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## THE EVOLUTIONARY GENETIC STATUS OF ICELANDIC EELS

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**Abstract.**—The Iceland population of *Anguilla* eels contains an elevated frequency of fish with vertebral numbers lower than those typical of European localities. Several distinct hypotheses have been advanced to account for these morphologically atypical fish: for example, they could represent (1) genetically “pure” American expatriates, (2) genetically “pure” European types with ontogenetic abnormalities, or (3) hybrids between American and European forms. Here we critically test these and other possibilities by examining the joint distributions of allozyme markers, mitochondrial DNA markers, and vertebral numbers in Icelandic eels. The particular patterns of association among the genetic and morphological traits demonstrate that the Iceland population includes, in low frequency, the products of hybridization between American and European eels. Approximately 2–4% of the gene pool in the Iceland eel population is derived from American eel ancestry. This hybrid zone is highly unusual in the biological world, because the mating events in catadromous eels presumably take place thousands of kilometers from where the hybrids are observed as maturing juveniles. The molecular data, in conjunction with the geographic distributions, strongly suggest that the differences in migrational behavior and morphology between American and European eels include an important additive genetic component. Evolutionary hypotheses are advanced to account for the original separation of North Atlantic eels into American and European populations, and for the presence of hybrids in Iceland.

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North Atlantic eels of two nominal species, *Anguilla anguilla* and *A. rostrata*, inhabit inland and coastal waters of Europe and North Africa, and the Americas, respectively. The only known morphological distinction between the two is number of vertebrae, which typically ranges from 103 to 110 in eels from North America (with an approximate normal distribution around mean 107.1), and from 110 to 120 in eels from Europe (approximate normal distribution around mean 114.7). Both forms apparently spawn in the tropical west-Atlantic Ocean (the Sargasso Sea—Fig. 1), yet disperse as larvae to “appropriate” continental waters, such that eels with high vertebral counts arrive in Europe and those with low vertebral counts predominate in the Americas (Table 1). On reaching sexual maturity, eels from both continents complete the catadromous life cycle by returning to the Sargasso Sea to reproduce.

The biological and taxonomic relationships of American and European eels have long been the subject of debate (review in Williams and Koehn, 1984). Tucker (1959) proposed that the vertebral count differ-

ences are entirely ecophenotypic (not genetically based), and arise in response to different ambient temperatures experienced along the different migration paths taken by larvae destined for America versus Europe; thus American and European forms would belong to a single (perhaps panmictic) population. The hypothesis of a single population became untenable with more recent demonstrations that American and European eels also differ significantly in frequencies of traits with unambiguous genetic basis: malate dehydrogenase (*Mdh-2*) allozymes (review in Williams and Koehn, 1984), and mitochondrial DNA (mtDNA) restriction sites (Avise et al., 1986). Early in this century, Schmidt (1925) had shown that newly produced larvae with high vertebral counts were concentrated northeast of the Lesser Antilles, while larvae with low numbers of vertebrae were found mainly to the west, between the Greater Antilles and Bermuda. The deduction, that spawning of American and European eels was largely allopatric, may help to account for the recent genetic data indicating two largely separate gene pools. However, McCleave et al. (1987)

have recently shown that overlap in spawning areas is probably greater than Schmidt (1925) had suspected.

Many questions remain concerning the possibility of hybridization and extent of gene flow (if any) between American and European eel populations. Particularly within northern Europe, a low proportion of eels (about 0.3%—Williams et al., 1984) exhibit vertebral counts normally characteristic of American forms (i.e., less than 110). Three distinct possibilities have previously been raised to account for these eels with low vertebral numbers in Europe (Boetius, 1980): (1) occasional straying of genetically "pure" American eel larvae into water masses bound for northern Europe, (2) occasional action of environmental influences that reduce vertebral numbers in "pure" European eels, and (3) occasional hybridization between American and European forms. Opportunities to address these and other possibilities become magnified in Iceland, where eel populations show predominantly European characteristics, yet exhibit an elevated frequency of low vertebral counts and of the *Mdh-2<sup>a</sup>* allele normally associated with the American form (Table 1).

