

Little Genetic Variation in the Oil Sardine, *Sardinella longiceps* Val., from the Western Coast of India

Maria R. Menezes

National Institute of Oceanography, Dona Paula, Goa 403004, India.

Abstract

Enzyme gene variability in the oil sardine, *Sardinella longiceps*, from three localities along the western coast of India was studied by starch-gel electrophoresis. Out of 19 loci scored, no locus was polymorphic by the 95% criterion. Seven loci were polymorphic at the $P=0.99$ level. The average heterozygosity ranged from 0.6% to 0.9%. These values are very low compared with those of other marine fish species.

Introduction

The Indian oil sardine, *Sardinella longiceps* Val. (Clupeidae), is an important species, contributing nearly a third of the total marine fish production of India in productive years (Bal and Rao 1984). Besides its usefulness as a table fish, it serves as a source of valuable by-products such as sardine oil for industry, fish-meal for cattle and poultry feed, and fertilizer for coconut, coffee and tea plantations. Its importance as a highly lucrative fishery in India is restricted to the southern and central regions of the western coast of India. *S. longiceps* occurs along the coasts of Somalia, Kenya, Seychelles, Gulf of Aden, Red Sea, Gulf of Oman, Persian Gulf, Pakistan, India, Sri Lanka, Andaman Sea, Malaysia, Indonesia, Philippines and Vietnam (Nair and Chidambaram 1951; Bal and Rao 1984).

Past studies of the species have concerned: size at first maturity (150-160 mm) (Antony Raja 1964; Bensam 1964; Dhulkhed 1964); spawning season (June to October) and spawning habits (fractional: once in a season, twice in a lifetime) (Antony Raja 1964); fecundity (about 38 000 ova per adult female and 750 million ova per tonne of adult female biomass) (Antony Raja 1964; Balan 1965); and age and growth (150-160 mm at the end of the first year, 170-180 mm at the end of the second year, and up to 220-229 mm thereafter) (Antony Raja 1970). The species is chiefly a surface/column filter-feeder on phytoplankton, with the feeding intensity depending on the relative abundance of food (Nair 1953; Dhulkhed 1962). Biometrical analysis of morphometric and meristic characters of *S. longiceps* specimens from Madras on the eastern coast of India and from several places on the western coast revealed a stability of the main external characters that suggested an absence of local races or brood differences (Hornell and Nayudu 1924). On the other hand, Davanesan and Chidambaram (1943) found different head lengths and tail lengths between samples from Aden, Muscat, Bombay-Karachi, Karwar and Malabar; they concluded that the Karwar specimens, which had the longest heads and the shortest tails, belonged to a distinct race and that the Aden specimens represented another race. Nair and Chidambaram (1951) suggested that this aspect requires further careful examination because the existence of distinct races in the oil sardine, if proved conclusively, has an important bearing on their biology and fishery.

Stocks of various species of fishes have been defined by geographic differences in meristic and morphological characters, by regional differences in population parameters, by physiology and behaviour, by the results of tagging experiments, and by physical oceanographic features (Ihssen *et al.* 1981; Iles and Sinclair 1982). With the development of protein electrophoresis and the restriction enzyme analysis of DNA, it has become possible to search for genetic differences between putative stocks that have been defined by non-genetic methods (Spanakis *et al.* 1989).

Electrophoresis of proteins has been widely applied in studies of genetic variation in fish populations (e.g. Stahl 1987; Menezes *et al.* 1990a; McElligott and Cross 1991); the fact that each protein is encoded by a single gene allows the identification of one or more loci with alleles and, hence, a description, useful for management, of the genetic structure of a population (Ryman and Utter 1987; Seeb *et al.* 1990). This paper is an initial attempt to estimate the degree of genetic variation, at protein-coding loci detected by electrophoresis, in *S. longiceps* from three localities along the western coast of India and to determine the structure of the population.

Materials and Methods

Specimens of *S. longiceps* were collected from the fish landing centres of Panaji (Goa) in December 1988, October 1990 and January 1991 and Malpe and Mangalore in January 1989 (Fig. 1). The fish were kept frozen at -20°C prior to analysis. The skeletal muscle, liver, heart and eye were dissected from individual specimens. The cell lysate obtained by freezing and thawing was subjected to starch-gel electrophoresis after the method of Menezes and Taniguchi (1988). The buffer system used was citric acid-aminopropylmorpholine at pH 6.0 (Clayton and Tretiak 1972). Detection of isozymes and locus nomenclature (Table 1) followed Shaklee *et al.* (1990).

