DIFFERENTIATION OF MITOCHONDRIAL DNA IN ATLANTIC HERRING, *CLUPEA HARENGUS*

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ABSTRACT

To investigate genetic relationships among spawning stocks of Atlantic herring, *Clupea harengus*, in the Gulf of Maine and Gulf of St. Lawrence, mitochondrial DNAs from ripe females at three localities were examined by restriction endonuclease analysis. Using seven variable restriction enzymes, mtDNAs from 69 completely characterized individuals produced 26 composite digestion patterns. The majority of individuals (65%) possessed composites which were common to two or more spawning localities; the other individuals displayed locality specific "unique" composites. Analysis of relationships among these unique composites suggested that some may have been derived from other areas. These results are not consistent with the idea that separate genetic stocks of Atlantic herring exist in the Gulf of Maine.

The relationships among discrete spawning stocks of Atlantic herring, *Clupea harengus*, are problematical. A large number of stocks and stock complexes are recognized throughout the eastern and western North Atlantic; these delineations are based largely on meristic characters, spawning time, and spawning location. Tagging studies in the western North Atlantic have shown extensive migration and mixing of stocks during nonreproductive periods (Creaser et al. 1984). More limited studies of spawning fish have demonstrated that some tagged individuals returned to their spawning locations (Wheeler and Winters 1984). Recent work has advanced the hypothesis that specific environmental attributes essential for growth and survival of larval herring largely determine where Atlantic herring will spawn (Iles and Sinclair 1982). The notion that spawning occurs near areas suitable for larval retention could explain the discontinuous or patchy distribution of spawning areas. Similarly, the occurrence of fall spawning and spring spawning Atlantic herring stocks may be a function of completion of larvae growth and metamorphosis constrained by resources within the larval retention area (Sinclair and Temblay 1984). There is thus a reasonable model to explain the existence of geographically or temporally discrete spawning stocks. However, the genetic structure among these different spawning groups is unresolved.

Implicit in the Atlantic herring stock concept is the idea that individual fish belong to defined groups by virtue of returning to specific spawning sites. If this is the case, there should exist high genetic continuity among individual Atlantic herring within stocks and relatively lower continuity among stocks. That is, genetic differences should be observable among stocks. Unfortunately, meristic characters useful for stock definition are under environmental influence and have a complex genetic basis. Electrophoretic characterization of allozyme variation should potentially permit identification of genetic discontinuities among stocks. However, despite the availability of a large number of polymorphic markers and adequate sample sizes, significant genetic heterogeneity among Atlantic herring stocks has not been demonstrated (Anderson et al. 1981; Kornfield et al. 1982; Grant 1984; Riviere et al. 1985). The inability of allozyme analysis to differentiate among herring stocks could occur for two alternative reasons. Herring stocks could have originated so recently that there has been insufficient time for stock specific allozyme variation to accumulate. Further, natural selection may be acting to homogenize allele frequencies that may characterize stocks. Thus, standard allozyme analyses may not be sufficiently sensitive to detect genetic variation which distinguishes stocks. Alternatively, herring stocks could be largely composed of individuals that do not return to natal spawning sites. Under this explanation, herring stocks would not represent discrete genetic groups but rather random assemblages of spawning individuals. Management of

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exploited herring stocks could differ dramatically depending upon which alternative is correct (MacLean and Evans 1981).

Restriction endonuclease analysis of mitochondrial DNA (mtDNA) has, in recent years, uncovered substantial genetic variation in natural populations (Brown 1983). The technique is potentially much more sensitive than conventional allozyme analysis for characterizing population structure and has been successfully exploited to discriminate groups not detectable with allozymes (e.g., Avise et al. 1986; R. W. Chapman unpubl. data). To further examine genetic relationships among herring stocks, restrictive enzyme digestion patterns of mtDNA were examined in individuals from three spawning localities in the northwestern Atlantic.