Here we critically evaluate several alternative hypotheses to account for atypical eels by examining the joint morphological and genetic characteristics of eels from Iceland. Genetic markers (*Mdh-2* and mtDNA) in conjunction with vertebral count numbers demonstrate that hybridization between American and European eels has indeed contributed to the Iceland eel gene pool. The genetic data also allow an estimate of the magnitude of flow of American eel alleles into Iceland, and motivate speculation about the evolutionary origins and mode of differentiation of the American and European eel populations.

#### MATERIALS AND METHODS

A total of 197 eels was collected from four sites in Iceland (Fig. 1) as follows: (1) Reykhólar ( $N = 48$ ), (2) Óxnalaekur ( $N = 70$ ), (3) Stokkseryi ( $N = 54$ ), and (4) Villingholtvatn ( $N = 25$ ). Frozen specimens were shipped to our laboratories at Stony Brook and Georgia, where assays of *Mdh-2* and mtDNA, respectively, were conducted.

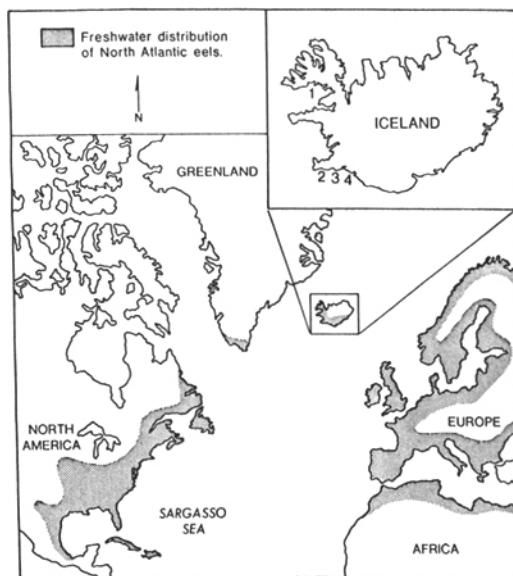


FIG. 1. Freshwater geographic distributions (shaded areas) of American and European eels; reproduction occurs in the Sargasso Sea area. Numbered collection locales in Iceland are described in Materials and Methods.

Numbers of vertebrae in the Iceland specimens were counted from X-ray photographs. In addition, 17 eels collected in Aarhus, Denmark, and 27 from Long Island, N.Y., were scored solely for mtDNA genotype.

*Mdh-2* genotypes were assayed by starch gel electrophoresis (Comparini and Rodino, 1980). mtDNA genotypes were determined from restriction digestion profiles revealed in Southern blot assays, using as a probe previously purified eel mtDNA (Avisé et al., 1986). The DNA isolation and Southern blotting procedures were similar to those described by Maniatis et al. (1982). For each specimen, either or both of two endonucleases, *Bgl*II and *Pvu*II, were used to digest total genomic DNA. These enzymes were chosen because each produces gel profiles that differ by at least two restriction site changes in American versus European eels (Avisé et al., 1986). Hence the possibility of misdiagnosis of an individual due to convergent mutations at multiple mtDNA sites is negligible.

For simplicity, in this paper the mitochondrial genotype "X" will refer to the pooled class of typically European mtDNA

TABLE 1. Characteristics that distinguish eel populations in North America from those in Europe. Also shown are frequencies of these traits in eels from Iceland.

