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Mitochondrial DNA differentiation in North Atlantic eels: Population genetic consequences of an unusual life history pattern

(*Anguilla*/population structure/dispersal/panmixia/catadromous fishes)

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ABSTRACT **A survey of restriction site polymorphism in the mitochondrial DNA (mtDNA) of the American eel *Anguilla rostrata* showed no genetic divergence among samples from a 4000-km stretch of North America coastline. Lack of geographic differentiation in mtDNA over such a large area contrasts sharply with results for terrestrial and freshwater vertebrates and is most likely attributable to the extraordinary life history of these catadromous fishes, which involves perhaps a single spawning population in the western tropical mid-Atlantic Ocean and subsequent widespread dispersal of larvae by ocean currents. However, samples of the European eel (nominally *Anguilla anguilla*) are highly distinct from *A. rostrata* in mtDNA genotype (distinguishable by 11 of 14 restriction endonucleases), contradicting some previous suggestions that the two forms belong to the same panmictic population. Results of this study emphasize the importance of life history in shaping population genetic structure.**

Anguilla eels exhibit an extraordinary life history pattern (review in ref. 1). Juveniles of two nominal species, *A. rostrata* and *A. anguilla*, inhabit coastal and inland waters of the Americas and Europe, respectively. During sexual maturation, they migrate to the western tropical mid-Atlantic Ocean, where spawning takes place. Leptocephalus larvae disperse to coastal regions, partly or largely through passive transport by ocean currents. Williams and Koehn (1) summarize the conventional view about the population consequences of this catadromous life cycle for either *A. rostrata* or *A. anguilla*: ". . . it is entirely possible that spawning is essentially panmictic. . . . it means that collections of juveniles from any locality are all samples of the same breeding population." If it is true that self-maintaining local populations are absent in *Anguilla*, any observed genetically based differences among geographically widespread collections of juveniles ". . . should represent what natural selection can accomplish within a single generation."

Predictions of this life history scenario have been used to interpret results of allozyme surveys of American eels collected from Newfoundland to Florida (2, 3). Mild but statistically significant geographic variation in electromorph frequencies at three loci was taken as evidence that selection pressures caused gene frequency changes of the order of 10% per generation (2). However, such conclusions are critically dependent upon the assumption of panmixia and random larval dispersal, which could be violated if adults from various continental locales mate assortatively (perhaps in subareas of the overall breeding grounds) and if larval dispersal has nonrandom or active aspects. For example, perhaps larvae of *A. rostrata* have a genetically based predisposition to swim west to the North American shore, whereas *A. anguilla* appropriately swim east to Europe. As noted by Williams and Koehn (1):

> If the possibility of larval behavioral mechanisms that direct larvae to one or the other side of the Atlantic is to be seriously considered, why stop at merely two such mechanisms? Why not endow some larvae with behavior that assures their arrival in North Carolina, similarly equip others for finding South Carolina, and so on? . . . the possibility of larval homing to the juvenile habitats of their parents needs careful examination. If real, it would support the concept of self-maintained local populations, invalidate our assumptions of panmixia, and force new interpretations of published data.

The purpose of this study is to use restriction site data on mitochondrial DNA (mtDNA) to reevaluate geographic differentiation in *Anguilla*, and its implications for the life history of North Atlantic eels. mtDNA is particularly appropriate because it is known to evolve rapidly (4) and to provide genetic markers that typically distinguish geographic populations of other vertebrates (reviews in refs. 5 and 6). Indeed, lack of mtDNA divergence among widely separated collections of eels would constitute a dramatic exception to conventional findings.

MATERIALS AND METHODS

Eels were collected from the locales shown in Fig. 1 and shipped live to our laboratory. Liver and heart were tissue sources for mtDNA, which was purified in closed-circular form by CsCl/ethidium bromide gradient centrifugation (7). Digestions by the 14 informative restriction endonucleases listed in Table 1 were carried out under conditions recommended by the vendor (New England Biolabs). [Four additional enzymes (*Bst*EII, *Cla* I, *Kpn* I, and *Sac* I) produced only zero or one cut in mtDNAs from all individuals and are not considered further.] Resulting fragments were radioactively end-labeled (8) with the appropriate [α-^{32}P]nucleotide(s) and separated by molecular weight through 1% (1.8% for *Dde* I) agarose gels (7). Autoradiography revealed the digestion profiles that constitute the raw data for this report. Molecular weight markers were provided by a combined *Hin*dIII digest of λ DNA and *Pvu* II–*Hin*cII double digest of pBR322 or by a 1-kilobase ladder standard available from Bethesda Research Laboratories.

