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Temporal Stability of Nuclear Gene (Allozyme) and Mitochondrial DNA Genotypes among Red Drums from the Gulf of Mexico

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Abstract.—Allelic variation in nine polymorphic nuclear genes and restriction-site variation in mitochondrial DNA (mtDNA) were assayed among 194 adult red drums *Sciaenops ocellatus* from the northern Gulf of Mexico. Data were combined with those of previous studies in order to examine patterns of temporal genetic variation among four year-classes (1984–1987) and individuals spawned prior to 1984. Tests of heterogeneity among year-classes in both nuclear gene allele frequencies and mtDNA haplotype frequencies were nonsignificant, and estimated fixation (F_{ST}) values were 0.009 (nuclear genes) and 0.002 (mtDNA). Estimates of Nei's unbiased genetic distance (nuclear genes) and nucleotide sequence divergence (mtDNA) among year-classes also indicated the absence of temporal genetic differentiation. Estimates of average heterozygosity (nuclear genes) and nucleon and nucleotide sequence diversities (mtDNA) indicated that levels of genome-wide variation within and among year-classes of red drum are equivalent to (or higher than) those in most marine fish species examined to date. Estimates of effective female population size suggest that the total size of the female red drum population in the northern Gulf of Mexico could be 10 million individuals.

The red drum *Sciaenops ocellatus* is an economically important, estuarine-dependent fish distributed throughout the northern Gulf of Mexico (Gulf) and along the Atlantic coast of the southeastern United States (Lux and Mahoney 1969; Matlock 1984; Reagan 1985). Historically, red drum supported both commercial and recreational fisheries in the northern Gulf (Swingle 1987). Declines in red drum abundance and recruitment, however, necessitated management measures to reduce overfishing (Swingle et al. 1984; Goodyear 1989). In part as a response to restrictions on red drum fishing, considerable effort has been expended to domesticate the species for aquaculture (Chamberlain et al. 1987). Questions of importance to present and future management of red drum include (1) Do reproducing subpopulations (stocks) occur within the Gulf? and (2) Have apparent declines in red drum abundance and recruitment (Matlock 1984; Reagan 1985; Goodyear 1989) affected the genetic diversity of the species?

In several recent studies, nuclear gene (allozyme) and mitochondrial DNA (mtDNA) variations have been used to test for the existence of spatial genetic heterogeneity among red drums from the northern Gulf (Bohlmeyer and Gold 1991; Gold and Richardson 1991; Gold et al. 1993, in press). More than 700 juvenile red drums, spawned in 1986 and 1987 and sampled from 11 geographic localities from the Lower Laguna Madre, Texas, to Sarasota Bay, Florida, have been surveyed for allozyme and mtDNA phenotypes. Little evidence for spatial genetic heterogeneity has been found, suggesting that red drums in the northern Gulf make up a single, randomly mating population. In addition, average heterozygosities in nuclear genes and mtDNA nucleon and nucleotide sequence diversities in Gulf red drums were equivalent to, or higher than, those in most other marine fishes examined to date. These data indicated that genetic variation among Gulf red drums has not been affected by apparent declines in abundance.

The purpose of this study was to examine temporal variation in nuclear gene and mtDNA haplotype frequencies among red drums in the northern Gulf. Significant temporal genetic differ-

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TABLE 1.—Locations and numbers of red drums examined from the northern Gulf of Mexico.

