Genetic and Morphometric Variation in the Pacific Sardine, *Sardinops sagax caerulea:* Comparisons and Contrasts with Historical Data and with Variability in the Northern Anchovy, *Engraulis mordax*

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ABSTRACT: Pacific sardines from five widely separated localities are found to have little genetic variation both within and between populations. Of the 32 allozyme-coding loci examined from a total of 149 fish, the proportions that are polymorphic within a population (P) range from 7% to 27% with a mean of 12%. Average proportions of heterozygous individuals per locus (H_e) range from 0.5% to 1.6% with a mean of 1.0% over the five populations. Pacific sardine populations are virtually genetically identical at the presumptive gene loci examined. For each locus that is polymorphic in more than one population, the same rare variant allele is shared at about the same frequency, suggesting strongly that there has been gene flow throughout the present range of the species. These results contrast with substantial genetic variation detected within and between northern anchovy populations from the California central stock (average P = 40%, average H_e = 7.5%) and with the significantly higher levels of genetic variation reported for other marine clupeoids. Despite a low level of genetic variation, the Pacific sardine shows a north-south cline in size-at-age that is as steep and as large as that seen in historical, precollapse populations. In the past, such differences were interpreted as evidence of genetically distinct subpopulations. Our results imply that rapid differentiation of growth rate among geographic populations, probably together with differentiation of correlated life history traits, is largely environmentally, and not genetically, determined. It appears that biological data from historical populations can safely be used for area-specific fisheries models of the recovering sardine stocks in California.

A variety of studies have suggested that, prior to its collapse in abundance, the Pacific sardine, *Sardinops sagax caerulea*, comprised two or more distinct subpopulations (morphometry and meristics: Hubbs 1925; Clark 1936, 1947; McHugh 1950; growth: Phillips 1948; Felin 1954; Clark and Marr 1955; Radovich 1962, 1982; movements of tagged fish: Clark and Janssen 1945; Clark and Marr 1955; spatiotemporal distribution of spawning: Ahlstrom 1954, 1959; erythrocyte antigens: Sprague and Vrooman 1962; Vrooman 1964; reviews by Marr 1957; Radovich 1982). Population structure likely played a role in the collapse of the fishery, perhaps directly, by virtue of differences among subpopulations in life history and resilience to fishing pressure (Wisner 1961; Murphy 1966; Radovich 1982) and more certainly, indirectly, by contributing to overestimations of stock size in the waning years of the fishery (MacCall 1979). With the return of substantial numbers of Pacific sardines to the California Current in recent years and the lifting of the fishing moratorium (Wolf et al. 1987) has come interest in management questions such as *which* sardine has recovered and what life history characteristics and yields can be expected (MacCall 1986).

We have made studies of protein and morphological variation in the Pacific sardine to shed light on such questions. Electrophoretic separation of proteins followed by chemical staining to reveal the locations of proteins or specific enzymes allows inferences to be made concerning variation in the genes encoding these proteins. This in turn provides a description, useful for management, of the genetic structures of exploited fish populations (see Ryman and Utter 1987). Several clupeoids, including the northern anchovy, *Engraulis mordax*, which co-occurs with the Pacific sardine in the California Current, have been shown to harbor considerable stores of electrophoretically detectable genetic variability (Hedgecock and Li 1983; Table 8). In this paper, we compare protein and allozyme variation in samples of Pacific sardines collected

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from five widely separated localities with that detected by us in nine trawl samples of northern anchovy taken from the central stock (sensu Vrooman et al. 1981).

MATERIALS AND METHODS

Sardinops sagax

Collections

Samples were collected from Guaymas, Sonora, Mexico (February 1985; Fig. 1, GUAYM; N = 48), Magdalena Bay, Baja California Sur, Mexico (May 1984; MAGDA; N = 37), the Southern California Bight (February–April 1986; Huntington Beach, CA, N = 8; San Pedro, CA, N = 28; pooled into one sample, SOCAL), Monterey Bay, CA (November 1984; MONTE; N = 29) and Tomales Bay, CA (December 1984; TOMAL; N = 5). Whole fish were frozen after collection and transported to the Bodega Marine Laboratory where they were kept at −70°C until thawed for morphometric measurements and dissection of tissue samples for electrophoresis.

Morphological Characters

Measurements of the following 12 morphometric traits were made on partially thawed specimens using either vernier caliper or mounted millimeter rule: a series of lengths measured from the snout to the 1) end of the hypural bones (standard length), 2) anterior margin of the orbit, 3) posterior edge of the maxillary, 4) posterior border of the supraoccipital, 5) posterior edge of the operculum, 6) dorsal-fin origin, and 7) vent, followed by measurements of 8) interorbital width, 9) maximum head width, 10) minimum body depth, 11)

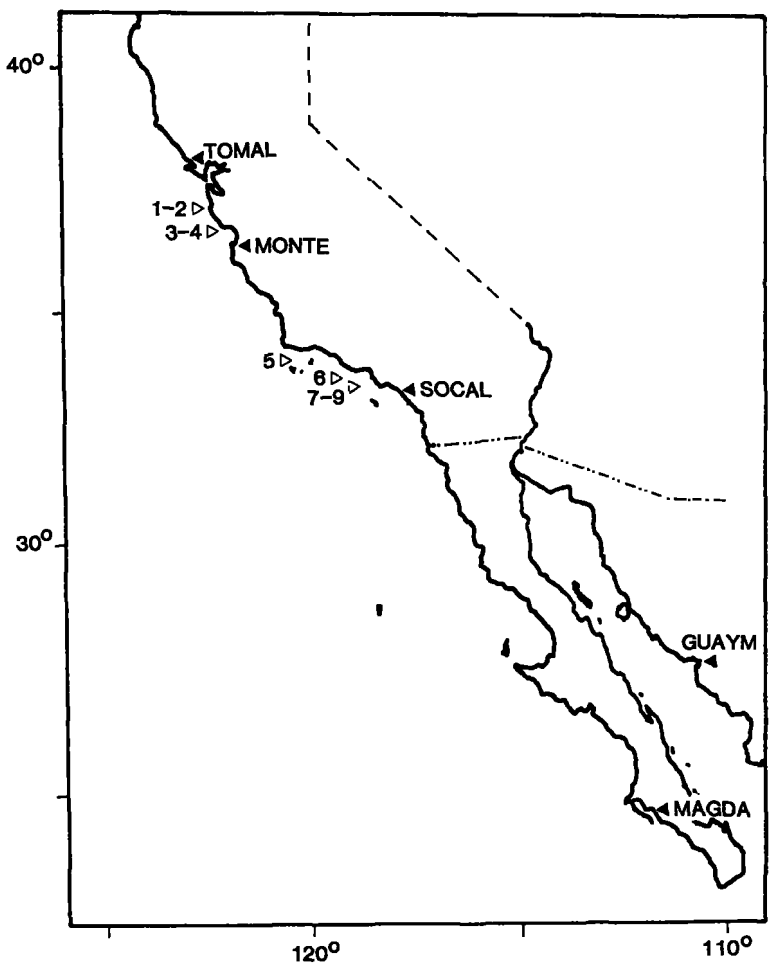

FIGURE 1.—Map showing locations of collections for Pacific sardine (solid arrowheads) and northern anchovy (open arrowheads).

anal-fin-base length, and 12) caudal-peduncle depth.

Tissue Samples

After morphological measurements were made, eye, heart, liver, and skeletal (epaxial) muscle tissues were dissected from each specimen for electrophoretic analyses. Tissue samples were kept in plastic well-trays on ice during dissection, then covered and stored frozen at −70°C for a period of several days prior to electrophoresis. The day before electrophoresis, tissue samples were thawed, equal volumes of 0.5 M Tris-HCl, pH 7.1 buffer were added to the samples, and the tissues were homogenized on ice, by hand, with a ground-glass pestle. Homogenized samples were then returned to the −70°C freezer overnight. On the day of electrophoresis, samples were allowed to thaw slowly on ice.