Because of poorer resolution and more intense background in the lower regions of the autoradiographs, no attempt was made to score fragments less than about 300–500 base pairs in size. Nearly all 138 collected eels were surveyed by 12 restriction endonucleases; fewer specimens were assayed by *Mbo* I and *Dde* I (Table 1). Estimates of nucleotide sequence divergence (p) between *A. rostrata* and *A. anguilla* were determined by the fragment comparison approach of Nei and Li (9). The final p estimate represents the weighted (by number of fragments) mean of values calculated separately for enzymes with 4-, 5-, and 6-base recognition sites.

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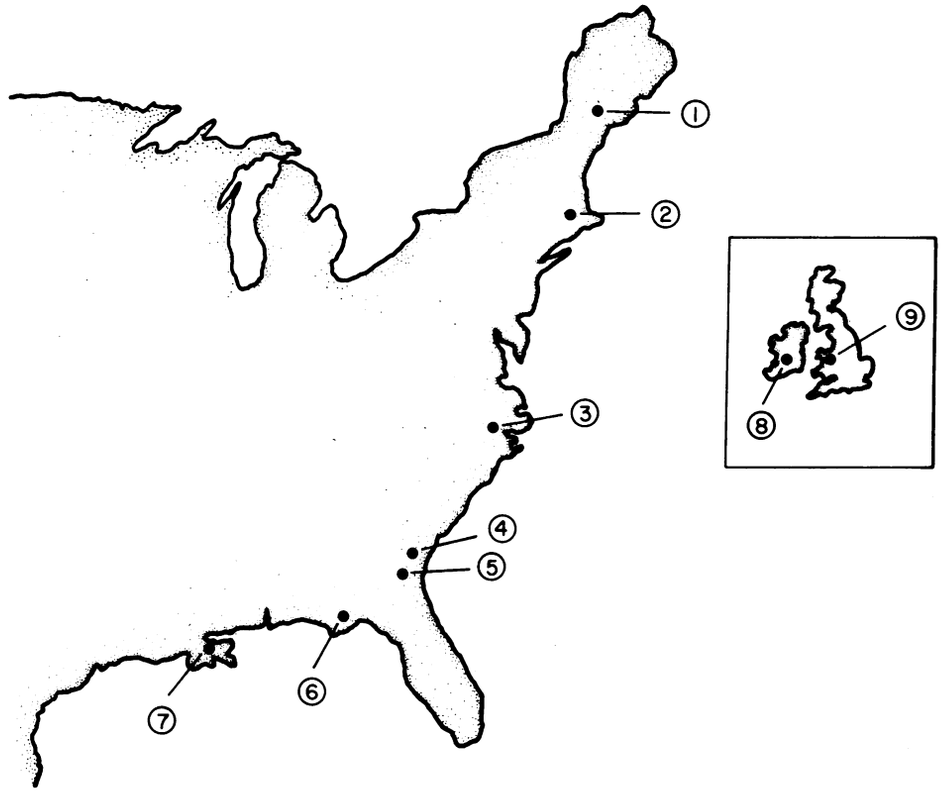

FIG. 1. Collection sites and sample sizes for North Atlantic eels. 1, Penobscot River, Maine (N = 10). 2, Annquatucket River, Rhode Island (N = 12). 3, Pamlico River, North Carolina (N = 13). 4, Altamaha River, Georgia (N = 11). 5, Satilla River, Georgia (N = 16). 6, Carabelle River, Florida (N = 16). 7, Lake Ponchartrain, Louisiana (N = 31). 8, River Shannon, Ireland (N = 14). 9, River Dee, England (N = 15).