Locality	Year spawned					
	1983[a]	1984	1985	1986[b]	1987[b]	Total
Florida						
Sarasota Bay		2	20	87	24	133
Riviera Bay				24	45	79
Apalachicola Bay	4			30	38	72
Mississippi						
Biloxi Bay				83	34	117
Black Bay				20		20
Louisiana						
Grand Isle	39	1		74	47	161
Texas						
Sabine Pass	4	1	7	25	18	55
West Bay	37	1		32	36	106
Pass Cavallo	23	3	5	13	18	62
Redfish Bay	21	2	3	35	21	82
Lower Laguna Madre		10	11	18	21	60
Total	128	20	46	441	302	947

[a] Individuals spawned in 1983 or earlier.
[b] These fish were also used by Gold et al. (1993, in press).

entiation might indicate the existence of stochastic effects due to small effective population sizes, or perhaps the occurrence of previously cryptic genetic subpopulations or stocks. In addition, we wished to determine whether juvenile red drums examined from the 1986 and 1987 year-classes reflected the genetic composition of reproductively active adults. Genetic differentiation between adults and juveniles could indicate the existence of either selection for specific nuclear gene alleles or mtDNA haplotypes, a decline in genotypic diversity due to reduced effective population size, or both. The existence of genetic differentiation would be of importance to both fishery management and aquacultural programs.

Methods

Appropriate tissues (liver and white muscle for allozyme electrophoresis and heart, spleen, and white muscle for mtDNA electrophoresis) were removed from 194 adult red drums representing the 1953–1985 year-classes and from 743 juveniles representing the 1986–1987 year-classes. All individuals were sampled from the northern Gulf (Table 1). Tissues were placed in liquid nitrogen for transport to Texas A&M University. Tissues were stored at −80°C. Fish were caught with trammel nets, haul seines, and hook and line. Ages of all individuals were determined from annuli on otoliths with methods described by Bumguardner (1991). The first annulus was considered to have formed 14–15 months after hatching (Bumguardner 1991). Adults were defined as individuals older than 3 years. Individuals spawned prior to 1984 were pooled, in part because of the low number of fish sampled from individual year-classes, and in part because collectively they are a sample of the adult population from which the 1986 and 1987 year-classes arose. Except for 1955, one to nine fish represent each year-class from 1953 to 1983. Age and length data for the fish used in this study are available upon request from the third author.

All individuals were assayed for allelic variation at nine polymorphic nuclear gene loci and for mtDNA restriction site variation. The nuclear gene loci were *sAAT** (which codes for aspartate aminotransferase, enzyme number 2.6.1.1 [IUBNC 1984], 4 alleles); *ACP-2** (acid phosphatase, 3.1.3.2, 3 alleles); *ADA** (adenosine deaminase, 3.5.4.4, 13 alleles); *ADH** (alcohol dehydrogenase, 1.1.1.1, 4 alleles); *EST-1** (esterase, 3.1.1-, 2 alleles); *GPI-B** (glucose-6-phosphate isomerase, 5.3.1.9, 3 alleles); *PEPB** (tripeptide aminopeptidase, 3.4.-.-, 3 alleles); *PEPD** (proline dipeptidase, 3.4.13.9, 4 alleles); and *PEPS** (peptidase-S, 3.4.-.-, 3 alleles). Techniques for vertical starch gel electrophoresis followed those of Siciliano and Shaw (1976) and Morizot and Siciliano (1984). Details of grinding and running buffers, starch composition of gels, and protein staining were described by Bohlmeyer (1989) and Bohlmeyer and Gold (1991). Banding patterns of proteins coded by each locus were interpreted according to sub-

unit composition of these proteins as described in the literature (e.g., Buth 1984). Designation of allelic variants was based on electrophoretic mobility relative to the most common allele (allele **100*).

Details of the assay of mtDNAs of individual fish were given by Gold and Richardson (1991). Thirteen type II restriction endonucleases were used to digest mtDNA molecules: *Bam*H I, *Bcl* I, *Eco*R V, *Hin*d III, *Nco* I, *Nsi* I, *Pst* I, *Pvu* II, *Sca* I, *Spe* I, *Stu* I, *Xba* I, and *Xmn* I. The total number of mtDNA restriction sites surveyed was 104 (Gold et al., 1993). Homology of fragments generated from single digestions of mtDNA molecules was tested by multiple, side-by-side comparisons. Variant patterns exhibiting only a single band of greater than 15 kilobases (kb) were tested for homology by using double digestions with *Bam*H I, as described by Gold and Richardson (1991).