MATERIAL AND METHODS

Samples of fall spawning Atlantic herring were obtained from two discrete localities in the Gulf of Maine: Jeffries Ledge, MA (lat. 42°50'N, long. 66°30'W; 9 September 1984) and Trinity Ledge, NB, Canada (lat. 45°20'N, long. 65°30'W, September 1984; 30 August 1985). A sample of spring spawning Atlantic herring was collected from off Pt. Escuminac, Gulf of St. Lawrence, NB (lat. 47°01'N, long. 64°40'W; 12 May 1985) (Fig. 1). All Atlantic herring were collected during peak reproduction. Samples were frozen in the field and stored at −80°C for up to 6 months prior to analysis.

Lansman et al. (1981) provided a useful review of the application of mtDNA to population studies. mtDNA was prepared from egg tissue (11-15 g/female) by the rapid phenol extraction procedure of Chapman and Powers (1984). After the final chloroform extraction, mtDNA in the aqueous phase was precipitated in 95% ethanol in the presence of 3 M sodium acetate, dried under vacuum and dissolved in 10 mM Tris, pH 7.5.

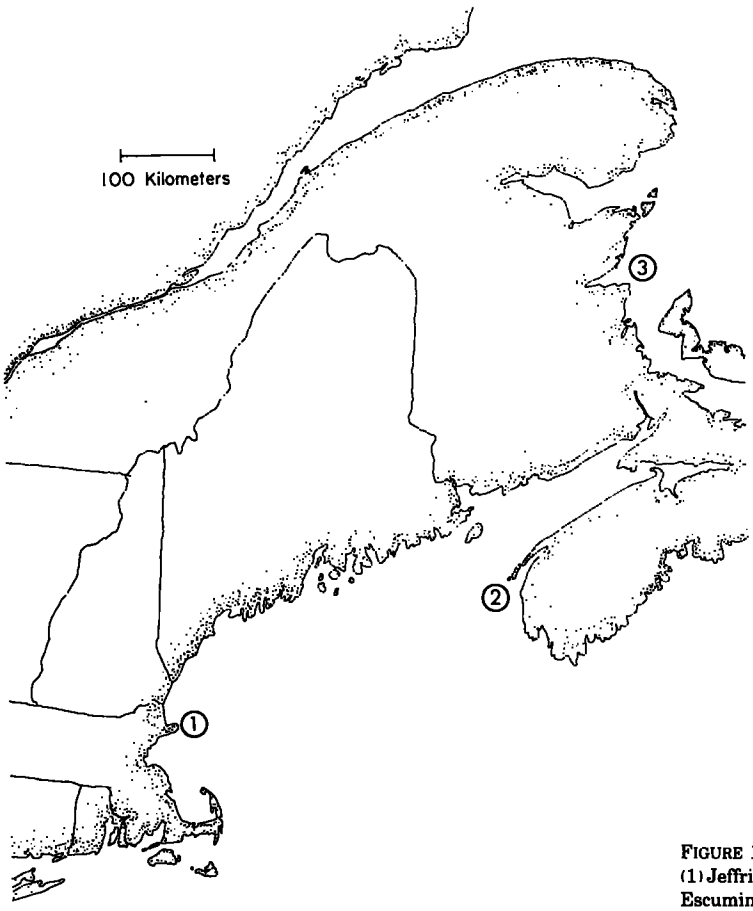

FIGURE 1.—Collection localities for Atlantic herring. (1) Jeffries Ledge, MA; (2) Trinity Ledge, NB; (3) Point Escuminac (St. Lawrence), NB.

Samples were digested with 16 six-base restriction endonucleases (Table 1) under conditions recommended by suppliers (Bethesda Research Labs, New England Biolabs). Just prior to addition of restriction enzymes, samples were incubated with Ribonuclease A (RNase) at 60°C for 5 minutes and allowed to cool to 37°C. Restriction fragments were separated by horizontal electrophoresis in 1% agarose gels. *Hin*dIII digests of lambda DNA were used as molecular weight standards on all gels. Gels were stained for 60 minutes with 0.5 g L^{-1} ethidium bromide and destained for 30 minutes in 5 mM $MgSO_4$ prior to photography. The relative mobilities of mtDNA and lambda fragments were measured from photographs with a stereomicroscope. Molecular weights of restriction fragments were calculated from least squares third order polynomial regressions of log-transformed lambda fragment mobilities.