RESULTS

A. rostrata (American Eel). One composite mtDNA genotype, representing 78 scored restriction sites and 389 base pairs of information, predominated in all geographic samples of the American eel (Tables 1 and 2). We designate this genotype 1C2C3C . . . 14C, where the numbers refer to the endonucleases as listed in Table 1 and the Cs denote the common pattern. Excluding *Dde* I and *Mbo* I (which were used to assay fewer specimens), the common genotype was observed in 73 of the 109 surveyed American eels (67%). Of 36 individuals remaining, 30 differed from the common genotype by a single restriction site gain or loss, and 6 differed by two restriction site changes (Table 2). Assuming that each restriction site change is due to one base substitution, the estimated maximum sequence divergence from the

Table 1. Informative restriction endonucleases employed in survey of *Anguilla* mtDNA

Endonuclease	Recognition sequence	Eels assayed, no.	Scored fragments in most common genotype, no.		
			rostrata, N_X	*anguilla*, N_Y	Shared, N_{XY}
6-Base sites					
1. *Bam*HI	GGATCC	137	2	1	0
2. *Bcl* I	TGATCA	137	2	2	2
3. *Bgl* I	GCCN$_5$GGC	136	3	2	2
4. *Bgl* II	AGATCT	136	2	2	0
5. *Eco*RI	GAATTC	134	4	6	2
6. *Hin*dIII	AAGCTT	138	5	5	3
7. *Pst* I	CTGCAG	136	4	4	4
8. *Pvu* II	CAGCTG	136	5	3	1
9. *Xba* I	TCTAGA	138	5	5	5
5-Base sites					
10. *Ava* I	CYCGRG	138	5	6	4
11. *Hin*cII	GTYRAC	135	8	7	4
4-Base sites					
12. *Msp* I	CCGG	133	10	9	5
13. *Mbo* I	GATC	33	10	7	5
14. *Dde* I	CTNAG	29	13	10	7
		Totals	78	69	44

Table 2. Frequency distributions of mutational steps from the composite mtDNA genotypes in *A. rostrata* and *A. anguilla**

A. rostrata		*A. anguilla*	
Restriction site changes from the common *A. rostrata* composite genotype, no.	Eels, no.	Restriction site changes from the *A. anguilla* composite genotype, no.	Eels, no.
0	73	0	0
1	30	1	10
2	6	2	6
3	0	3	9
4	0	4	2
5	0	5	1
6	0	6	1
Totals	109		29

*Not including limited data from *Dde* I and *Mbo* I.

common mtDNA genotype is $p \approx 0.006$, and the overall mean divergence from this pattern is $\bar{p} \approx 0.001$.

Considering the fragment patterns from enzymes individually, the 14 endonucleases produced 26 variant mtDNA genotypic patterns listed in Table 3. Each variant pattern could be attributed to a single-site gain (12 instances) or loss (14 instances) from the common type (for example, a single-site gain is inferred from the appearance in a digestion profile of two new fragments whose summed molecular weight equals that of a fragment present in the common but not in the variant pattern). Among the 26 genotypic variants, 21 were observed in a single individual and, hence, in a single geographic locale. The five remaining variants, present in two or more individuals, were geographically widespread (Table 3). Thus, the *Bam*HI genotype 1B was observed in Georgia and Louisiana; *Pvu* II 8A in Louisiana and Maine; *Msp* I 12B in Florida and Rhode Island; *Dde* I 14D in Louisiana, Rhode Island, and Maine; and *Hinc*II 11A in Louisiana, Florida, Georgia, Rhode Island, and Maine. Only the *Hinc*II 11A variant was common enough to permit a statistical test; it was not significantly heterogeneous in frequency among our samples from seven collection locales (G-test for heterogeneity, $G_H = 9.65$; degrees of freedom, df = 6; $0.5 > P > 0.1$).

Table 3. mtDNA genotypic variants (expressed as departures from the most common composite pattern 1C2C3C . . . 14C) observed in the American eel *A. rostrata*

Endonuclease	Variant genotype	Characterization	Eels, no.	Geographic location
*Bam*HI	1B	Site loss	2	Georgia (site 4)
			2	Louisiana
Bcl I	2D	Site gain	1	Maine
Bgl I	3B	Site loss	1	Louisiana
	3D	Site gain	1	North Carolina
*Eco*RI	5B	Site loss	1	Louisiana
	5D	Site loss	1	North Carolina
*Hin*dIII	6B	Site gain	1	Florida
	6D	Site loss	1	Florida
Pst I	7B	Site loss	1	Georgia (site 5)
Pvu II	8B	Site loss	1	Louisiana
	8D	Site loss	1	Louisiana
	8A	Site loss	1	Louisiana
			1	Maine
Xba I	9B	Site loss	1	Georgia (site 4)
Ava I	10B	Site gain	1	Georgia (site 4)
*Hinc*II	11B	Site gain	1	Florida
	11D	Site loss	1	Georgia (site 5)
	11A	Site gain	6	Louisiana
			3	Florida
			4	Georgia (site 5)
			2	Rhode Island
			2	Maine
Msp I	12B	Site gain	1	Florida
			1	Rhode Island
	12D	Site loss	1	Rhode Island
	12A	Site gain	1	Maine
Mbo I	13D	Site gain	1	Florida
	13B	Site loss	1	Louisiana
	13A	Site gain	1	Maine
	13E	Site loss	1	Georgia (site 5)
Dde I	14D	Site gain	1	Louisiana
			1	Rhode Island
			2	Maine
	14B	Site gain	1	Georgia (site 5)