Allozyme data.—Tests of Hardy–Weinberg equilibrium expectations, Wright's F_{ST} statistic (the standardized variance of allele frequencies among samples), and Nei's (1978) genetic identity and unbiased distance indices were carried out with the computer program BIOSYS-1 (Swofford and Selander 1981). Deviations from Hardy–Weinberg expectations were tested with pooled genotypes and chi-square statistic with 1 df. Significance testing of allele-frequency heterogeneity was accomplished with the *G*-statistic (Sokal and Rohlf 1969) applied to contingency tables of allele counts via the BIOM-PC program (Rohlf 1983), and with the *V*-statistic (DeSalle et al. 1987) applied to arcsin, square-root-transformed allele frequencies. Significance levels for all multiple tests performed simultaneously were adjusted according to Cooper (1968). Nei's unbiased genetic distances were clustered by the unweighted pair-group method with arithmetic averages (UPGMA). The computer program used for UPGMA clustering also computes standard errors for phenogram nodes following equations in Nei et al. (1985).

mtDNA data.—Nucleon diversity values were estimated according to Nei and Tajima (1981). Significance testing of haplotype frequencies was carried out with the *G*- and *V*-statistics as described above for allozyme data and a randomization (bootstrap) procedure developed by Roff and Bentzen (1989). Bootstrapping employed the MONTE program in the Restriction Enzyme Analysis Package (REAP) developed by McElroy et al. (1992). Significance levels for multiple tests performed simultaneously were adjusted according to Cooper (1968). We calculated F_{ST} values after Weir and Cockerham (1984) using computer programs described by Weir (1990). Estimates of (intrapopulational) nucleotide sequence diversity within year-classes and (interpopulational) nucleotide sequence divergence among year-classes were generated with equations of Nei and Tajima (1981). Intrapopulational values represent the average nucleotide sequence difference between any two individuals drawn at random from one sample or population. Interpopulational values represent the average nucleotide sequence difference between any two individuals drawn at random from two samples or populations. Interpopulational nucleotide sequence divergence values were clustered by using UPGMA and the program that computes standard errors for individual branches in the phenogram (see above).

Results

Spatial Subdivision among Adults

The low numbers of adults sampled at some localities (Table 1) permitted tests of spatial homogeneity only among (pooled) individuals spawned in 1983 or earlier at four localities: Grand Isle, Louisiana; West Bay, Texas; Pass Cavallo, Texas; and Redfish Bay, Texas. All homogeneity tests for both nuclear-gene allele and mtDNA haplotype frequencies were nonsignificant ($P > 0.05$). Results of these tests for spatial homogeneity are not presented, but may be obtained from the first author.

Temporal Subdivision

Allele frequencies at the nine polymorphic nuclear gene loci are summarized in Table 2 by year-class and include data from Gold et al. (in press). When corrected for multiple tests, no significant deviations from Hardy–Weinberg equilibrium expectations were found in any year-class. Mean heterozygosity-per-locus values, based on direct-count estimates and averaged over all nine loci, ranged from 0.219 ± 0.073 (SE) in individuals spawned in 1983 or earlier to 0.267 ± 0.077 in individuals from the 1984 year-class (Table 3). Mean heterozygosity values among year-classes did not differ significantly at the 5% level (i.e., by ≥ 2 SE). This indicates that levels of genetic variability, as measured by nuclear genes, are equivalent among year-classes. If the 33 loci found to be monomorphic by Bohlmeyer and Gold (1991) are monomorphic in all individuals surveyed, overall average heterozygosity ($\bar{H}$) values are 0.047, 0.057, 0.051, 0.049, and 0.047 for individuals spawned in 1983

TABLE 2.—Allele frequencies at nine polymorphic loci for year-classes of red drum from the northern Gulf of Mexico. Data for the 1986 and 1987 year-classes are from Gold et al. (in press).