Trait	America	Europe	Iceland	Reference
<b>Vertebral number</b>				
Range	103–110	110–120	108–118	Williams and Koehn (1984) and references therein
Freq. $\leq 110$	0.994 ( $N = 1,609$ )	0.003 ( $N = 15,854$ )	0.033 ( $N = 241$ )	Present study
Freq. $\leq 110$	—	—	0.056 ( $N = 197$ )	Present study
<b>Malate dehydrogenase</b>				
Freq. <i>Mdh-2<sup>a</sup></i>	0.958 ( $N = 696$ )	0.100 ( $N = 1,079$ )	0.129 ( $N = 241$ )	Williams and Koehn (1984) and references therein
Freq. <i>Mdh-2<sup>a</sup></i>	—	—	0.109 ( $N = 197$ )	Present study
<b>Mitochondrial DNA</b>				
Freq. "C" genotype	1.00 ( $N = 109$ )	0.00 ( $N = 29$ ) <sup>1</sup>	—	Avise et al. (1986)
Freq. "C" genotype	1.00 ( $N = 27$ )	0.00 ( $N = 17$ ) <sup>2</sup>	0.036 ( $N = 197$ )	Present study

<sup>1</sup> England and Ireland.

<sup>2</sup> Denmark.

genetic markers (Avise et al., 1986), while mtDNA "C" will refer to American mtDNA genotypes. Similarly, *Mdh-2<sup>b</sup>* will refer to a pooled class of typically European electromorphs, distinct from *Mdh-2<sup>a</sup>* which is the common allele in North America (Table 1).

## RESULTS

### *Genetic Status of Fish with Low Vertebral Counts*

In the current sample of 197 fish from Iceland, 11 (or 5.6%) exhibited vertebral counts of 110 or less. This value represents roughly a 17-fold increase in the frequency of such "low count" eels compared to other European locales, but is consistent with previous estimates for the Iceland population (Table 1). The following comparisons are based on data summarized in Table 2 and Figure 2. These data involve genotype frequencies for mtDNA and *Mdh-2*, their associations with one another (Table 2), and their associations with vertebral count numbers (Fig. 2). Since Boetius' (1980) hypotheses refer specifically to such low count fish, we will initially focus attention on the genetic status of eels in this lower end of the frequency distribution of vertebral numbers (Fig. 2). We will then examine properties of the pooled collections of Icelandic eels.

*Hypothesis 1: Icelandic eels with low vertebral counts are American expatriates.* If the Icelandic eels with low vertebral numbers are "pure" American eels that happen

to have settled in Iceland, they should represent a random draw from the American gene pool. They do not. Six of the 11 eels with 110 or fewer vertebrae possess mtDNA genotype "X" that has not yet been observed in any American locale (Table 1). Of the remaining five eels with low vertebral numbers, none is homozygous for *Mdh-2<sup>a</sup>/Mdh-2<sup>a</sup>*, the prevalent allozyme genotype in the Americas. One of these latter individuals is homozygous for *Mdh-2<sup>b</sup>/Mdh-2<sup>b</sup>*, a genotype expected with frequency 0.002 in America, while the other four are heterozygotes (*Mdh-2<sup>a</sup>/Mdh-2<sup>b</sup>*). The probability that any single eel from America is a heterozygote is 0.08; the probability that four such eels would all be heterozygous is  $4 \times 10^{-5}$ . Clearly then, eels with low vertebral counts in our Iceland sample are not American expatriates collectively, nor with high likelihood are they pure American eels individually. Hypothesis 1 also seems unlikely on morphological grounds alone, since all 11 Icelandic eels with "low" vertebral numbers have counts above the American mean.

*Hypothesis 2: Icelandic eels with low vertebral counts are ontogenetic abnormalities within the European gene pool.* If the Icelandic eels with low vertebral numbers are "pure" European eels, they should provide a random sample (with respect to genes not involved in vertebral development) from the European gene pool. They do not. Five of the 11 eels with 110 or fewer vertebrae possess mtDNA genotype "C" that has not yet

TABLE 2. Numbers (in parentheses) and frequencies of the joint *Mdh-2*/mtDNA genotypes in eels from Iceland. The cytonuclear associations (and their standard errors) are summarized using the allelic ( $D$ ) and genotypic ( $D_1, D_2, D_3$ ) disequilibrium statistics of Asmussen et al. (1987).