Otoliths

Sagittal otoliths were removed from specimens and cleared overnight in a 2% KOH solution. They were then rinsed in deionized water for one or more days, air dried, placed by pairs in gelatin capsules with the specimen number, and stored in envelopes labeled by population. Specimen identification for the Magdalena Bay sample was lost.

The age of each specimen was determined by counting otolith annuli following the methods of Collins and Spratt (1969). Each pair of otoliths was placed for examination under water in a separate well (1 cm in diameter and painted black) drilled into a strip of plexiglass. All annuli were counted under a binocular dissection microscope with incident illumination by one of us (F. L. Sly). His recounts agreed with his initial counts (98% consistency); in a comparison test, 80% of his counts were in agreement with those of California State Department of Fish and Game otolith readers.

Electrophoretic Protocol, Genetic Interpretation, and Allozyme Nomenclature

Methods for horizontal starch-gel electrophoresis, protein assays, and genetic interpretation of zymograms were substantially the same as those described previously (Ayala et al. 1973; Tracey et al. 1975; Utter et al. 1987). The protocol used to separate and resolve 20 enzymes or proteins inferred to be encoded by a total of 32 genes is summarized in Table 1. Nomenclatures for proteins, for genes inferred to encode these proteins, and for alleles at these genes are detailed by Utter et al. (1987). Proteins are referred to by the capitalized abbreviations given in Table 1 and the corresponding genes by these same abbreviations italicized in upper and lower case. Numerical suffixes distinguish among isozymes or multiple proteins in order of increasing anodal migration. Alleles are symbolized by italicized numerals obtained by adding or subtracting the number of millimeters separating variants from the most common electromorphs observed for each protein. Alleles encoding common electromorphs are arbitrarily designated *100*. Specimens from several populations were included in every electrophoretic run so that repeated comparisons of relative mobilities of their allozymes were made.

Allozyme Data Analysis

Maximum-likelihood estimates of allelic frequencies and observed proportions of heterozygous genotypes at each locus scored in at least two population samples were computed from numbers of individuals in allelic or genotypic categories and the total numbers of genomes ($2N$) or individuals (N) sampled, respectively. Observed and expected proportions of heterozygous genotypes at each locus were averaged over loci to obtain means (Nei's [1978] unbiased estimates of H_o and H_e, respectively). The proportion of genes for which any electrophoretic variation was detected in a population sample was defined as P; for the population sample sizes used, this criterion of polymorphism is close to the frequently used definition that the most common allele cannot exceed a frequency of 0.99 for a polymorphic locus. Averaging of P and H over population samples was done using angular transformation of these proportional values followed by back-transformation of means and confidence limits. Owing to the nature of the results, no further genetic statistics were calculated.

Morphometric Data Analysis

The BMDP multivariate statistical software package (Dixon 1981) was used to perform discriminant function (P7M) and principal component (P4M) analyses on log-transformed morphometric data. Standard settings were used in the discriminant analysis for tolerance (0.01), F-to-

TABLE 1.—Starch-gel electrophoretic protocols used to reveal allozyme variation.

			Pacific sardine			Northern anchovy		
	Enzyme or protein	E.C. no.	Tissue	Buffer[1]	No. loci	Tissue	Buffer[1]	No. loci
AAT	aspartate aminotransferase	2.6.1.1	—	—	—	E	A	1
ADA	adenosine deaminase	3.5.4.4	L	A	1	L	A	1
ADH	alcohol dehydrogenase	1.1.1.1	E	B	1	—	—	—
ADK	adenylate kinase	2.7.4.3	M	D	2	M	D	1
ALDO	aldolase	4.1.2.13	—	—	—	M	B	1
CK	creatine kinase	2.7.3.2	M	D	1	M	D	1
EST	esterase[2]	3.1.1.–	L	A,B	1	L	A	2
FBP	fructose biphosphatase	3.1.3.11	L	B	1	—	—	—
FUM	fumarate hydratase	4.2.1.2	M	C,D	1	M	D	1
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	M	C	2	M+E	C	2
GL	dipeptidase[3]	3.4.13.11	E	B	1	—	—	—
GPDH	glycerol-3-phosphate dehydrogenase	1.1.1.8	M	D	1	M	D	1
GPI	glucose-6-phosphate isomerase	5.3.1.9	L,M	D,B	1	L	B	1
HBDH	3-hydroxybutyrate dehydrogenase	1.1.1.30	E+L	B	2	E+L	B	2
HK	hexokinase	2.7.1.1	—	—	—	M	B	1
IDH	isocitrate dehydrogenase	1.1.1.42	H+L	D	2	H	D	1
LAP	leucine aminopeptidase	3.4.11.1	—	—	—	L	A	1
LDH	lactate dehydrogenase	1.1.1.27	L+M	B	2	L+M	B	2
LGG	tripeptidase[4]	3.4.13.4	E	B	1	E,L	B	1
LT	dipeptidase[5]	3.4.13.11	E	B	1	E+L	B	2
MDH	malate dehydrogenase	1.1.1.37	H	D	2	H	D	2
ME	NADP-dependent malate dehydrogenase[6]	1.1.1.40	M	B	1	—	—	—
6PGDH	6-phosphogluconate dehydrogenase	1.1.1.44	E,M	C	1	E,M	C	1
PGM	phosphoglucomutase	2.7.5.1	M	A	1	M	A	1
PNP	purine nucleoside phosphorylase	2.4.2.1	—	—	—	L	E	1
PP	dipeptidase[7]	3.4.13.9	L	B	1	—	—	—
PROT	general proteins[8]	general	E+M	A	4	E+M	A	9
SOD	superoxide dismutase[9]	1.15.1.1	L	B	1	L	B	1
TPI	triosephosphate isomerase	5.3.1.1	—	—	—	M	D	1
XDH	xanthine dehydrogenase	1.1.1.204	—	—	—	L	B	1
	Totals[10]		20 proteins,		32 loci	24 proteins,		39 loci

[1]Buffers A, B, C, and D as described by Tracey et al. (1975); buffer E is the lithium borate discontinuous buffer system 2 of Selander et al. (1971).
[2]α-naphthyl butyrate and β-naphthyl acetate as substrates.
[3]L-glycyl-leucine as substrate.
[4]L-leucyl-glycyl-glycine as substrate.
[5]A 1:1 mixture of L-leucyl-valine and L-leucyl-tyrosine as substrates.
[6]Known as malic enzyme.
[7]L-phenylalanyl-proline as substrate.
[8]Stained with Coomassie blue.
[9]Usually scored on gels stained for HBDH.
[10]Di- and tripeptidases counted as one enzyme in totals.

enter (4.000), F-to-remove (3.996), and prior probabilities (equal).

Engraulis Mordax

Collections

Samples of 48 anchovies were obtained from each of nine midwater trawl stations (Fig. 1) occupied between the nights of 24 January and 1 February 1982, by CalCOFI cruise 8202 aboard the RV *David Starr Jordan* of NOAA Southwest Fisheries Center, La Jolla. CalCOFI grid coordinates (CalCOFI Atlas No. 1, 1963) for these collections and place names assigned to them for convenience were as follows: 1) Half Moon Bay, 63.3:51.0; 2) Half Moon Bay, 63.3:52.0; 3) Santa Cruz, 66.7:49.0; 4) Santa Cruz, 66.7:50.0; 5) Santa Barbara, 81.3:42.4; 6) Point Dume, 85.0:44.0; 7) Santa Monica Bay, 85.8:34.0; 8) Santa Monica Bay, 85.8:36.0; 9) Santa Monica Bay, 85.8:41.0. Whole fish were frozen individually aboard ship at −70°C and then packaged in plastic bags labeled by locality. Frozen packages were shipped by air to the Bodega Marine

Laboratory where they were kept in a −70°C freezer until dissection.