RESULTS

Restriction digests of mtDNAs prepared by the rapid phenol extraction procedure well resolved, repeatable digestion patterns (Fig. 2). Two enzymes, *Bam*H1 and *Sal*I did not digest the herring mtDNA molecule. Variant digestion patterns were noted for the majority of enzymes examined (Table 1) and were common both within

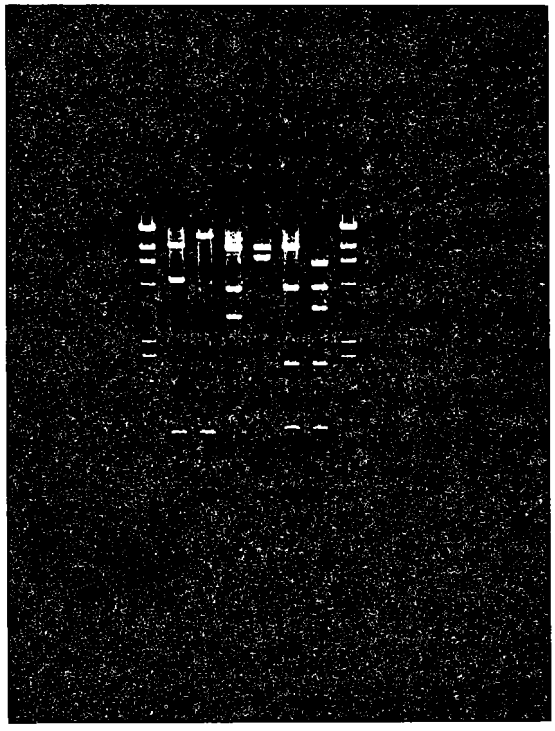

FIGURE 2.—Ethidium bromide stained agarose gel of mtDNA digestion patterns of Atlantic herring, *Clupea harengus* (lanes 2-8). Samples were digested with *Bst*EII (lanes 2, 3; phenotypes B, C), *Eco*RI (lanes 4, 5; phenotypes A, C), and *Bgl*II (lanes 6, 7; phenotypes A, B). Standard (lanes 1, 8) is a *Hin*dIII digest of lambda DNA.

TABLE 1.—Digestion patterns of Atlantic herring mtDNA produced by six-base restriction endonucleases[1]. Superscripts denote homologous fragments, measured independently.

*Apa*I						*Bst*EII					*Bgl*II			
A	B	C	D	H	I	A	B	C	D	E	A	B	C	D
8,800[a]	8,800[a]	7,200[e]	6,350	8,800[a]	7,350	11,830[d]	10,820[d]	15,160	12,200	11,700[d]	9,940	6,590[d]	14,000	6,600[d]
7,200[e]	6,100[c]	5,300	6,100[c]	7,200[e]	6,100[c]	4,670[a]	4,670[a]	1,150[c]	4,700[a]	3,650	4,130[a]	4,180[a]	1,990[b]	3,900
900[b]	960[d]	3,450	2,450[h]	1,100	2,450[h]	550[b]	1,160[c]	550[b]		1,340	1,990[b]	3,230[e]	1,270[c]	3,250[e]
	900[b]	900[b]	960[d]		900[b]		550[b]			550[b]	1,270[c]	1,960[b]		1,970[b]
			900[b]									1,220[c]		1,220[c]
16,900	16,760	16,850	16,760	17,100	16,800	17,050	17,200	16,860	16,900	17,240	17,330	17,180	17,260	16,940