Locus, allele, and sample size	Year-class				
	1983[a]	1984	1985	1986	1987
*sAAT**					
**120*	0.000	0.000	0.000	0.001	0.004
**110*	0.074	0.150	0.109	0.111	0.104
**100*	0.918	0.850	0.880	0.881	0.886
**90*	0.008	0.000	0.011	0.007	0.006
N	(128)	(20)	(46)	(449)	(269)
*ACP-2**					
**125*	0.000	0.000	0.000	0.000	0.003
**115*	0.059	0.075	0.109	0.100	0.089
**100*	0.941	0.925	0.891	0.900	0.908
N	(128)	(20)	(46)	(456)	(297)
*ADA**					
**150*	0.000	0.000	0.000	0.001	0.002
**130*	0.023	0.025	0.065	0.040	0.029
**125*	0.375	0.200	0.402	0.353	0.341
**118*	0.000	0.025	0.000	0.001	0.005
**115*	0.051	0.075	0.120	0.083	0.116
**113*	0.004	0.000	0.000	0.001	0.003
**110*	0.023	0.075	0.022	0.070	0.020
**100*	0.461	0.550	0.336	0.383	0.432
**90*	0.023	0.050	0.000	0.019	0.013
**85*	0.016	0.000	0.022	0.017	0.017
**78*	0.004	0.000	0.000	0.001	0.002
**75*	0.016	0.000	0.033	0.023	0.017
**65*	0.004	0.000	0.000	0.008	0.003
N	(128)	(20)	(46)	(464)	(298)
*ADH**					
−100	0.520	0.600	0.446	0.500	0.543
−75	0.425	0.350	0.489	0.459	0.430
−50	0.047	0.025	0.065	0.039	0.020
−20	0.008	0.025	0.000	0.002	0.007
N	(128)	(20)	(46)	(458)	(298)
*EST-1**					
**100*	0.930	0.950	0.913	0.933	0.921
**95*	0.070	0.050	0.087	0.067	0.079
N	(128)	(20)	(46)	(464)	(297)
*GPI-B**					
−110	0.000	0.000	0.000	0.002	0.002
−100	0.957	0.975	0.924	0.962	0.963
−50	0.043	0.025	0.076	0.036	0.035
N	(128)	(20)	(46)	(459)	(298)
*PEPB**					
**115*	0.004	0.025	0.000	0.012	0.012
**100*	0.973	0.950	1.00	0.985	0.983
**85*	0.023	0.025	0.000	0.003	0.005
N	(128)	(20)	(46)	(459)	(298)
*PEPD**					
**115*	0.004	0.050	0.000	0.011	0.012
**100*	0.930	0.900	0.946	0.952	0.946
**85*	0.066	0.050	0.054	0.035	0.042
**75*	0.000	0.000	0.000	0.002	0.000
N	(128)	(20)	(46)	(457)	(298)
*PEPS**					
**105*	0.023	0.100	0.022	0.038	0.037
**100*	0.969	0.900	0.978	0.960	0.961
**95*	0.008	0.000	0.000	0.002	0.002
N	(128)	(20)	(46)	(457)	(298)

[a] Individuals spawned in 1983 or earlier.

TABLE 3.—Mean heterozygosity per locus (direct-count estimate from nine polymorphic loci) for year-classes of red drum from the northern Gulf of Mexico. Data for the 1986 and 1987 year-classes are from Gold et al. (in press).

Year-class	Heterozygosity ± SE
1983[a]	0.219 ± 0.073
1984	0.267 ± 0.077
1985	0.237 ± 0.073
1986	0.227 ± 0.078
1987	0.221 ± 0.075

[a] Individuals spawned in 1983 or earlier.

or earlier, 1984, 1985, 1986, and 1987, respectively.