Data Taken on Specimens

Eye, heart, liver, and skeletal muscle tissue samples were taken from each of the 432 northern anchovy used for this study. For all specimens but those from population sample 8, standard length and sex were recorded and otoliths were taken. Annuli were counted by one of us (F. L. Sly) in a manner similar to that described for the sardine, following the methods of Collins and Spratt (1969).

The electrophoretic protocol used to separate 24 proteins encoded by 39 scorable genes is given in Table 1. These proteins were assayed in tissue samples from an average of nearly 46 specimens (minimum of three) from each of the 9 population samples. Genetic interpretation and allozyme nomenclature were as described above for the sardine analysis with the following additions: 1) Gels for polymorphic enzymes were scored independently by the authors D. Hedgecock and Gang Li, and any discrepancy between the two scores was resolved by reexamination and negotiation; and 2) problems with the resolution of certain allozymes from liver tissue became apparent. A pattern of missing IDH-2, missing EST-5, and blurred LDH-3 and HBDH-2 phenotypes—the last mimicking the 100/105 polymorphism—was subsequently associated with degenerated liver tissues in individual specimens or even entire population samples that were, perhaps, not frozen soon enough after trawling. IDH-2 appeared most sensitive to this and was eliminated from the study, except as an indicator of degenerate liver tissue. To correct for potential bias in *Hbdh-2* data, individual HBDH-2 scores were omitted if (i) any other element of the above composite, degenerate-liver zymogram was observed in that individual *and* (ii) missing elements (i.e., IDH-2 and EST-5) were observed in at least one other specimen from the same population sample (to prevent a missing or failed enzyme assay from causing data rejection). Mean number of individuals assayed for HBDH per population sample was thus reduced to 33 ± 3.

Allozyme Data Analysis

Single-individual genotypes were recoded as paired alphabetical characters and submitted to the BIOSYS-1 program of Swofford and Selander (1981) for calculations of allelic frequencies, average proportions of heterozygous individuals per locus (H_o and H_e as defined above), proportions of polymorphic genes (P, where a locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99), chi-square goodness-of-fit tests to Hardy-Weinberg-Castle (H-W-C) equilibrium genotypic proportions using Levene's (1949) correction for small sample sizes, Wright's (1978) F-statistics, and Nei's (1978) unbiased estimates of average genetic identity (I) and genetic distance (D). Averaging of P and H over population samples was done using angular transformation followed by back-transformation of means and confidence limits. Spearman rank correlations of angular-transformed allelic frequencies with the sines of latitude of collection localities, log-likelihood ratio (G) tests of the independence of allelic frequency and locality, and analyses of allelic frequencies cross-classified by locality, sex, and age (ACCCD; Fienberg 1980) were used to evaluate sources of genetic heterogeneity.

RESULTS

Sardinops sagax caerulea

Genetic Variation

We detected electrophoretic variation in the zymograms of seven proteins, including three di- and tripeptidases, from Pacific sardines, (EST-6, FBP, GPDH, IDH-2, LGG, LT, PP, 6PGDH, SOD; Table 2B). There is, however, remarkably little protein polymorphism and individual heterozygosity at the total 32 loci examined in 149 Pacific sardines (Table 2A). The proportion of polymorphic genes ranges from 7.4% in each of the samples from Tomales and Magdalena Bays to 26.9% in the Guaymas sample, with an average of 12.3% (95% C.L.: 6.4–19.6%). Average heterozygosities range from 0.5% in the Magdalena Bay sample to 1.7% in the Guaymas sample, with a mean over all population samples of 1.0% (95% C.L.: 0.6–1.5%). Estimates of genetic variation are probably best for the Guaymas sample, for two reasons: 1) There were generally larger numbers of individuals sampled per gene, which accounts for the finding of rare heterozygotes at *Idh-2*, *Lgg*, and *Pp*, loci that were not well sampled elsewhere. 2) We sampled two moderately polymorphic loci, *Est-6* and *Fbp*, that were not scored in any other large population sample. There are no significant differences between

TABLE 2.—Allozyme variation in five population samples of Pacific sardine.

	Tomales Bay	Monterey Bay	S. Calif. Bight	Magdalena Bay	Guaymas
A. Summary statistics					
No. of fish (*N*)	5	29	30	37	48
No. of loci	27	27	23	27	26
P (as percent)	7.4	14.8	8.7	7.4	26.9
H_o (as percent)	1.5	1.0	0.6	0.5	1.7
H_e (as percent)	1.5	1.0	0.6	0.5	1.6

B. Polymorphic enzymes[1]

	Variants	No. of heterozygotes in samples of size (*N*)				
EST-6	(103)	0 (5)	—	—	—	3 (48)
FBP	(102)	1 (5)	—	—	—	7 (47)
GPDH	(102)	0 (2)	1 (29)	0 (30)	0 (34)	0 (12)
IDH-2	(93)	0 (5)	0 (22)	1 (29)	0 (23)	1 (48)
LGG	(96)	0 (5)	—	0 (30)	0 (7)	1 (48)
LT	(97)	0 (5)	—	0 (30)	0 (7)	1 (48)
PP	(103)	0 (5)	3 (28)	0 (30)	1 (37)	4 (48)
6PGDH	(103+98)	1 (5)	3 (29)	3 (30)	4 (37)	3+1 (48)
SOD	(93)	0 (5)	1 (29)	0 (30)	0 (37)	0 (48)

[1]Zymogram banding patterns of presumptive heterozygotes at the loci inferred to encode these proteins conform to those expected on the basis of known subunit structures: tetrameric structure for FBP; dimeric structure for GPDH, IDH, LGG, 6PGDH, and SOD; monomeric structure for EST-6, LT and PP (Darnall and Klotz 1975; Harris and Hopkinson 1976; Ruth and Wold 1976; Koehn and Eanes 1978; Utter et al. 1987).

observed and expected average heterozygosities in any of the population samples. For no individual locus is it possible to test H-W-C expected genotypic proportions in a population sample, owing to the low frequencies of variant alleles and the relatively small sample sizes.

In addition to the low genetic variation within each of the samples of Pacific sardines, there is almost no variation among populations in the frequencies of allozymes (Table 2B). Except for 6PGDH, for which a third allozyme was inferred from observation of a single 98/103 heterozygous phenotype in the Guaymas sample, all polymorphic allozymes are represented by just two alleles. For each locus that is polymorphic in more than one population, the same rare variant allele is shared at about the same frequency (Table 2B). Thus, the five, widely separated populations of Pacific sardine sampled in our study are virtually genetically identical at the 32 loci examined.

Ageing from Otolith Annuli

Under the assumption that an annulus represents a yearly growth ring and that fish with one ring are one year old, three age classes, 1's, 2's, and 3's, predominate in all five population samples. There are no statistically significant differences in distribution of ages among the four larger samples $\chi^2_{9df} = 10.7083$, $0.1 < P < 0.5$; data in Table 3). Nor is there a difference between the small Tomales Bay sample and the others (Fisher's exact test on Tomales Bay data versus all other data combined, $P > 0.90$).

TABLE 3.—Distributions of ages in five population samples of Pacific sardine.