*Dra*I		*Eco*RI			*Eco*RV				*Hin*dIII	*Kpn*I		*Pst*I
A	B	A	B	C	A	B	C	D	A	A	B	A
7,600	11,370	9,460[a]	14,020	9,400[a]	8,680[a]	8,200	8,600[a]	14,500	13,500	15,200	17,000	10,790
3,700	2,530[a]	4,220	3,130[b]	7,250	6,000	6,490	8,200	2,270[b]	2,820	1,910		5,850
2,570[a]	2,290[b]	3,010[b]			2,230[b]	2,030			1,000			
2,310[b]						1,000						
16,170	16,190	16,690	17,150	16,650	16,910	17,720	16,800	16,770	17,320	17,110	17,000	16,640

*Pvu*II			*Sac*I	*Xba*I	*Xho*I		*Xmn*I
A	B	C	A	A	A	B	A
7,480	12,840	13,600	9,800	5,350	14,000	16,500	7,940
5,590	1,550[a]	1,560[a]	7,300	4,880	2,690		3,780
1,580[a]	1,420[b]	1,400[b]		2,890			2,330
1,420[b]	850[c]			2,060			1,810
900[c]				2,050			1,320
16,970	16,660	16,560	17,100	17,230	16,690	16,500	17,180

[1]Two additional enzymes, *Bam*HI and *Sal*I produced no (or one) cuts.

and among population samples. For polymorphic restriction enzymes, all digestion profiles of variants were consistent with the hypothesis of single nucleotide substitutions. No mtDNA size variants, resulting from additions or deletions of DNA, and recently found in a number of fish groups (Bermingham et al. 1986; R. W. Chapman[2]), were observed.

The mean mtDNA genome size, found by averaging the sums of all digestion patterns (Table 1), was 16,990 bp (base pairs) ± 620 bp (SD). In quantifying molecular size from ethidium bromide stained agarose gels, two constraints must be noted. First, variation associated with measurement of fragment mobilities is inevitable. Because of the nonlinear relationship between fragment mobility and molecular size, slight measurement errors can produce large variations in estimated sizes, particularly for fragments with low mobilities. As a consequence, homologous cleavage fragments (those which consistently exhibit the same mobility on a single agarose gel) may yield different molecular size estimates, e.g., *Apa*I fragment "a", Table 1. Second, mtDNA cleavage fragments less than 500 bp could not be routinely scored on ethidium bromide gels because of their low absolute staining intensities and fluorescent background in this region (Fig. 2). Regardless of the above constraints, individual cleavage fragment phenotypes could be consistently determined.

Seven polymorphic restriction endonucleases (*Apa*I, *Bgl*II, *Bst*EII, *Eco*RI, *Eco*RV, *Pvu*II, and *Xho*I) were used to generate composite digestion patterns for individual Atlantic herring. Twenty-six unique composite digestion patterns were observed in 69 completely characterized individual Atlantic herring. The distribution of these composites with respect to spawning locality is given in Table 2; five common composites (nos. 1-5) were observed to occur at all three spawning localities.

Shared fragment similarity was calculated pairwise for all composites and was used to generate estimates of p, percent sequence divergence (Upholt 1977). Estimated sequence divergence among composites varied considerably, mean = 1.66% +/− 0.91 (SD), range 0.19% − 4.37% (Table 3). Phenetic relationships among composites were examined by UPGMA (Unweighted Pair Group Method of Arithmetic averaging) clustering (Sneath and Sokal 1973) of sequence divergence (Fig. 3). Two major clusters were noted: one involving three composites (5, 17, 26) and the other including all other composites. Composites from both clusters were present in all spawning populations.

A network of relationships among composites was constructed by connecting composites in increments of single site gains or losses to minimize the total number of restriction site changes required (Fig. 4). Sixteen equally parsimonious networks requiring 29 steps were generated. Composite number 1 can be considered central because it is the most common pattern observed and also occurs in the Eastern Atlantic (S. M. Bogdanowicz unpubl. data).