No evidence for temporal genetic subdivision among year-classes was found by either *G*- or *V*-tests; that is, no significant heterogeneity (5% level) in allele frequencies among year-classes was detected after correction for multiple tests at any of the nine loci. The effective absence of genetic subdivision among year-classes was also reflected in the estimated F_{ST} value of 0.009.

The matrix of Nei's unbiased genetic distances among year-classes was clustered with the UPGMA algorithm. The standard error of the most distant node in the phenogram was greater than the distance between the first and last nodes, effectively collapsing all nodes (Figure 1). These estimates of Nei's unbiased distance were based only on nine (polymorphic) loci, and inclusion of the 33 monomorphic loci surveyed by Bohlmeyer and Gold (1991) would have substantially decreased unbiased genetic distances.

Mitochondrial DNA fragment patterns from single digestions with the 13 restriction enzymes generated 48 composite mtDNA genotypes (haplotypes) among the adults assayed. No evidence for mtDNA size variation was observed. One individual (1973 year-class, sampled near Grand Isle, Louisiana) was heteroplasmic for a single mtDNA restriction site involving the enzyme *Xmn* I. Methods used to verify heteroplasmy were the same as those employed by Gold and Richardson (1990). Thus far, 114 mtDNA haplotypes have been found among red drums from the northern Gulf (Appendix Table A.1). The distribution of mtDNA haplotypes among adults and juveniles from the 1986 and 1987 year-classes by locality is available upon request from the first author. Not included in Table A.1 are 15 haplotypes found only among red drums from the Atlantic coast of the southeastern USA and three haplotypes identified by restriction enzymes not used in this study

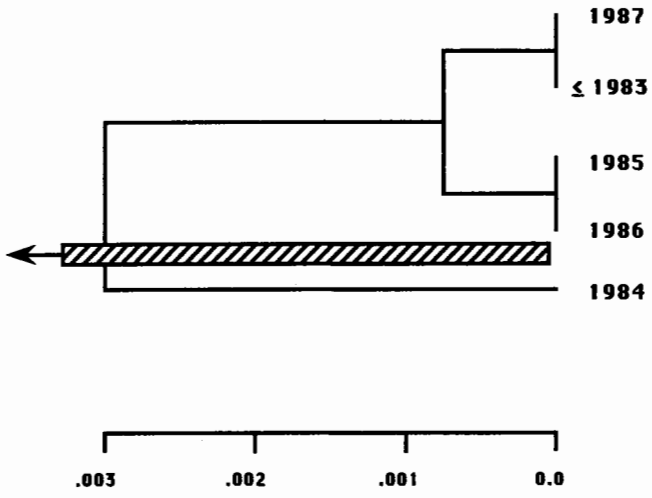

FIGURE 1.—Cluster analysis (UPGMA) of Nei's unbiased genetic distances for red drum. Operational units are year-classes from the Gulf of Mexico. Hatched bar is the standard error of the last node in the phenogram.

(Gold and Richardson 1991; Gold et al. 1993). Eleven of the haplotypes (numbers 122–132, Table A.1) have been found only among adult red drums. Five haplotypes (12, 20, 52, 90, and 91) found among adult red drums had been previously documented only from the Atlantic coast.

Mitochondrial DNA nucleon diversities ranged from 0.945 to 0.965 and were essentially identical across year-classes; mtDNA nucleotide sequence diversities across year-classes ranged from 0.523 to 0.596 and were within 1 SD of one another (Table 4). No evidence for temporal genetic subdivision among year-classes was found by *G*- or *V*-tests of mtDNA haplotype frequencies or in bootstrap analysis. The estimated F_{ST} value among year-classes was 0.002, also indicating the absence of genetic subdivision. Uncorrected interpopulational nucleotide sequence divergence values (in percent) among year-classes ranged from 0.540 (1985 versus 1986) to 0.578 (1984 versus 1987) and were essentially identical to nucleotide sequence diversities within year-classes (Table 4). In UPGMA clustering of the matrix of interpopulational values, the standard error of the most distant node was greater than the distance between the first and last nodes, effectively collapsing all nodes (Figure 2).