Age	Tomales Bay	Monterey Bay	S. Calif. Bight	Magdalena Bay	Guaymas
1	0	9	10	19	21
2	4	14	14	13	22
3	1	3	5	1	3
4	0	0	1	0	0
Sample size:	5	26	30	33	46
Mean:	2.2	1.8	1.8	1.5	1.6

Morphological Variation

In contrast to the similarity of age class compositions among population samples, size distributions among sites are grossly different, with sardines from California being much larger than those from Mexico (Fig. 2). Stepwise discriminant function analysis (DFA) of the 12 log-transformed morphometric variates reveals, after 10 steps, significant differences among the five population samples (approximate F = 22.085 with 32, 499 df, $P << 0.001$). However, discrimination is based primarily on log of standard length which enters the discriminant function first with F = 243.49 ($P << 0.001$; 4, 142 df). A principal component analysis (PCA) of those traits contributing to between-group variance in the DFA produces a single factor, heavily and positively loaded by all traits and accounting for 97% of the variance in data space (minimum factor eigenvalue set to 1.0); such a factor is generally interpreted to represent variance in size (Humphries et al. 1981). The evident geographic cline in size apparently reflects a cline in growth rate; at the extremes, fish of the same age from central California and from the Gulf of California can differ in standard length by nearly 100 mm (see Figure 2).

Two further analyses do indicate minor but significant morphological variation attributable to shape differences among sardines from different geographic areas. First, two factors extracted in a PCA of log-transformed variates standardized by subtraction of the log of standard length show complementary patterns of factor loadings suggestive of different allometries of head size relative to body size among populations (Fig. 3A). The separation of populations along the factor 1 axis (Fig. 3B) is *inversely* related to standard length; i.e, larger California fish are on the left of smaller Mexican fish, owing to the negative allometry of head dimensions relative to standard length. This result is consistent with observations on postlarval Pacific sardines (McHugh 1950) as well as with a general geographical pattern in fish morphology (Jensen 1944; Martin 1949).

Second, comparisons of pairs of population samples for which there is considerable overlap in the sizes of specimens (Southern California

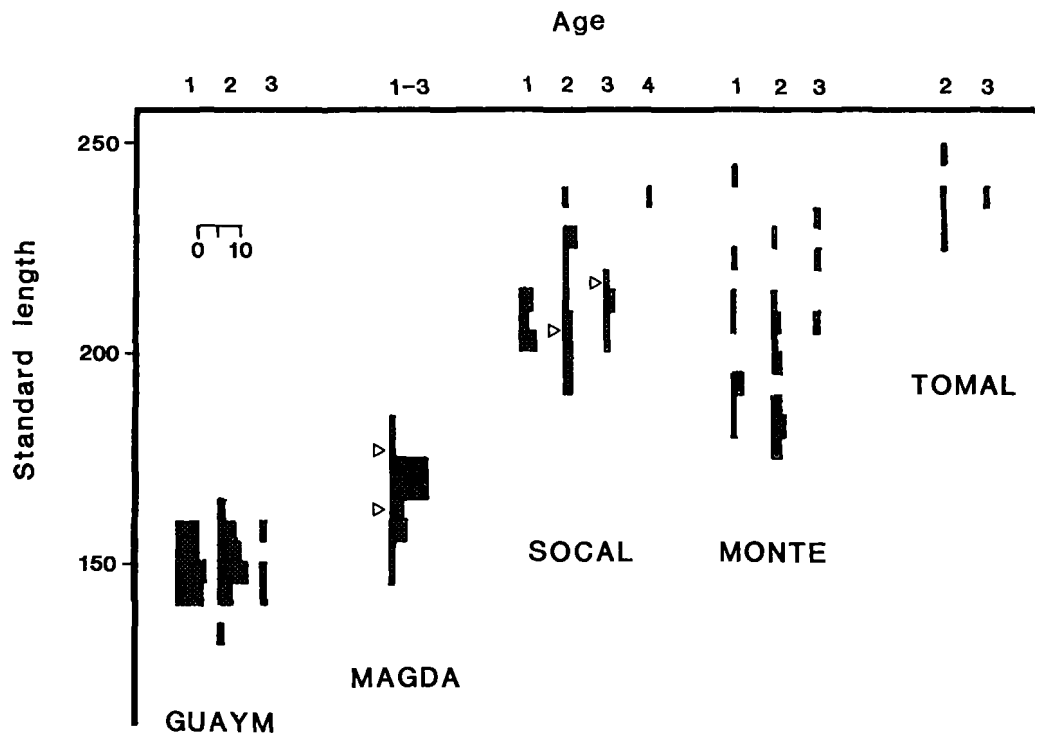

FIGURE 2.—Histograms of standard lengths for various ages of Pacific sardines in five population samples. Open arrowheads along the baselines of the Magdalena Bay and Southern California Bight population samples indicate the mean sizes of two- and three-year-old fish in the 1961–62 sardine catches of Baja California and California, respectively (from Vrooman 1964).

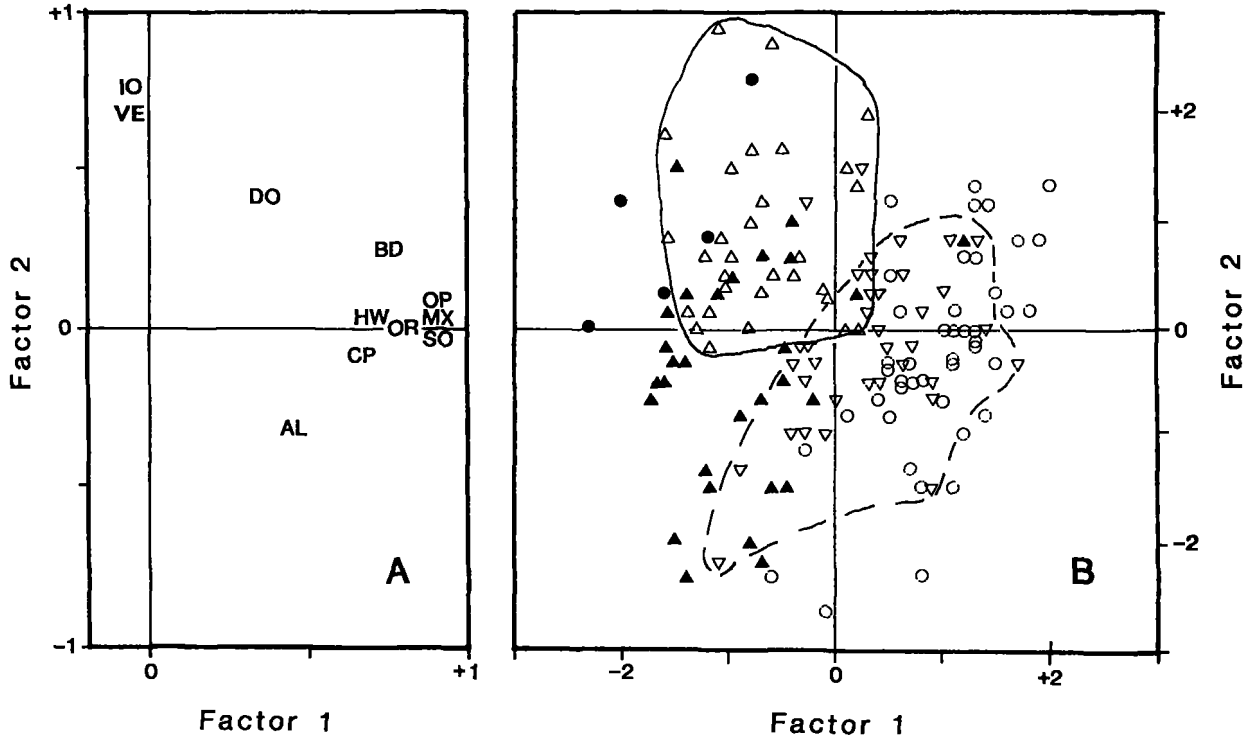

FIGURE 3.—(A) Loadings on the first two factors from a principal components analysis of size-standardized morphometric traits measured on 147 Pacific sardines; I0 = interorbital width, VE = snout to vent length, DO = snout to dorsal-fin origin, AL = anal-fin base length, CP = caudal-peduncle depth, HW = maximum head width, BD = minimum body depth, OR = snout to orbit length, OP = snout to operculum length, MX = snout to maxillary length, SO = snout to supraoccipital length. (B) Scores for individual sardines on these first two factors. Open triangles, solid line: SOCAL; open inverted triangles, dashed line: MAGDA. Other populations are solid circles, TOMAL; solid triangles, MONTE; open circles, GUAYM (see Figure 1).