DISCUSSION

Based on the occurrence of geographically disjunct spawning groups and homing of some tagged individuals, it has been tacitly accepted that Atlantic herring stocks are reproductively isolated. The basic analytical premise of this study was that restriction endonuclease analysis

[2]R. W. Chapman, Chesapeake Bay Institute, The Johns Hopkins University, Shandy Side, MD 20764, pers. commun. December 1985.

TABLE 2.—Distribution of mtDNA composite digestion patterns in samples of Atlantic herring.

Composite designation	Composite mtDNA digestion pattern[1]	Jeffries Ledge	Trinity Ledge 1984	Trinity Ledge 1985	St. Lawrence
1	AAAAAAA	5		2	5
2	AABAAAA	2	2	2	6
3	BAAAAAA	2		3	6
4	AABAAAB	1	1	3	1
5	ABAABAA	1	1	2	1
6	BABAAAA	1			
7	AABACAA			1	1
8	AAAADAB	1			
9	IAAAAAA				2
10	DAAAAAA				1
11	CAAAABA				1
12	BAEAAAA				1
13	HABAAAA				1
14	AAAAABB		1		
15	CAAAAAA		1		
16	BABAAAB		1		
17	ABABBAA		1		
18	ABBACAA			1	
19	AAACCAA			1	
20	IABAAAA			1	
21	AACAAAA			1	
22	BADAAAA			1	
23	CABAAAA			1	
24	AADAAAA			1	
25	ACDAAAA			1	
26	ADDABAA			1	
		13	8	22	26

[1]Letters (from left to right) are digestion patterns for *Apa*I, *Bgl*II, *Bst*EII, *Eco*RI, *Eco*RV, *Kpn*I, and *Xho*I (Table 1).

TABLE 3.—Estimated percent sequence divergence among mtDNA composites in Atlantic herring.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1	—	0.42	0.42	0.91	1.40	0.86	0.91	0.97	0.73	0.87	0.91	0.87	0.14	0.97	0.73	1.40	2.04	1.40	0.97	1.19	0.59	0.91	1.19	0.45	0.97	2.47
2		—	0.87	0.42	1.54	0.69	0.42	1.49	0.87	1.33	1.40	1.68	0.27	1.49	0.87	1.19	1.78	0.87	1.49	0.69	0.42	0.73	0.40	0.29	0.77	2.62
3			—	1.40	1.92	0.40	1.40	1.49	0.56	0.40	1.40	0.40	0.56	1.49	0.87	0.87	2.62	1.92	1.49	1.00	1.06	0.42	1.32	0.91	1.49	3.09
4				—	2.62	0.87	0.91	0.97	1.40	1.92	2.47	1.92	0.73	0.97	1.78	0.42	3.47	1.40	1.74	1.19	0.91	1.63	0.87	1.12	1.74	3.46
5					—	2.90	2.62	1.49	2.32	2.90	3.09	2.90	1.92	3.31	2.32	3.76	0.42	1.54	1.89	3.38	2.62	3.61	2.90	2.47	2.80	0.73
6						—	0.56	2.04	0.69	0.82	1.92	1.13	1.00	2.04	1.32	0.40	3.24	1.33	2.04	0.53	0.87	0.56	0.82	1.06	1.63	3.76
7							—	1.74	1.40	1.92	2.04	1.92	0.73	2.17	1.40	1.40	2.47	0.42	0.97	1.19	0.91	1.63	0.87	1.12	1.74	3.47
8								—	1.89	2.04	2.64	2.04	1.49	0.32	1.49	1.49	2.64	2.32	1.42	2.47	1.74	2.17	2.04	1.58	2.32	3.16
9									—	0.40	1.06	1.00	1.19	1.89	0.87	1.19	3.09	1.92	1.89	0.13	1.40	1.06	1.00	1.26	1.89	3.61
10										—	1.19	1.13	1.33	2.04	1.00	1.33	3.24	2.46	1.63	0.53	1.19	0.56	1.13	1.06	1.63	3.24
11											—	2.32	1.78	1.34	0.42	3.09	3.47	2.62	2.17	1.92	1.63	2.04	0.87	1.49	2.17	4.04
12												—	1.33	2.47	1.33	1.33	3.24	2.46	2.04	1.46	1.19	0.87	1.81	1.40	2.04	3.76
13													—	1.49	0.87	1.19	3.09	1.19	1.49	1.00	0.73	1.40	0.69	0.91	1.49	3.09
14														—	1.49	1.12	3.73	1.89	2.32	2.47	1.74	2.17	2.04	1.58	2.32	4.37
15															—	1.54	2.18	1.92	1.49	1.33	1.06	1.40	0.40	0.91	1.49	3.09
16																—	3.09	1.92	2.80	1.00	1.40	1.06	1.33	1.63	2.32	4.19
17																	—	2.18	2.64	4.33	3.47	4.04	3.76	3.89	3.73	1.26
18																		—	1.49	1.68	1.40	2.18	1.33	1.63	1.89	1.78
19																			—	2.47	1.74	2.17	2.04	1.58	2.32	3.16
20																				—	1.54	1.19	0.82	1.40	2.04	3.76
21																					—	1.26	0.87	0.77	1.34	2.95
22																						—	1.54	0.45	0.97	2.47
23																							—	1.06	1.63	3.24
24																								—	0.47	1.89
25																									—	1.73
26																										—