Discussion

Previous genetic studies of variation in nuclear genes and mtDNAs have indicated that spatial genetic heterogeneity among red drums in the northern Gulf of Mexico is absent (Ramsey and Wakeman 1987; Bohlmeyer and Gold 1991; Gold and Richardson 1991; Gold et al. 1993, in press). Results of this study indicate the absence of significant temporal genetic heterogeneity as well. Both allele frequencies at polymorphic nuclear gene loci and mtDNA haplotype frequencies appear to be stable among the 1984–1987 year-classes and a pooled sample of adults hatched between 1953 and 1983. These data also indicate that juveniles from the 1986 and 1987 year-classes do not differ

TABLE 4.—Mitochondrial DNA nucleon and (intrapopulational) nucleotide sequence diversities among year-classes of red drum from the northern Gulf of Mexico. Data for the 1986 and 1987 year-classes are from Gold et al. (1993).

Year-class	Number of individuals	Number of haplotypes	Nucleon diversity	Nucleotide sequence diversity (±SD)[a]
1983[b]	126	39	0.945	0.570 ± 0.311
1984	19	13	0.965	0.596 ± 0.313
1985	46	20	0.947	0.523 ± 0.291
1986	392	74	0.952	0.576 ± 0.304
1987	302	66	0.949	0.574 ± 0.310
Overall	885	114	0.949	0.572 ± 0.308

[a] Values are in percent. Standard deviations are used instead of standard errors because of the large number of pairwise comparisons used to generate mean values.
[b] Individuals spawned in 1983 or earlier.

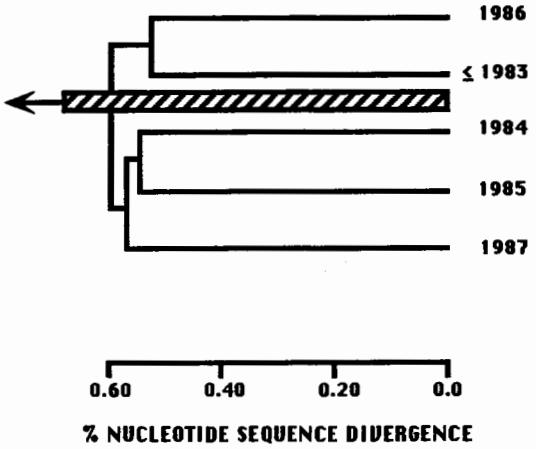

FIGURE 2.—Cluster analysis (UPGMA) of (uncorrected) nucleotide sequence divergence values (in percent) for red drum. Operational units are year-classes from the Gulf of Mexico. Hatched bar is the standard error of the last node in the phenogram.

genetically from samples of adults from which the juveniles were drawn, and thus reinforce earlier conclusions that red drums in the northern Gulf form a single, randomly mating population.

Average heterozygosity of nuclear genes and mtDNA nucleon and nucleotide sequence diversities indicate that both spatial and temporal genetic variation shown by red drum are equivalent to, or perhaps greater than, those observed for many other marine fishes surveyed to date (Smith and Fujio 1982; Waples 1987; Gold and Richardson 1991; Avise 1992; Richardson and Gold 1993). These data indicate that perceived declines in red drum abundance and recruitment (Matlock 1984; Reagan 1985; Goodyear 1989) have not affected either the genetic variability or the long-term adaptive potential of red drum in the northern Gulf of Mexico. The latter conclusion is based on the concept that levels of genetic variability affect probabilities of survival and fitness within populations (Soulé 1980; Frankel and Soulé 1981) and that allelic variations in nuclear genes and mtDNA are appropriate measures of genome-wide variation.

