. Within the hybrid population, mtDNA and allozyme genotypes were associated approximately at random.

The distinct genetic markers provided by the mtDNA genomes provided an improved test of the possibility of within-individual mtDNA polymorphism because true heteroplasmicity could readily be distinguished from results of incomplete digests. Nonetheless, in somatic or germ cells, no individual bluegill exhibited mtDNA heteroplasmicity (at 5% or greater level) that could be explained by paternal mtDNA transmission.

Additional samples from Louisiana to Florida tentatively confirm that the geographic distributions of the two distinct mtDNA genomes are highly concordant with the previously described ranges of *L. m. macrochirus* and *L. m. purpurescens* defined by morphology and allozymes. Overall, the results on mtDNA variability in bluegill conform to and strengthen some of the more straightforward expectations about the pattern of evolution of uniparentally-transmitted genomes in sexually reproducing populations.

Acknowledgments

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Corresponding Editor: Wesley M. Brown