***A. anguilla* (European Eel).** European eels were less intensively sampled, but exhibited a higher level of mtDNA polymorphism than did *A. rostrata* (Tables 2 and 4). Considering the endonucleases individually, a total of 20 variant genotypes was observed (Table 4), and with two exceptions (*Eco*RI patterns 5W and 5V) these could be attributed to single-site gains (9 instances) or losses (9 instances). No individual possessed the composite genotype that would represent the combination of the most common digestion profiles for each enzyme, and on average an individual differed from this expected composite genotype by 2.34 assayed mutational steps (Table 2).

Nonetheless, we have little convincing evidence for geographic differentiation between our England and Ireland samples. Thus most of the common mtDNA variants (*Bam*HI 1C, *Bgl* I 3Z, *Eco*RI 5Y, *Pvu* II 8Z, and *Msp* I 12W) were observed in both locales, the only exception being *Msp* I 12Y that was present in 4 of 15 specimens from England but was not observed in 14 specimens from Ireland ($G_H = 5.87$; df =

Table 4. mtDNA genotypic variants (expressed as departures from the common composite pattern 1X2C3X4X5X6X7C-8X9C10X11X12X13X14X*) observed in the European eel *A. anguilla*

Endonuclease	Variant genotype	Characterization	Eels, no.	Geographic location
*Bam*HI	1C	Site gain	9	England
			5	Ireland
	1Y	Site gain	1	England
Bcl I	2B	Site gain	2	England
Bgl I	3Y	Site loss	1	England
	3Z	Site gain	1	England
			1	Ireland
*Eco*RI	5Y	Site loss	4	England
			7	Ireland
	5W	≥2 site differences	1	Ireland
	5V	≥2 site differences	1	Ireland
Pvu II	8Z	Site loss	7	England
			4	Ireland
Ava I	10C	Site loss	1	England
*Hinc*II	11Y	Site loss	2	Ireland
Msp I	12W	Site gain	6	England
			1	Ireland
	12Y	Site gain	4	England
	12U	Site loss	1	England
	12Z	Site loss	2	Ireland
	12R	Site loss	2	Ireland
	12Q	Site gain	1	Ireland
	12O	Site loss	1	Ireland
Mbo I	13Y	Site gain	1	England
	13W	Site gain	1	England

*In this composite designation, Xs denote the common fragment patterns that differ from those observed in any *A. rostrata*; the Cs denote genotypes identical to those most common in *A. rostrata*; numbers refer to the endonucleases listed in Table 1.

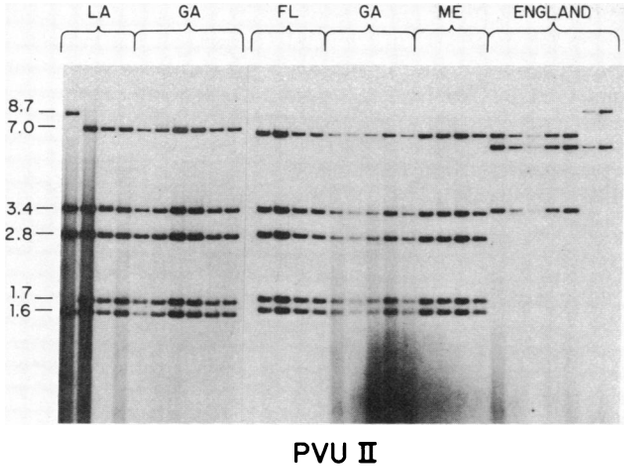

FIG. 2. *Pvu* II digests (from two gels) of *A. rostrata* from Louisiana (LA), Florida (FL), Georgia (GA), and Maine (ME) and of *A. anguilla* from England. *Pvu* II exemplifies the pattern of mtDNA genotypes observed with many enzymes—a common profile shared by American eels from all collection locales, with occasional variants usually differing by one restriction site (see the left-most lane); a profile shared by most European eels, with more common variants from it (see the right-most two lanes). There is a clear distinction between the profiles for the two species. Sizes in kb are indicated at left.