Mito- chondria	<i>Mdh-2</i>			Total
	a/a	a/b	b/b	
C	0.000 (0)	0.030 (6)	0.005 (1)	0.035 (7)
X	0.000 (0)	0.188 (37)	0.777 (153)	0.965 (190)
Total	0.000 (0)	0.218 (43)	0.782 (154)	1.000 (197)

$D = 0.011 \pm 0.005$   
 $D_1 = 0.000 \pm 0.000$   
 $D_2 = 0.022 \pm 0.009$   
 $D_3 = -0.022 \pm 0.009$

been observed in any European locale (Table 1). Of the remaining six eels with low vertebral count numbers, five are heterozygous for *Mdh-2<sup>a</sup>/Mdh-2<sup>b</sup>*, an allozyme genotype observed in Europe with frequency 0.18. Hence the probability that any of these latter eels is "pure" European is less than 20%, and the probability that all five are European is  $2 \times 10^{-4}$ . Clearly, eels with low vertebral counts in our Iceland sample do not possess typical European genotypes.

**Hypothesis 3: Icelandic eels are part of a selection-mediated cline of genotype frequencies in one Atlantic eel gene pool.** This possibility, not considered by Boetius (1980), suggests that the intermediate allele and vertebral count frequencies in Iceland eels (Table 1) represent selection-mediated responses to an Icelandic habitat that is somehow intermediate to most American and European locales. If so, allele frequencies at several loci might indeed be shifted concordantly. However, within the Iceland population, there should be no particular pattern of association among unlinked (and nonepistatic) genes, or between such genes and vertebral count numbers. But such associations do exist (Fig. 2). In a  $2 \times 2$  test of independence (Sokal and Rohlf, 1969), involving the mtDNA genotypes "C" and "X" and the vertebral count categories  $\leq 110$  versus  $> 110$ , there is a highly significant association between the American-type mtDNA and low vertebral count number

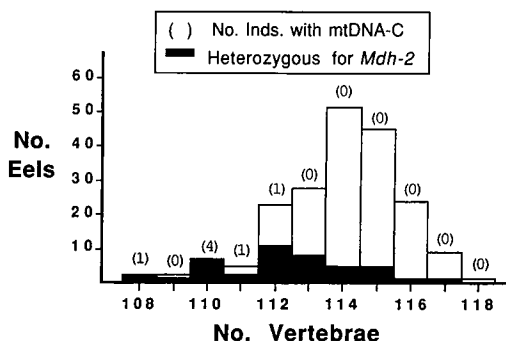


FIG. 2. Frequency distribution of vertebral count numbers in the current sample of eels from Iceland. Shaded areas of the histogram indicate fish with the heterozygous genotype *Mdh-2<sup>a</sup>/Mdh-2<sup>b</sup>* (no *Mdh-2<sup>a</sup>/Mdh-2<sup>a</sup>* homozygotes were observed). In parentheses are the numbers of individuals with various vertebral counts that exhibited the mtDNA-C genotype normally characteristic of American eels.

( $G = 24.1, P \ll 0.01$ ). In a similar test involving *Mdh*, there is a comparably strong association between *Mdh-2<sup>a</sup>* and low numbers of vertebrae ( $G = 21.4, P \ll 0.01$ ). (Associations between the *Mdh* and mtDNA genotypes will be discussed later.)

**Hypothesis 4: Icelandic eels represent the founder stock for both American and European eel populations.** Under this hypothesis, the Icelandic gene pool contains American and European genotypes by virtue of retention of polymorphisms from an ancestral stock in Iceland. This possibility seems very unlikely since Iceland became habitable only about 10,000 years ago. Furthermore, any retained polymorphisms at unlinked and nonepistatic loci are not expected to be associated within particular individuals. But as discussed beyond, such associations between mtDNA and *Mdh-2* do exist, as do associations between these genotypes and vertebral count numbers.