Bight vs. Monterey Bay and Magdalena Bay vs. Guaymas) also show variation in head : body-size allometry. A standard length range of 192–240 mm defines a subset of the Monterey Bay sample comprising 17 individuals whose mean length is identical to that of the 30 southern California specimens. DFA of log-transformed variates takes two steps to produce significant between-group variance (F = 13.344, $P << 0.001$; 2, 44 df) and an average percent correct assignment in a posteriori classifications of 77% (Table 4A). The two characters used in the classification functions are interorbital width and head width (Table 4A). Between subsets of similarly sized fish (145–162 mm) from Guaymas (N = 37) and Magdalena Bay (N = 11), DFA of nine log-transformed variates (three were discarded to keep the number of variates less than N = 11 for Magdalena Bay) produces significant variance in three steps (F = 17.568, $P << 0.001$; 3, 44 df) and 90% correct classification (Table 4B). Maxillary length, interorbital width, and length of anal-fin base contribute to the classification functions for these two Mexican populations (Table 4B).

Engraulis mordax

Genetic Variation Within Populations

Electrophoretic variation was detected in all northern anchovy proteins examined except ALDO and CK. From this variation in protein phenotypes, we infer that our samples of northern anchovy populations contain substantial levels of individual genetic variation (Table 5). Over the 39 loci examined, the average number of alleles per locus per population is 1.61 ± 0.02, ranging from 1.49 ± 0.13 in the inshore Half Moon Bay sample to 1.72 ± 0.16 in the offshore Santa Cruz sample. Proportions of polymorphic loci per population range from 33.3% in the inshore Half Moon Bay sample to 46.2% in the middle station of the Santa Monica Bay transect; mean P over the nine samples is 39.8% (95% C.L.: 37.2–42.5%). Average expected hetero-

TABLE 4.—Classification functions and a posteriori (jackknifed) classifications from discriminant function analyses of similarly sized Pacific sardines from (A) California and (B) Mexican population samples.

A. California's classification functions:

Variate	Monterey	S. Calif. Bight
log interorbital	−668.284	−597.504
log head width	2437.560	2357.524
constant	−1311.788	−1274.079

Population sample (192–240 mm)	% correct classification	Cases classified in sample		
		Monterey	S. Calif. Bight	*N*
Monterey Bay	82.4	14	3	17
S. Calif. Bight	73.3	8	22	30

B. Mexico's classification functions:

Variate	Guaymas	Magdalena Bay
log maxillary	3061.400	2871.099
log interorbital	−449.694	−537.635
log anal-fin base	521.755	591.291
constant	−2006.101	−1949.291

Population sample (145–162 mm)	% correct classification	Cases classified in sample		
		Guaymas	Magdalena Bay	*N*
Guaymas	89.2	33	4	37
Magdalena Bay	90.9	1	10	11

zygosities range from 6.9% in the inshore Half Moon Bay and Point Dume samples to 8.0% in the middle Santa Monica Bay sample, with a mean over the nine samples of 7.5% (95% C.L.: 7.1–7.9%). There are no significant differences between observed and expected average heterozygosities in any of the samples.

Sample sizes and levels of polymorphism permit goodness-of-fit tests to H-W-C genotypic proportions in 27 cases, involving five loci—*Fum*, *Hbdh-2*, *Lgg*, *Pgm*, and *Xdh*. Prior to 14 of these tests, rare alleles were pooled and the frequencies of composite genotypic classes recalculated accordingly (Pamilo and Varvio-Aho, 1984). The probability assigned to a significant deviation from the H-W-C (null) hypothesis, $P_{\alpha 0.05}$, was adjusted for multiple testing by dividing $\alpha_{0.05}$ by the number of populations over which a given locus was simultaneously tested (Cooper 1968). None of the 27 tests is significant at the adjusted $\alpha_{0.05}$ level, although chi-square values for *Hbdh-2* in the offshore Santa Cruz sample and for *Lgg* in the Point Dume sample come close.

Deviations from H-W-C equilibrium for *Hbdh-2* and *Lgg* tend towards excess heterozygotes. Wright's (1978) fixation indices (F_{IS}) for *Hbdh-2* in population 4 and for *Lgg* in populations 4, 6, and 8 (four chi-square tests with $P \leq 0.06$) are −0.49, −0.37, −0.36, and −0.19, respectively. Averaged over populations and alleles, F_{IS} for *Hbdh-2* and *Lgg* is −0.13 and −0.175, respectively; the weighted average F_{IS} over all loci is −0.045 (see Table 7). There is, owing to these excesses of heterozygotes within population samples, an overall excess of heterozygotes in the total population sampled (mean F_{IT} over all loci and populations is −0.012), despite divergence among populations (next section), which is expected to reduce heterozygosity (Wahlund 1928).

Genetic Variation Between Populations

Eleven loci were polymorphic enough throughout the nine population samples (Table 6) to permit analyses of geographical heterogeneity in allelic frequencies. Rare alleles were pooled into one class for all but the *Lgg* and *Xdh* loci, for which two rare-allele classes were formed. The resulting two- or three-by-nine matrices were tested for r × c independence by log-likelihood

TABLE 5.—Summary statistics for three measures of genetic diversity in northern anchovy.

Population sample (CalCOFI grid no.)[1]	Mean sample size per locus (SE)	Mean no. alleles per locus (SE)	% loci polymorphic	Mean heterozygosity	
				Observed (SE)	Expected (SE)
1. Half Moon Bay (63.3:51.0)	44.1 (1.4)	1.49 (0.13)	33.3	7.6 (2.6)	6.9 (2.3)
2. Half Moon Bay (63.3:52.0)	45.7 (1.0)	1.59 (0.14)	35.9	7.9 (2.6)	7.3 (2.3)
3. Santa Cruz (66.7:49.0)	45.4 (1.1)	1.51 (0.11)	41.0	7.1 (2.3)	7.6 (2.4)
4. Santa Cruz (66.7:50.0)	46.3 (0.8)	1.72 (0.16)	41.0	8.70 (2.4)	8.0 (2.5)
5. Santa Barbara (81.3:42.4)	45.9 (1.3)	1.64 (0.17)	35.9	7.9 (2.5)	7.3 (2.3)
6. Point Dume (85.0:44.0)	46.6 (1.1)	1.59 (0.14)	41.0	6.9 (2.5)	6.9 (2.3)
7. Santa Monica Bay (85.8:34.0)	46.3 (0.8)	1.64 (0.14)	43.6	8.1 (2.6)	7.8 (2.4)
8. Santa Monica Bay (85.8:36.0)	42.6 (1.7)	1.67 (0.13)	46.2	8.7 (2.5)	8.8 (2.4)
9. Santa Monica Bay (85.8:41.0)	46.8 (0.4)	1.62 (0.14)	41.0	6.9 (2.2)	7.3 (2.3)
Averages: (SE or 95% C.L.)	45.5 (0.5)	1.61 (0.02)	39.8 (37.2-42.5)	7.8 (7.3-8.2)	7.5 (7.1-7.9)

[1]California Cooperative Oceanic Fisheries Investigations (1963).