1; $0.05 > P > 0.01$). For all other enzymes, common digestion profiles were identical in these two locations. Estimated mtDNA sequence divergence within *A. anguilla* was also low—maximum divergence (observed in one specimen) from the composite European genotype was $p \approx 0.020$, and mean divergence was $\bar{p} \approx 0.008$.

Comparison of *A. rostrata* and *A. anguilla*. In contrast to the genetic uniformity among geographic samples of *A. rostrata* (or, with less certainty, *A. anguilla*), striking differences in mtDNA genotype existed between the two nominal species. Eleven of 14 endonucleases (79%) produced distinct digestion profiles (Fig. 2), and for only three enzymes (*Bcl* I, *Pst* I, and *Xba* I) were the common *multifragment* digests shared by *A. rostrata* and *A. anguilla* (Table 1). Many of the enzymes produced profile differences that could not be attributed to single-site gains or losses (Fig. 2), so a direct tally of restriction site differences could not be made. However, overall proportion of shared fragments in the composite mtDNA genotypes for American versus European eels was $F = 0.60$, which translates into a weighted estimate of nucleotide sequence divergence, $p \approx 0.037$. Although the absolute magnitude of p may be questioned [due to a limited number of assayed fragments and to reservations about assumptions underlying the conversion of F to p (9)], there can be no doubt that *A. rostrata* and *A. anguilla* are very readily distinguishable by mtDNA genotype.

DISCUSSION

Lack of Geographic Differentiation in *A. rostrata*. An important objective of empirical population genetics is to provide an adequate description of the genetic structure of natural populations. Such information is prerequisite to an understanding of evolutionary factors, including life history, responsible for a given genetic architecture. In vertebrates as diverse as freshwater fishes and terrestrial rodents, the rapidly evolving mtDNA molecule (4, 10) has proven to be a rich source of such information. Typically, restriction site surveys have uncovered extensive mtDNA sequence differences among conspecific but allopatric samples, suggesting that for most species the geographic spread of mtDNA genotypes by dispersal is not sufficient to override historical patterns of population subdivision (5, 6). Very few important exceptions to the rule of extensive geographic structuring of mtDNA genotypes have been reported (see refs. 5, 6, 11, and 12).

Here we report a well-documented example of the lack of mtDNA differentiation among widely separated conspecific samples. In the American eel (for which our sampling was most extensive), we found no evidence of mtDNA divergence among collections along a 4000-km stretch of shoreline from Maine to Louisiana, as indicated by four results. (*i*) One mtDNA genotype, composed of 78 assayed restriction sites, dominated all collections. (*ii*) Variant genotypes observed in two or more individuals were not confined to specific locales. (*iii*) One strong polymorphism (provided by *Hinc*II) was geographically widespread and not significantly different in frequency among collections. (*iv*) Overall mtDNA sequence diversity was low ($\bar{p} \approx 0.001$). All of these findings are consistent with the prevailing hypothesis that the life history of *A. rostrata* involves a single mating population with widespread random dispersal of larvae. Our results thus add considerable strength to the contention that self-maintaining local populations are indeed absent in *A. rostrata* (at least over the range of the assayed populations), and they appear to exemplify the profound influence that life history can play in shaping population genetic structure.

However, two important caveats apply to this interpretation. First, we cannot prove the null hypothesis that no genetic differentiation exists among geographic samples of *A. rostrata*. An ardent supporter of the concept of local population maintenance in eels could always argue that the critical genetic character(s) documenting population structure had not yet been surveyed. For example, as pointed out by Vladykov (13), it has been (and remains) somewhat of an enigma why *A. rostrata* occurs in the eastern Gulf states and the Caribbean, given the ocean currents and the spawning area proposed by Schmidt (14). Perhaps separate and as yet undiscovered breeding populations are maintained (15). However, it is also possible that the spawning region of the known population extends farther south than is currently recognized, that ocean currents are incompletely understood (15, 16) and may passively transport some larvae into the Gulf from the known spawning area, or that larval dispersal has some active aspects. In any event, even if distinct mating populations are eventually found to exist and to contribute larvae to separate regions of North America, our mtDNA data argue that such breeding population separation must have been of extremely recent evolutionary occurrence.