**Hypothesis 5: Icelandic eels with low vertebral counts include  $F_1$  hybrids between American and European forms.** The most common genotypes expected in  $F_1$  hybrids between American and European eels are *Mdh-2<sup>a</sup>/Mdh-2<sup>b</sup>/mtDNA-C*, and *Mdh-2<sup>a</sup>/Mdh-2<sup>b</sup>/mtDNA-X*, depending on whether an American or European eel was the female parent in the cross. (From Mendelian calculations, the likelihood that one or the other of these genotypic classes is present in an

$F_1$  is 0.86.) Among the 11 eels with low vertebral numbers, 5 were *Mdh-2<sup>a</sup>/Mdh-2<sup>b</sup>/mtDNA-C*, and 5 were *Mdh-2<sup>a</sup>/Mdh-2<sup>b</sup>/mtDNA-X*. This finding suggests that these individuals may indeed be first generation hybrids, and furthermore that the crosses involved in their production took place in both directions with respect to sex.

Nonetheless, an analysis of markers from multiple nuclear genes would be required to firmly establish that these individuals are  $F_1$  as opposed to later generation hybrids. For example, a first-generation backcross to a European eel would be expected to possess either of the two *Mdh*/mtDNA genotypes listed above with probability 0.52. In fact, there are two initial lines of evidence to suggest that our Icelandic eel sample as a whole includes some non- $F_1$  hybrids. First, one individual with low vertebral count number exhibited *Mdh-2<sup>b</sup>/Mdh-2<sup>b</sup>*, a genotype expected in an  $F_1$  with probability only 0.04. Second, even after removal of the 11 eels with vertebral counts  $\leq 110$  from our Iceland sample, significant associations remain between lower vertebral count numbers (in this case 111–113) and both the *Mdh-2<sup>a</sup>* and mtDNA-C frequencies ( $G = 17.2$  and  $16.8$ , respectively;  $P \ll 0.01$ ).

Thus overall, there is strong genetic evidence for hybridization between American and European eels. Most of the eels in Iceland with low vertebral count numbers are likely  $F_1$  or other early generation hybrids. In addition, there is a probable invasion of American genes (via backcrossing) into the predominantly European gene pool of Icelandic eels. We will now estimate the relative contributions of American and European alleles to the Icelandic stock.

#### *Gene Flow into the Iceland Eel Population*

The eel stock in Iceland clearly contains a mixture of alleles from America and Europe. The contribution of European genes to the Icelandic gene pool can be estimated by

$$M = (q_h - q_a)/(q_b - q_a) \quad (1)$$

(Wallace, 1968 p. 81), where  $q_a$ ,  $q_b$ , and  $q_h$  represent the frequencies of the *Mdh-2<sup>a</sup>* (or mtDNA-C) allele in America, Europe, and Iceland, respectively. Using the *Mdh-2* al-

lele frequencies summarized in Table 1 (with  $q_h$  the weighted average of *Mdh-2<sup>a</sup>* in the two surveys),  $M = 0.977$ . This value agrees closely with the estimate of  $M = 0.964$  obtained from a similar analysis of the mtDNA haplotype frequencies (Table 1). Thus Iceland eels exhibit European genes predominantly, and both *Mdh-2* and mtDNA indicate that only about 2–4% (i.e.,  $1 - M$ ) of the gene pool in the Iceland eel population is derived from the American eel ancestry. Interestingly, the percentage shift in the mean vertebral count number in Icelandic eels toward the American population mean (9%) appears generally consistent with these conclusions.