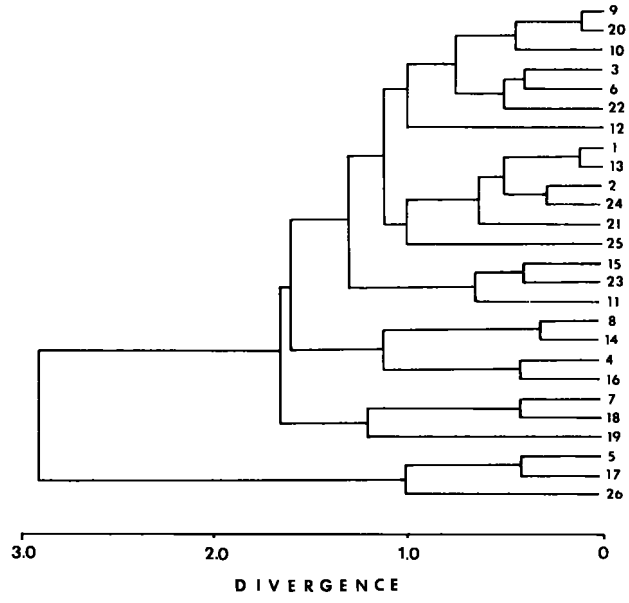

FIGURE 3.—Phenetic relationships among mtDNA composite cleavage patterns of Atlantic herring. Estimates of sequence divergence were clustered by UPGMA.

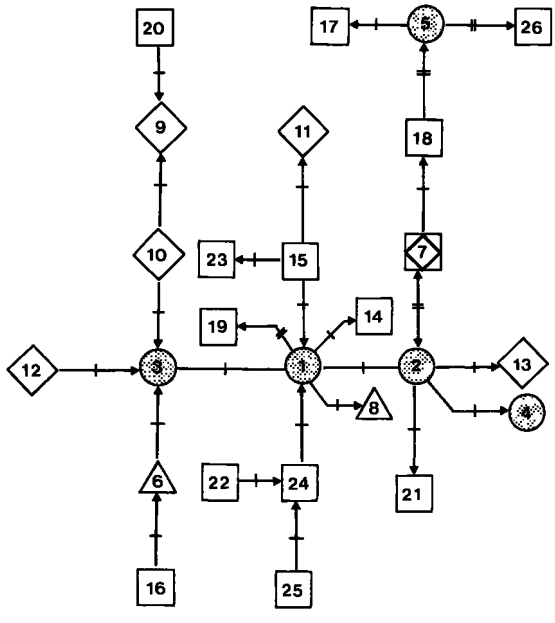

FIGURE 4.—Cladistic relationships of 26 composite cleavage patterns of Atlantic herring mtDNA. Composites are connected parsimoniously to minimize the number of restriction site changes required. Shaded numbers refer to composites observed at all three spawning locations. Crossbars on connecting lines indicate minimum number of site changes required to connect adjacent composites; arrows indicate direction of site losses. Locality symbols: square - Trinity Ledge; triangle - Jeffries Ledge; diamond - St. Lawrence.