A second reservation in interpreting our data is that little comparative information on mtDNA differentiation is available for other marine species. Thus a distinct (and testable) possibility exists that species inhabiting the potentially more continuous marine realm may *generally* exhibit less geographic differentiation in mtDNA genotype than appears typical for terrestrial and freshwater faunas (12). Graves *et al.* (17) report no consistent differences in mtDNA genotype (nine endonucleases, about 40 restriction sites) between small samples of skipjack tuna collected in Hawaii, Puerto Rico, and Brazil. Skipjack tuna lack discrete spawning areas and their pelagic larvae are found circumtropically (18). Graves *et al.* (17) suggest that interoceanic gene flow (perhaps around the Cape of Good Hope in Africa) has been sufficient to prevent genetic differentiation of Atlantic and Pacific samples. The mtDNA findings for *A. rostrata* (and the skipjack tuna) provide a strong rationale for continued genetic analysis of other marine species exhibiting a variety of life history patterns.

Genetic Divergence Between *A. rostrata* and *A. anguilla*. Despite intense effort, it has previously proven difficult to distinguish American from European eels. A single quanti-

tative morphological trait, the number of vertebrae (genetic basis unknown), alone serves to separate most specimens of eels from the two continents. Tucker (19) proposed that vertebral counts are influenced by water temperatures during development, and that eels in Europe are merely expatriates from what is really a single American population. In allozyme composition at 15 loci, genetic divergence between the species is low (1) and only a single locus (*Mdh-2*) can distinguish most specimens [frequencies of the *Mdh-2a* allele are 0.96 and 0.10 in North America and Europe, respectively (20)]. Williams and Koehn (20) interpreted all available evidence as indicating that *A. rostrata* and *A. anguilla* should be considered conspecific but with largely allopatric spawning areas as suggested by other workers (14, 21, 22).

The mtDNA restriction site data provide the first convincing evidence that American and European eels are well differentiated genetically. Among the digestion profiles for 14 endonucleases, about 40% of the fragments were different between the nominal species, and sequence divergence was estimated to be $p \approx 0.037$. What inferences can be made about the biology and taxonomic status of American and European eels on the basis of this new information?

Since mitochondria are asexually transmitted through female lineages, some caution must be exercised in inferring reproductive relationships solely from mtDNA genotype. For example, situations are known in which individuals with grossly different mtDNA genotypes ($\bar{p} \approx 0.085$) coexist within a single random-mating population (23). However, an analogous situation is very unlikely to hold true for North Atlantic eels. Suppose mtDNA genotypes characteristic of *A. rostrata* and *A. anguilla* were to be present in a single panmictic assemblage (perhaps as long-retained female lineages in one gene pool or as more recent admixtures of formerly separated gene pools). The mtDNA genotypes would then almost certainly be in gametic phase equilibrium with various nuclear genotypes, so that any active dispersal behavior taking larval *A. rostrata* to North America and *A. anguilla* to Europe would have to be encoded or determined cytoplasmically, probably by the mtDNA itself! From our genetic data, two other possibilities thus appear far more reasonable: either (*i*) *A. rostrata* and *A. anguilla* spawn sympatrically but do not interbreed, in which case larval dispersal must have some active aspects such that American and European eels end up on appropriate continents; or (*ii*) most *A. rostrata* and *A. anguilla* belong to geographically separate breeding populations such that larval dispersal, even if passive, leads to appropriate continental distributions. Taking all available information in account (14, 20–22), this latter suggestion seems most likely.

Although our data demonstrate that most American and European eels do not now belong to the same randomly interbreeding population, the question of taxonomic species status remains thorny. mtDNA differences as large as that estimated between American and European eels ($\bar{p} \approx 0.035$) are commonly observed among conspecific vertebrates, including fishes (6). Suppose that *A. rostrata* and *A. anguilla* do indeed occupy largely allopatric spawning regions. Since biological species status depends in part on the biological potential for genetic exchange in sympatry, which is unknown in this case, the current mtDNA data should not necessarily be used to make a final taxonomic decision.

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