Additional information can be extracted from the joint distributions of mtDNA and *Mdh-2*, as summarized by allelic ( $D$ ) and genotypic ( $D_1$ ,  $D_2$ , and  $D_3$ ) cytonuclear disequilibria defined by Asmussen et al. (1987). The parameter  $D$  measures the association between alleles at a nuclear and a cytoplasmic locus, and  $D_1$ ,  $D_2$ , and  $D_3$  measure associations between two cytotypes and the three respective genotypes at a diallelic nuclear locus. These four measures of disequilibria are expected to exhibit certain patterns in a hybrid zone depending on a variety of factors such as mating behavior, selection, and migration. Table 2 shows the cytonuclear disequilibria calculated for Icelandic eels. Using the  $G$ -test approach described in Asmussen et al. (1987), the hypothesis that all four disequilibria equal zero can be rejected ( $G = 13.65$ ,  $P = 0.001$ ), while the hypothesis that  $D_1$  alone equals zero cannot ( $G = 0.04$ ,  $P = 0.84$ ). (Because of the small expected counts in some cells of Table 2, we confirmed that the  $G$ -value of 13.65 was significant by an exact test that involved calculating the multinomial probabilities of all possible tables with the same marginal counts as those exhibited by Table 2.) We conclude that there are significant associations between mtDNA and *Mdh-2* in the Iceland population.

To explain the pattern of cytonuclear association in the context of migration of eels into Iceland, we can apply the models developed by Asmussen et al. (1989). As applied to the current situation, we can view the Iceland stock as being composed of some

fraction of pure European and American eels, and some fraction of hybrids stemming from random mating in the hybrid zone. We further assume that the censusing takes place after mating, migration of pure American and European eels to Iceland occurs at a fixed rate per (nonoverlapping) generation, and genotypes are neutral with respect to fitness. Recursions for the cytonuclear genotypic frequencies under this model are given by Equations (B4) in Asmussen et al. (1989). The model was fitted to the data of Table 2 by the method of maximum likelihood, under the assumption that the Iceland eel population is at genetic equilibrium. The best goodness-of-fit ( $G = 8.08$ ,  $P = 0.044$ ) was obtained when the per-generation migration rates of pure American ( $m_1$ ) and pure European ( $m_2$ ) eels into the Iceland population were 0.02 and 0.70, respectively. Thus by this approach, the accumulated proportion of Icelandic alleles with European ancestry is  $M = m_2/(m_1 + m_2) = 0.972$ . This estimate is virtually identical to the values obtained above from a consideration of the mtDNA and nuclear genotypes separately ( $M = 0.964$  and  $0.977$ , respectively).

#### *Spatial Structure of Genotypes*

American and European eels have distinct dispersal patterns that take them to the appropriate continent. In the current study, we also have the first evidence for nonrandom settlement of eels on a microgeographic scale. Among the four collection sites in Iceland, there was significant heterogeneity in the frequencies of both the *Mdh-2<sup>a</sup>* and mtDNA-C alleles ( $G_H = 19.7$  and  $20.7$ , respectively, both  $P < 0.01$ ), and in frequencies of fish with  $\leq 110$  versus  $> 110$  vertebrae ( $G_H = 14.3$ ,  $P < 0.01$ ). For example, all seven individuals carrying mtDNA-C were present in the Reykhólar collection, 37 of the 43 *Mdh* heterozygotes were observed in the Reykhólar and Öxnalaekur collections, and all 11 fish with  $\leq 110$  vertebrae were in Reykhólar and Öxnalaekur. Such nonrandom spatial distributions of genotypes and morphologies raise the possibility that specific subsets of eel reproduction (products of certain spawns or sets of spawns) may occasionally tend to stay to-

gether and settle jointly. Nonetheless, any particular distribution of genotypes on a fine spatial scale would likely be ephemeral, changing with each round of reproduction and current-mediated migrational influx. If this is true, the microspatial differences would not be expected to accumulate through time, and hence are not necessarily inconsistent with the absence of dramatic macrogeographic heterogeneity of gene frequencies within regions such as North America or Europe (Avisé et al., 1986; Williams and Koehn, 1984). In the future, it would be of interest to monitor temporal variation in genotype frequencies at particular locales.