TABLE 6.—Frequencies of allozymes for 11 polymorphic enzymes[1] in northern anchovy samples.

Locus (*N*) Alleles	Population samples								
	1	2	3	4	5	6	7	8	9
Est-5									
(*N*)	44	48	37	47	44	48	41	22	40
98	0.011	0.0	0.0	0.011	0.011	0.01	0.061	0.0	0.013
100	0.966	0.948	0.973	0.957	0.955	0.948	0.915	0.909	0.925
101	0.0	0.0	0.0	0.011	0.0	0.021	0.012	0.0	0.025
102	0.023	0.042	0.027	0.021	0.023	0.021	0.012	0.091	0.038
103	0.0	0.01	0.0	0.0	0.011	0.0	0.0	0.0	0.0
Fum									
(N)	48	48	48	48	48	48	48	47	48
96	0.021	0.0	0.01	0.01	0.0	0.0	0.01	0.0	0.0
100	0.5	0.615	0.479	0.573	0.458	0.51	0.573	0.585	0.49
104	0.479	0.385	0.51	0.417	0.542	0.49	0.417	0.415	0.51
Gpi									
(*N*)	48	48	48	48	48	48	48	47	48
90n	0.0	0.0	0.0	0.0	0.0	0.01	0.01	0.0	0.0
96	0.0	0.0	0.0	0.01	0.021	0.01	0.01	0.0	0.01
100	0.979	0.969	0.958	0.958	0.958	0.969	0.958	0.798	0.927
103	0.021	0.021	0.042	0.01	0.01	0.01	0.0	0.202	0.042
105	0.0	0.01	0.0	0.021	0.01	0.0	0.021	0.0	0.021

TABLE 6.—*Continued.*

Locus (*N*) Alleles	Population samples								
	1	2	3	4	5	6	7	8	9
Hbdh-1									
(*N*)	48	48	48	48	48	48	48	48	48
90	0.0	0.01	0.0	0.0	0.0	0.0	0.0	0.0	0.0
92	0.01	0.0	0.0	0.0	0.01	0.0	0.0	0.0	0.0
96	0.042	0.0	0.0	0.021	0.042	0.042	0.0	0.0	0.02
98	0.01	0.031	0.021	0.021	0.01	0.0	0.031	0.021	0.0
100	0.938	0.948	0.979	0.958	0.927	0.958	0.969	0.979	0.979
103	0.0	0.01	0.0	0.0	0.01	0.0	0.0	0.0	0.0
Hbdh-2									
(*N*)	28	29	32	32	41	48	31	17	40
100	0.821	0.741	0.547	0.516	0.732	0.927	0.597	0.765	0.837
105	0.179	0.259	0.453	0.484	0.268	0.073	0.403	0.235	0.162
Ldh-1									
(*N*)	48	48	48	48	48	48	48	48	48
96	0.115	0.208	0.188	0.125	0.198	0.198	0.125	0.146	0.135
100	0.885	0.792	0.813	0.875	0.802	0.802	0.875	0.854	0.865
Lt-1									
(*N*)	48	48	48	48	48	48	48	48	48
96	0.0	0.021	0.0	0.021	0.01	0.0	0.0	0.021	0.01
100	0.948	0.958	0.948	0.938	0.948	0.99	0.969	0.885	0.938
103	0.052	0.021	0.052	0.042	0.042	0.01	0.031	0.094	0.052
Lgg									
(*N*)	48	48	48	48	48	48	48	48	48
97	0.021	0.021	0.083	0.01	0.042	0.021	0.031	0.052	0.0
100	0.646	0.66	0.74	0.604	0.708	0.583	0.563	0.583	0.688
104	0.333	0.319	0.177	0.385	0.25	0.385	0.365	0.365	0.313
107	0.0	0.0	0.0	0.0	0.0	0.01	0.042	0.0	0.0
6Pgdh									
(*N*)	48	48	48	48	48	48	48	48	48
98	0.021	0.01	0.021	0.052	0.0	0.0	0.0	0.01	0.021
100	0.979	0.979	0.969	0.948	0.99	1.0	0.969	0.958	0.979
104	0.0	0.01	0.01	0.0	0.01	0.0	0.031	0.031	0.0
Pgm									
(*N*)	48	48	48	47	48	48	48	48	48
96	0.0	0.01	0.0	0.01	0.0	0.01	0.0	0.0	0.0
98	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.01	0.0
100	0.708	0.677	0.74	0.667	0.771	0.76	0.813	0.833	0.844
103	0.292	0.313	0.26	0.323	0.229	0.229	0.188	0.156	0.156
Xdh									
(*N*)	48	48	48	48	48	48	48	39	48
98	0.177	0.125	0.031	0.167	0.156	0.281	0.135	0.0	0.365
100	0.792	0.844	0.854	0.823	0.823	0.719	0.792	0.744	0.604
102	0.031	0.031	0.115	0.01	0.021	0.0	0.073	0.256	0.031

[1]Zymogram banding patterns of presumptive heterozygotes conform to those expected on the basis of known subunit structures: tetrameric structure for FUM and LDH-1; dimeric structure for GPI, HBDH-1, HBDH-2, LGG, and 6PGDH; monomeric structure for EST-5, LT-1 and PGM; XDH not separated well enough to confirm tetrameric structure (see Table 2 for references).

ratios (G, Table 7). For five of the 11 loci, allelic frequencies are significantly nonindependent of locality; significant heterogeneity of allelic frequencies corresponds to F_{ST} values ≥ 0.019. Considering just the 11 polymorphic loci, Nei's (1978) unbiased genetic identity and distance statistics for 36 pairs of population samples average 0.992 ± 0.001 and 0.008 ± 0.001, respectively; over all 39 loci these statistics average 0.998 ± 0.0003 and 0.002 ± 0.0003, respectively.

Further analyses show that some of the heterogeneity in allelic frequencies is geographically patterned, some is associated with genetic differences between the sexes and among age classes, but that most is not associated with any obvious environmental or biological factor. Allelic frequencies at two of the five variable loci, *Pgm* and *Xdh*, are significantly correlated with latitude (Fig. 4). An attempt was made to examine the dependence of gene frequencies on sex and age as well as locality, but analyses of cross-classified data are made difficult by small sample sizes and uneven distributions of sexes and age classes over localities. In tests of three-factor (LOCALITY × SEX × ALLELIC FREQUENCY) log-linear models for *Hbdh-2*, *Lgg*, *Pgm* and *Xdh*, sex was found to be associated with ALLELIC FREQUENCY only for *Hbdh-2*, for which a fully saturated model with all three pairwise interactions among factors appeared to be the best fit. In 15 tests of log-linear models of AGE × SEX × ALLELIC FREQUENCY within individual population samples, only four could *not* be fit by the model of complete factor independence (*Hbdh-2* in population 9, *Lgg* in population 4, *Pgm* in populations 3 and 4). AGE and ALLELIC FREQUENCY were associated only for *Pgm* in the inshore Santa Cruz sample, for which, again, a fully saturated model was the best fit. Interestingly, interactions of AGE and SEX independent of (or only conditionally associated with) ALLELIC FREQUENCY were significant in all four cases; an association of older males with younger females appeared to be responsible for this.

TABLE 7.—F-statistics and log-likelihood ratio (G) tests of allelic frequency x locality independence for polymorphic loci in nine population samples of northern anchovy.