Avise et al. (1988) presented theoretical models whereby estimates of evolutionary effective female population size ($N_{f(e)}$ values) of a species (or a distinct subpopulation or stock of a species) can be generated from estimates of mtDNA intrapopulational nucleotide sequence diversity. With these models, $N_{f(e)}$ values for Gulf red drum are estimated to be slightly less than 100,000. As discussed by Avise et al. (1988) and Avise (1992), $N_{f(e)}$ estimates based on mtDNA data appear to significantly underestimate the present-day female population or census sizes. Nonetheless, $N_{f(e)}$ values and estimates of census size appear to be highly correlated, and $N_{f(e)}$ values generally tend to be from one to three orders of magnitude less than reasonable estimates of census size (Avise 1992). If similar considerations pertain to red drum (i.e., that estimated $N_{f(e)}$ values are, on average, roughly two orders of magnitude less than census size), the estimated $N_{f(e)}$ values of slightly less than 100,000 red drum females suggest that the total size of the female red drum population in the northern Gulf is about 10 million individuals. Given the similarity of mtDNA (intrapopulational) nucleotide sequence diversities within year-classes and given that $N_{f(e)}$ values reflect adult, reproductively active females, the implications are that the red drum population in the northern Gulf is both large and genetically stable.

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Appendix: Mitochondrial DNA Haplotypes of Red Drum

TABLE A.1.—Distribution of mtDNA composite digestion patterns for year-classes of red drum from the northern Gulf of Mexico. Data for the 1986 and 1987 year-classes are from Gold et al. (1993).

Haplotype number	Composite mtDNA digestion pattern[a]	Year-class				
		1983[b]	1984	1985	1986	1987
1	ABAAAAAAAAAAA	16	2	5	26	31
2	ABCCAAAAAAAAA	4	1	2	12	9
3	ABBACAAAAAAAA	2	1		10	12
4	EAAAAABAAAAAA				4	1
5	BAAAACBAAAAAA				1	
6	CBAAAAAAAAAAA				6	4
7	AAABAAAAAAAAA	1			4	4
8	AAAAAABAAAAAA	18	2	5	53	40
9	BAAAAAAAAAAAA	9	2	3	30	21
10	BBAAAAAAAAAAA	4		5	19	11
11	AAAAAAAAAAAAA	8	2	5	32	24
12	CBAAAABAAAAAA	1				
13	ABCAAACAAAAAA				1	2
16	ACAAAAAAAAAAA	2	1	1	12	7
18	ABAACAAAAAAAA	2			11	4
20	ABBAAAAAAACAA	1				
21	BABAAAAAAAAAA	5		2	12	13
22	BAAAAABAAAAAA	5	2		14	7
23	AAAABAAAAAAAA	3		5	15	13
24	AAAAAAAAAAAAC	1		2	10	4
25	ADCCAAAAAAAAA	1			4	3
26	BABABAAAAAAAA	1	1	2	2	4
29	AAAAABABAAAAA	6	2		20	17
30	EAAAAAAAAAAAA				1	
31	DBCAAAAAAAAAA	8			7	8

TABLE A.1.—Continued.