#### DISCUSSION

We have shown that the Reykhólar and Öxnalaekur samples of eels in Iceland include, in low frequency, hybrids between American and European forms. This hybrid situation is highly unusual, because the location in which the hybrid animals are found as juveniles and young adults is presumably far removed from where the mating events take place (some 5,000 km away, in the Sargasso Sea). What could account for the original genetic separation of American and European eels, and for the occurrence of hybrids in Iceland? The following scenarios are based on available genetic and life history data for eels, and should be viewed as hypotheses requiring further evaluation.

At some time in the past, all North Atlantic eels must have belonged to a single population that subsequently became separated into American and European forms. The current nucleotide sequence divergence in mtDNAs between American and European populations (after correction for within-continent divergence) is approximately  $p = 0.03$  (Avisé et al., 1986). Under a "conventional" mtDNA clock calibration of 2% sequence divergence per million years (Brown et al., 1979; Shields and Wilson, 1987), a separation time of approximately 1.5 million years ago is suggested. The multilocus allozyme distance [Nei's (1972) measure] is  $D = 0.11$  codon substitutions per locus (Williams and Koehn, 1984), which also suggests a divergence time of perhaps 1–2 million years under some "con-

ventional" protein clock calibrations (see Avise and Aquadro, 1982). While the mtDNA and protein estimates of divergence times are provisional, they both point to a time of separation in the Pleistocene.

Ancestral eels in the North Atlantic probably exhibited a catadromous life cycle [no species of *Anguillidae* is known to pass its entire life history in freshwater, and all other *Anguilliformes* are strictly marine (Marshall, 1966; Moyle and Cech, 1982)]. These ancestral eels may have bred as one population, producing larvae destined for one continent or geographic region. The following "dispersal" scenario will assume that juveniles of the ancestral population occurred in Europe and secondarily colonized North America, but the arguments hold equally well if the direction of continental colonization is reversed.

Suppose that during the Pleistocene, shifting oceanic currents brought some larvae normally destined for Europe within reach of the North American coastline, and that some of these larvae settled "prematurely" (North Atlantic current patterns are thought to have varied widely in the Pleistocene, due to global climatic changes associated with glacial advances and retreats—e.g., Keffer et al., 1988). After maturing in North America, and on returning to the sea to reproduce, these eastward-swimming eels would likely have first encountered "suitable" waters for spawning in the western portion of the Sargasso Sea area, while eels returning westward from Europe would first encounter suitable spawning waters in the eastern Sargasso Sea area. Hence there may have been an initial tendency for assortative mating by continental origin due simply to the geographic positioning of returning adults.

Suppose further that these oceanic currents persisted, and that there was some initial genetic variation with respect to larval settlement times. Today, American eels settle in about 1 year and European eels settle after 2 years or more (Williams and Koehn, 1984). Initially, as now, there must have been strong disruptive selection favoring larval settlement on one continent or the other, eventually leading to the bimodal distribution of larval settlement times. Concomitantly, this selection pressure should

have provided a fitness advantage to any genetically based behavioral tendencies (migration routes or mating preferences *per se*) leading to assortative mating among eels from the two continents. Thus once set in motion by an initial continental colonization event, a selection-driven feedback process should have reinforced any original tendency for premating isolation between the two eel forms. An important component of this premating isolation is no doubt the continuing spatial separation within the Sargasso Sea of the major concentrations for breeding of American versus European eels (Schmidt, 1925), but homotypic mating preferences in sympatry may also be at work (see McCleave et al., 1987).

Alternatively, a "vicariant" scenario might be entertained to account for the original separation of American and European eels. Perhaps a single ancestral population produced larvae that dispersed to north-central Atlantic coasts, particularly Greenland, Iceland, and northern Scandinavia. With cooling during Pleistocene glacials, this northern region became uninhabitable, and forced a southward retreat and disjunction of spawning areas. Two separate breeding grounds may then have produced larvae destined for North America and Europe, respectively. Adaptive choice of spawning region would again be accompanied by shifts in behavior of larvae enabling them to reach appropriate temperate coasts. With the subsequent retreat of sea ice and the reemergence of suitable habitats northward, spawning areas may then have expanded and secondarily overlapped, leading to the current hybrid situation.