Locus	F_{IS}	F_{IT}	F_{ST}	G	df	Sig.[1]
Est-5	0.083	0.095	0.012	5.53	8	ns
Fum	0.006	0.019	0.013	11.21	8	ns
Gpi	0.069	0.123	0.057	31.14	8	***
Hbdh-1	−0.040	−0.027	0.012	8.05	8	ns
Hbdh-2	−0.130	−0.037	0.082	57.66	8	***
Ldh-A	0.043	0.051	0.008	7.28	8	ns
Lt-1	0.033	0.046	0.013	13.06	8	ns
Lgg	−0.175	−0.153	0.019	37.98	16	**
6Pgdh	−0.036	−0.021	0.015	11.19	8	ns
Pgm	−0.067	−0.047	0.019	16.68	8	*
Xdh	0.006	0.067	0.062	130.15	16	***
Means:	−0.045	−0.012	0.032			

[1]Probability levels for significance of G-tests are * = $0.01 < P < 0.05$; ** = $0.001 < P < 0.01$; *** = $P < 0.001$.

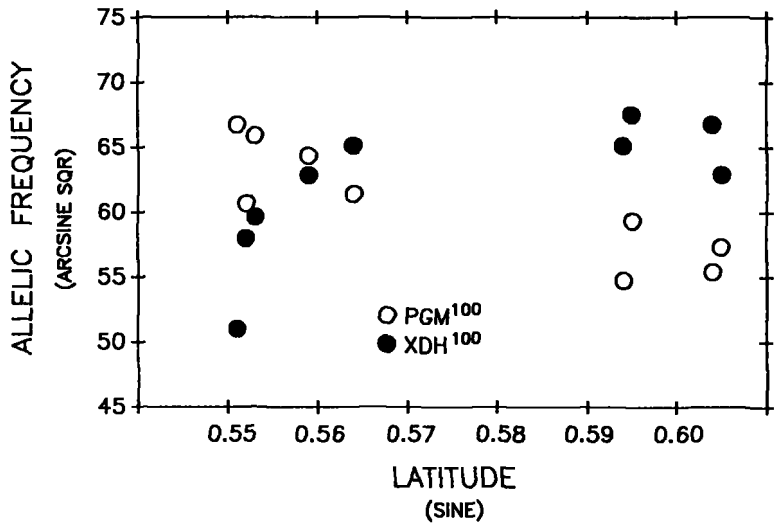

FIGURE 4.—Frequencies of the common alleles for *Pgm* and *Xdh* (arcsine-square-root transformed) plotted against the sine of latitude of collection site. Spearman rank correlation coefficients for the two loci are $r_S = -0.783$, $t = -3.330$ for *Pgm* and $r_S = 0.812$, $t = 3.681$ for *Xdh*.

DISCUSSION

Low Genetic Variation in the Pacific Sardine

Relative to other clupeoids, the Pacific sardine, *Sardinops sagax*, is depauperate in allozyme variation. Direct comparison with the northern anchovy, *Engraulis mordax*, in this study shows that the Pacific sardine has less than 25% of the average heterozygosity of the northern anchovy (Table 2A vs. Table 5). The northern anchovy, not the Pacific sardine, appears to have levels of variation typical of those reported in allozyme studies of clupeoids (Table 8). Average heterozygosity for 15 marine species of the order Clupeiformes is 7.1% (95% C.L.: 6.0–8.2%). The average expected heterozygosity for the Pacific sardine, 1.0%, and even the slightly greater heterozygosity found in the Guaymas population sample, 1.6% fall significantly below the clupeoid distribution ($z = -4.02$, $P < 0.000033$ and $z = -3.39$, $P < 0.0003$, respectively).

There is so little variation within and between Pacific sardine populations that it is not possible to test whether distributions of genotypes conform to the expectations of random mating or whether allelic frequencies are heterogeneous throughout the range of populations sampled. That sardines in widely separated localities have the same rare alleles (Table 2B) suggests strongly, however, that there has been substantial gene flow among contemporary populations (Slatkin 1985).

TABLE 8.—Allozyme variation reported for marine species of the order Clupeiformes.

Species	No. of pops studied	No. of loci studied	Mean no. alleles per locus	Percent loci polymorphic[1]	% mean heterozygosity Obs.	% mean heterozygosity Exp[2]	References[3]
Clupeidae							
Clupeinae							
Opisthonema							
bulleri	2	29	1.4	20.7	5.4	5.5	(1)
medirastre	2	29	1.3	27.6	8.5	7.7	(1)
libertate	5	29	1.6	31.0	6.3	6.7	(1)
Sardinops							
sagax	5	27	1.4	14.5	1.0	1.0	this study
melanosticta	1	22	—	22.7	6.4	—	(2)
Clupea							
harengus	6	40	—	47.5	—	6.5	(3)
harengus	3	25	1.8	38.8	—	7.0	(4)
harengus	14	42	—	21.3	4.8	—	(5)
pallasi	21	40	—	65.0	—	8.3	(6)
pallasi	1	17	—	29.4	5.8	—	(2)
Sprattus							
antipodum	2	13	1.7	50.0	9.6	11.2	(7)
sp.	1	13	1.6	46.2	5.5	5.7	(7)
Dussumieriinae							
Spratelloides							
gracilis	2	16	1.5	24.7	4.0	—	(8)
sp.	2	14	1.5	32.1	4.6	—	(8)
Engraulidae							
Engraulinae							
Engraulis							
japonicus	1	22	—	36.4	6.7	—	(2)
capensis	31	31	—	48.3	—	11.5	(9)
mordax	9	39	1.6	39.8	7.7	7.5	this study
Stolephorus							
heterolobus	3	18	1.7	22.2	10.1	—	(8)
devisi	3	19	1.7	17.1	6.8	—	(8)

[1]Polymorphism defined either as $P_{0.99}$ or inclusive of all observed variation.
[2]H_e are either unbiased estimtes (Nei 1978) or simple averages.
[3](1) Hedgecock et al. 1988; (2) Fujio and Kato 1979; (3) Grant 1984; (4) Andersson et al. 1981; (5) Kornfield et al. 1982; (6) Grant and Utter 1984; (7) Smith and Robertson 1981; (8) Daly and Richardson 1980; (9) Grant 1985a.

By contrast, the substantial allozyme polymorphism in the northern anchovy allows tests of both random mating and spatial homogeneity of allelic frequencies. Chi-square goodness-of-fit tests detect no significant departures from the genotypic proportions expected under random mating, although substantial excesses of heterozygotes are found at the *Hbdh-2* and *Lgg* loci. While liver-tissue degradation may have contributed to this result for *Hbdh-2* (see Materials and Methods), this explanation cannot hold for *Lgg*, which was scored reliably from both liver and eye zymograms. Differences in allelic frequencies either among age classes or between sexes can produce excess heterozygosity, and significant interaction of sex and allelic frequency is detected by fitting of log-linear models to *Hbdh-2* data. For *Lgg*, on the other hand, sex and allelic frequency are independent given locality. It must be remembered that the chi-square test of Hardy-Weinberg-Castle equilibrium has little power to detect failure of its basic assumptions, notably no selection at the locus and an infinite, unsubdivided population (Wallace 1958; Lewontin and Cockerham 1959).

The northern anchovy, again in contrast to the Pacific sardine, appears to have a complex population structure as evidenced by significant heterogeneity of allelic frequencies at 5 of 11 polymorphic loci (Table 7), correlations of some alleles with latitude (Fig. 4), and dependence of some allelic frequencies on sex and age. This heterogeneity is unexpected. All samples were collected within the area occupied by the central stock, which has been considered a single, randomly mating population, primarily on the basis of transferrin-allele frequencies and meristic data (Vrooman et al. 1981; see MacCall et al. 1983). Differences among populations within this area have nevertheless been described for growth and age composition (Parrish et al. 1985), size-adjusted otolith weight (Spratt 1972), size at age (Collins 1969; Mais 1974; Mallicoate and Parrish 1981), seasonality of spawning (Mais 1974; Parrish[1]), and migration patterns (Haugen et al. 1969; Mais 1974), together with between-year variation in many of these life history traits. Similar genetic heterogeneity of anchovy stocks has been described for *Engraulis encrasicholis* (Altukhov et al. 1969a, b; Dobrovolov 1978), although homogeneity of allelic frequencies was reported by Grant (1985b) for *E. capensis*.