of mtDNA should have been able to differentiate among such reproductively isolated populations. However, the spawning groups studied were not fully distinguishable by composite mtDNA digestion patterns generated by six-base restriction endonucleases; no absolute stock markers were present. Six of the twenty-six composite designations, representing more than 65% of all individuals, were shared by at least two geographically distinct spawing groups.

The occurrence of common composites in all spawning populations could occur for at least two reasons: First, commonality could reflect recent and/or ongoing gene exchange among populations. Consistent with this idea, there is no association between frequencies of common composites (nos. 1-5) and spawning locality ($G = 6.29$, p 0.5, Sokal and Rohlf 1981). As is generally acknowledged, small numbers of individuals migrating among populations are sufficient to homogenize different groups (Allendorf 1983). The absence of two common composites (1 and 3) in the 1984 Trinity Ledge sample might be due to the stochastic effect of small sample size, though this observation could also imply some element of temporal instability in composition. Second, and alternatively, these common composites could represent ancestral mtDNAs which were widespread prior to any genetic isolation of populations. That is,

the occurrence of common composites need not imply current gene exchange (Avise et al. 1984; Neigel and Avise 1986); population sizes are sufficiently large to support the co-occurrence of common ancestral composites and their derivatives.

The presence of 20 composites which were specific to spawning groups suggests that there may be some degree of genetic isolation among stocks. Given the limited number of individuals sampled, it is difficult to know whether "unique" composites are actually restricted to specific stocks. For example, rather than increasing the abundance of previously observed unique composites, the second sample from Trinity Ledge generated additional composites. There is thus little indication that "unique" composites may be useful in defining stocks. The great composite diversity displayed in the samples of Atlantic herring most probably reflects the very large population sizes involved.

In the absence of gene flow among spawning populations, we would expect a unique composite to be found in the same population as its most probable precursor. In three out of seven instances, precursors of unique composites occurred in different spawning populations (this result holds for all other equally parsimonious networks). For example, composite 9, which only occurs in the St. Lawrence sample, is the immediate ancestor of composite 20 from Trinity Ledge. In addition, the two unique composites which were maximally divergent (12 steps) occurred in the same population (Trinity Ledge 1985). These considerations, as well as the absence of any consistent geographic pattern of unique composites are consistent with the idea of gene flow.

Evidence for the ability of mtDNA analysis to detect subtle population differentiation is compelling (Avise et al. 1979; Lansman et al. 1981; Wilson et al. 1985; Bermingham and Avise 1986). However, since differentiation of mtDNA restriction patterns is a time dependent process (Kessler and Avise 1985), it is possible that there has been insufficient time to accumulate population specific differences in Atlantic herring (Grant 1984, 1985). Atlantic herring stocks, as they currently exist, can not predate the origin of the Gulf of Maine following glacial withdrawal 18,000 years ago (Kellogg 1980). In addition, since the effective population sizes of Atlantic herring stocks are very large, they would be expected to diverge only very slowly by lineage sorting (Neigel and Avise 1986).

Consistent, significant genetic differences among spawning groups of Atlantic herring is a sufficient, but not a necessary, condition to regard populations as discrete stocks. Our results do not support the hypothesis that discrete Atlantic herring stocks exist throughout the Gulf of Maine; however, the absence of such differences does not allow us to rigorously conclude that there is gene flow among the populations in question. More comprehensive sampling of mtDNA composites within and among populations in the western North Atlantic may better allow resolution of this problem. Regardless, for the sake of preserving variability, resources like the Atlantic herring should be managed under the assumption that every spawning group is a semi-discrete genetic entity.

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