In any event, Iceland occupies a position longitudinally intermediate to North America and Europe (Fig. 1). Since we now know that some hybrid eels occur in Iceland, it seems reasonable to speculate that this is the result of an intermediate hybrid migrational behavior. This behavior could reflect larval dispersal along unique currents stemming from the geographically intermediate spawning grounds in the Sargasso Sea where American and European eels presumably overlap, and/or an intermediate settlement time from ocean currents sweeping by Iceland. A general behavioral intermediacy of hybrid animals is not unusual (Brown, 1975;



Lamb, 1987), and there is at least one known precedent for the intermediacy of hybrids with respect to migratory behavior: Berthod and Querner (1981; see also Berthod, 1988) found that hybrids between migratory and nonmigratory European warblers exhibit patterns of migratory restlessness intermediate to those of their parents.

Nonetheless, there are some potential problems with this simple hypothesis for the elevated frequency of hybrid eels in Iceland. Surface currents of the Gulf Stream, after crossing the North Atlantic, are thought to pass the British Isles before swinging west in higher latitudes and reaching Iceland (this presumably accounts for the great preponderance of European genotypes in the Icelandic eel population). Yet England does not appear to exhibit an elevated frequency of American vertebral count numbers. However, ocean currents and eddies in the North Atlantic, particularly at various depths, are not well understood (Richardson, 1985), and it is quite possible that certain eddies reach Iceland via a more direct route from the Sargasso Sea, carrying with them hybrid larvae from an overlap zone in the breeding area. Or perhaps young eels actively swim north from the Gulf Stream, at an intermediate settlement time, to reach Iceland.

What have been the genetic consequences of hybridization in eels?—are the hybrids an evolutionary dead-end, or have they provided an avenue of gene exchange between the American and European forms? For example, Boetius (1980) found that mean numbers of vertebrae are homogeneous over most of the European range of eels, but are shifted slightly toward American values in localities such as Scotland and Sweden, a result that could be interpreted as evidence for introgression of American genes into the northern European gene pool (sample sizes are not yet large enough to evaluate the possibility of a comparable shift in northern Europe in frequencies of *Mdh-2* or mtDNA genetic markers). Answers to questions about the magnitude and pattern of genetic introgression depend in large part on assumptions about the ancestral condition of the American and European gene pools. Suppose, as an extreme example, that American and European eels were at one time fixed for alternate alleles at *Mdh-2*. An

application of Equation (1) to the *Mdh-2* data in Table 1 then suggests that about 10% of the nuclear genes in Europe are of American ancestry, and that about 4% of the nuclear genes in America are of European ancestry. But an alternative interpretation is that the presence of both *Mdh-2* alleles on the two continents represents the parallel retention of an ancestral polymorphism in the absence of any introgression. With very large genetic samples of European eels, it might be possible at least partially to evaluate these competing hypotheses: if hybridization and introgression were predominantly recent, genetic disequilibrium might remain among alleles at unlinked loci, and associations could exist between such marker genes and particular morphological features such as vertebral counts.

Overall, we have shown that the Icelandic eel stock includes, in low frequency, the products of hybridization between American and European eels. Associations between *Mdh-2* and mtDNA genotypes, morphologies, and locations strongly imply that there is an important additive genetic component to intermediate (hybrid) eel vertebral count numbers and migrational behavior. While the firm documentation of hybrids in Iceland eliminates some of the hypotheses previously advanced to account for the unusual properties of Icelandic eels, the findings also raise many new questions, particularly about the specific location of the hybrid breeding grounds, and the migrational routes taken by hybrid larvae. Further field studies, perhaps in conjunction with genetic tests, will be required.

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