Haplotype number	Composite mtDNA digestion pattern[a]	Year-class				
		1983[b]	1984	1985	1986	1987
32	BBBACAAAAAAAA				1	1
33	AAAAABABBAAAA				1	
34	AABAAAAAAAAAA				4	
35	ABBAAAAAAAAAA	1		1	2	2
36	ABADAAAAAAAAA	1			1	1
37	EAAAAABAAADAA				2	2
38	AAAAAAAAAAACA				1	
39	ABEAAEAAAAAAA				1	
40	AAAAAAAAAAABA				1	
41	AAAABAAADAAAA	3			6	4
42	BAAACAAAAAAAA				1	
43	AEAAABABAAAAA				4	1
44	BADAAAAAAAAAA				1	
45	BABAAABAAAAAA				1	1
46	ABEAAAAAAAAAA			1	4	1
47	BBAAAFAAEAAAA	1	1		2	3
48	AAEAAAAAAAAAA	7			2	4
49	CBBAAAAAAAAAA				3	
51	ABCAAAAAAAAAA				2	1
52	BBAAAAACAABAB	1				
53	ABBAAAAAAAFAA				3	
58	BBAAAFAAAAAAA				3	1
62	BBBAAAAAAAAAA				1	2
64	AAAEAABAAAAAA				4	2
65	AAAAAABBAAAAA				1	
66	BBADAAAAAAAAA	1		1	3	1
67	AADAAAAAAAAAA	1			2	
68	BBAEAAAAAAAAA				1	
69	AFAAAABAAAAAA				2	2
70	ACAAAAAACAAAA				1	
71	AAAFAAAAAAAAA				1	
72	AAAAAADAAAAAA				1	1
73	BBAAAAAAAAADA				1	
74	ABCAEAAAAAAAA			1	1	1
75	DBBACAAAAAAAA				1	
76	BAAAAAAAABAAA				2	
77	ABAAAGFAAAAAA				1	
78	BBEAAAAAAAAAA					1
79	GBADAAAAAAAAA	2				1
80	DBBACAAAAAADA					1
81	BAAAAAFAAAAAA					1
82	ABAAAAFAAAAAA	1			2	2
83	IAAAAAAAAAAAA				1	
84	BAAAAAAAAAAAC				1	2
85	ABAAAABAAAAAA				1	
86	AAAAABAAAAAAA	1				1
87	BBAAAFBAAAAAA					1
88	AAIABAAAAAAAA				2	
90	BAAAAAGAEAAAA	1				
91	AAAAAAABAAAAA	1				
93	AAAFGAAAEAAAA				1	
94	AAAAAABAAAADA			1		1
95	BAAAAHAAAAAAC					2
96	BCAAAAAAAAAAA					1
97	HBAAAAAAAAAAA					1

TABLE A.1.—Continued.

Haplotype number	Composite mtDNA digestion pattern[a]	Year-class				
		1983[b]	1984	1985	1986	1987
98	BAAABAAAAAAAA	1				2
99	BBBAAAAAAAFAA					1
100	AAIAAABAAAAAA					1
101	ABCCAAFAAAAAA					1
102	BABAAAEAAAAAA					1
103	ABBAAAHAAAFAA					1
104	ABCAAAFAAAAAA				1	
105	AEAAABAAAAAAA				1	
106	AAAAIAAAAAAAC					1
107	BAAAABABAAAAA					2
108	ABAAAAAAAAAAF				1	
109	ABCCAAAAFAAAA				1	
110	ABCCAAAAAABAA					1
111	ABAAAAAACAAAA					1
112	BBBAAAAAAAFCA					1
113	AGAAAAAAFAAAA				1	
114	ACBAAAAAAAAAA					1
115	BAAAAAAAAADAA				1	
116	AAAAAABAAAFAA				1	
117	GBAAAAAAAAAAA				1	1
118	AAKBAAAAAAAAA					1
120	ABBAAAACAABAB					1
121	ABADAAAADAAAA				1	
122	BABAAABDAAAAA		1			
123	AAAAHBABAAAAA			1		
124	BBAAAAHAAAAAA			1		
125	BBAAAAAAEAAAA			1		
126	AABAACBAAAAAA		1			
127	BAAAAABAAABAA	1				
128	AAEAAHAAAAAAA	1				
129	BBAAAABAAAAAA	1				
130	BBAAAAAAAAAAD	1				
131	AAAAAABAAAAAE			1		
132	LBAAAAAAAAAAA	1				

[a] Letters (from left to right) are digestion patterns for *Nco* I, *Bcl* I, *Sca* I, *Pvu* II, *Spe* I, *Xba* I, *Xmn* I, *Hin*d III, *Stu* I, *Bam*H I, *Eco*R V, *Pst* I, and *Nsi* I. Fragment sizes (restriction sites) of individual digestion patterns are available upon request.

[b] Individuals spawned in 1983 or earlier.