For loci polymorphic over the nine population samples, Wright's (1978) measure of average genetic variance among populations, F_{ST}, and Nei's (1978) average genetic distance D—two measures that are maximized by allele replacement among populations—are both relatively small: 0.032 and 0.008, respectively. Significant heterogeneity of allelic frequencies without substantial allele replacement may reflect population subdivision and differentiation resulting from ecological, rather than historical processes. We will explore the causes of this paradoxical genetic heterogeneity in subsequent reports drawing on much larger sets of allozyme, sex, age, and morphological data for northern anchovy collected between 1982 and 1985.

Why does the Pacific sardine have low genetic variation? One possibility is that this species originally had levels of variation typical of clupeoids, but that much of this was lost in the collapse of the California sardine fishery in the 1950's and early 1960's. That this fishery collapse did *not* constitute a population genetic bottleneck, however, appears likely for several reasons. First, the genetically effective population size during the bottleneck would have had to have been very small, on the order of 10 or fewer individuals, in order to account for the current low level of heterozygosity (Chakraborty and Nei 1977). Second, sardine populations in southern Baja California and in the Gulf of California were unaffected by the collapse of the California fishery (Murphy 1969; Sokolov 1974), yet these populations today show low variation also. Finally, by analogy, the Japanese sardine, *Sardinops melanosticta*, which also experienced a severe fishery collapse in the 1940's but has since recovered (Kondo 1980), does not have reduced levels of genetic variation (Fujio and Kato 1979; Table 8).

Alternatively, a restriction in population size in the more distant past might explain low variation in the Pacific sardine. The historical record of scale deposits in varved, anaerobic marine sediments of the Santa Barbara Basin, southern California, does show that, relative to northern anchovy and Pacific hake, Pacific sardines were always less abundant and much more frequently absent (Soutar 1967; Soutar and Isaacs 1969, 1974). Over the past 1,850 years, the Pacific sardine was abundant during 12 periods, each lasting from 20 to 150 years. Intervals between these periods of abundance

[1]Parrish, R. H. 1983. Evidence for a fall spawning anchovy stock. Paper presented at 1983 CalCOFI Conference.

lasted, on average, 80 years and ranged in duration from 20 to 200 years (Soutar and Isaacs 1969). One or more of these periods of low abundance could have been a severe enough bottleneck to cause loss of variation, but this hypothesis may be difficult to falsify. According to Fitch (1969), fossil remains of *Sardinops* are absent from samples of California Pliocene and Pleistocene sediments, whereas evidence of five other pelagic species (*Clupea pallasi*, *Engraulis mordax*, *Merluccius productus*, *Scomber japonicus*, and *Trachurus symmetricus*) is present in at least some samples. This raises the possibility that *Sardinops sagax caerulea* may be a recent arrival in the California Current System and that the low variation is attributable to a small number of founders rather than to a subsequent bottleneck.

Other than such historical hypotheses, we can pose no ecological explanation of low genetic variation (such as provided for decapod crustaceans by Nelson and Hedgecock 1980, for example); the ecology of the Pacific sardine does not appear to be unique relative to those clupeoids having higher levels of variation (Blaxter and Hunter 1982). So we are left at present with no compelling hypothesis to explain the observation of low genetic variation in the Pacific sardine.

Structures of Historical and Contemporary Populations of the Pacific Sardine

Sprague and Vrooman (1962) and Vrooman (1964) described three genetically distinct subpopulations of *Sardinops sagax caerulea* on the basis of significantly different frequencies of a C-positive blood factor (13.6% in samples taken from California waters, 6.0% in samples taken from Baja California, and 16.8% in fish from the Gulf of California). Regrettably, though understandably, electrophoretic separation of allozymes has completely supplanted immunological methods for studying population structure. Data comparable in quantity and quality to historical data on serotype frequencies would be difficult to gather today. A considerable drawback to the immunological method is the requirement for fresh blood, whereas allozymes can be readily obtained from fresh or fresh frozen, muscle or visceral tissues. Moreover, allozyme methods allow a much larger survey of genes than does blood typing; this, in turn, provides for statistical analyses of genetic diversity that take into account the large component of variance among loci (Nei 1978).

Our finding of low genetic variation across a widespread sampling of Pacific sardines contradicts the hypothesis that there are currently genetically different, geographic subpopulations of Pacific sardine. Combined with the recency of the Pacific sardine's reexpansion into the California Current (Wolf et al. 1987), our observations support the alternative hypothesis that this species comprises a single, homogeneous gene pool. Examination of the distributions and abundances of sardine eggs and larvae (Ahlstrom 1954, 1957) does suggest the possibility of dispersal around the tip of Baja California, particularly during cold-water (anti-El Niño) years.[2] Our data on the sharing of rare alleles by widely separated populations support this conjecture by implying a high rate of gene flow throughout the range of the species (Slatkin 1985).

The present study does not falsify the subpopulation hypothesis for historical sardine populations, but our data show that it is unlikely. The former hypothesis requires that only a single southern subpopulation survived the fishery collapse to repopulate the Gulf of California, the Pacific coast of Baja California Sur, and more recently, the California Current. Data on the frequency of C-positive blood type in contemporary sardine populations would be useful. However, morphological and life history data also played an important role in past inferences concerning the structure of historical sardine populations (Radovich 1982). The implications of our data on morphological variation among contemporary populations are discussed next.

Morphological and Life History Variation Among Historical and Contemporary Pacific Sardine Populations

Life history traits, such as the schedules of age-specific growth, mortality, and reproduction, and the covariances among these traits, determine responses by fish populations to exploitation (Cushing 1973; Nelson and Soulé 1987). Indeed, the historical biology of the California sardine fishery and its demise provides an elegant example of this axiom. An important

[2]R. A. Schwartzlose, Centro de Investigaciones Biologicas de Baja California sur and Scripps Institution of Oceanography, La Jolla, CA 92093, pers. commun. 1988.

feature of the collapse of the sardine fishery was its north-to-south progression, which owed greatly to the interaction of underlying life history variation (in particular, steep north-south clines in size-at-age, age of first reproduction, maximum size, and the schedule of natural mortality), geographical shifts in fishing pressure, and natural between-year variation in recruitment (Murphy 1966; Radovich 1982). That life history variation was built upon genetic differences among geographic populations, however, now appears unlikely from the results of our study.

The single, most obvious component of morphological variation in Pacific sardines today is a geographic cline in size-at-age that is as steep and as large as that seen in historical populations (Fig. 2). In the past, such differences were used by several authors to infer the existence of genetically distinct subpopulations, yet the differences have been reestablished in genetically homogeneous, contemporary populations within just a few generations. This implies that rapid differentiation of growth rate among geographic populations—probably together with differentiation of correlated life history features such as age at first reproduction, maximum size, and age-specific mortality (Clark and Marr 1955; Blaxter and Hunter 1982)—is largely environmentally, and not genetically, determined. This is not to say that there are not genes that determine life history traits; but variation of these genes cannot be responsible for geographic variation in life history. The genotype of the Pacific sardine must instead provide for remarkable plasticity in life history phenotype.

One must now be skeptical of the interpretation that life history differences among historical sardine populations were conditioned by genetic differences among subpopulations or races. The question of which sardine stock is now recovering is moot. More importantly, it appears that information on the biology of sardine populations prior to the collapse of the fishery can safely be used for area-specific fisheries models of the recovering stocks.

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