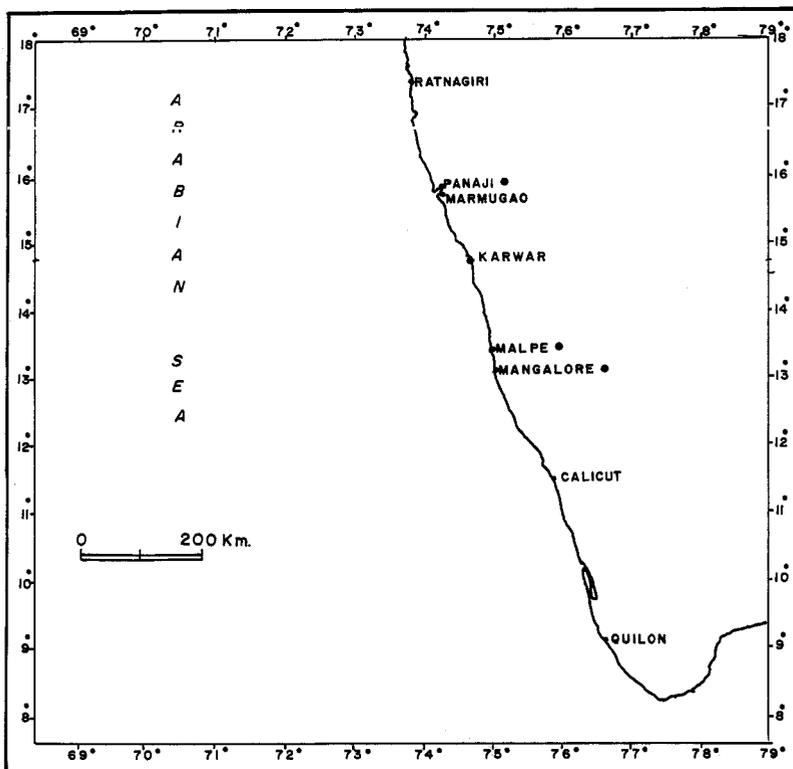


Fig. 1. Map of the western coast of India, showing sampling localities (●).

Table 1. *Sardinella longiceps*. Enzymes examined, loci identified and tissues assayed

Enzyme	Enzyme No.	Locus	Tissue
Alcohol dehydrogenase (ADH)	1.1.1.1	<i>ADH*</i>	Liver
Aspartate aminotransferase (AAT)	2.6.1.1	<i>AAT*</i>	Liver
Creatine kinase (CK)	2.7.3.2	<i>CK*</i>	Skeletal muscle
Glycerol-3-phosphate dehydrogenase (G3PDH)	1.1.1.8	<i>G3PDH-1*</i> <i>G3PDH-2*</i>	Skeletal muscle Liver
Isocitrate dehydrogenase (IDHP)	1.1.1.42	<i>IDHP-1*</i> <i>IDHP-2*</i>	Skeletal muscle Liver
Lactate dehydrogenase (LDH)	1.1.1.27	<i>LDH-A*</i> <i>LDH-B*</i> <i>LDH-C*</i>	Skeletal muscle Skeletal muscle, heart Eye
Malate dehydrogenase (MDH)	1.1.1.37	<i>MDH-1*</i> <i>MDH-2*</i> <i>MDH-3*</i>	Skeletal muscle Skeletal muscle Skeletal muscle
Malic enzyme (MEP)	1.1.1.40	<i>MEP*</i>	Skeletal muscle
Phosphoglucomutase (PGM)	5.4.2.2	<i>PGM*</i>	Skeletal muscle
Phosphogluconate dehydrogenase (PGDH)	1.1.1.44	<i>PGDH*</i>	Liver
Haemoglobin (HB)		<i>HB*</i>	Heart
General protein (PROT)		<i>PROT-1*</i> <i>PROT-2*</i>	Skeletal muscle Skeletal muscle

Allelic variants were designated according to their relative mobility, the most common allele being designated 100 and other alleles being given numbers that indicated their mobility relative to that of the most common allele. Genetic identity and distance were calculated from the formula proposed by Nei (1972). Hardy-Weinberg statistical analysis followed Hartl (1980). Average heterozygosity was determined by summing the number of observed heterozygotes for each locus, dividing this by the total number of individuals in the samples, and averaging over all loci.

Results

Of the 19 loci detected from 10 enzymes, a general protein and haemoglobin, no locus was polymorphic by the 95% criterion. No locus showed sufficient variation to be of use in the analysis of population structure. However, seven loci were polymorphic at very low degrees of variability.

The genetic variabilities observed in *S. longiceps* from the three localities and in the total pooled sample are given in Table 2. Among the loci investigated, polymorphism ($P \leq 0.99$) was observed for five (*G3PDH-1**, *IDHP-1**, *MEP**, *PGDH** and *AAT**) in the Panaji sample, three (*LDH-B**, *G3PDH-1** and *G3PDH-2**) in the Malpe sample, and two (*IDHP-1** and *PGDH**) in the Mangalore sample. Allele frequencies and χ^2 values for the differences between observed and expected Hardy-Weinberg numbers of phenotypes of

Table 2. Estimates of genetic variability in *S. longiceps*

Locality	Number of alleles per locus	Proportion of loci polymorphic		Mean heterozygosity per locus
		$P = 0.95$	$P = 0.99$	
Panaji	1.26	0	0.263	0.006 ± 0.011
Malpe	1.18	0	0.176	0.009 ± 0.020
Mangalore	1.12	0	0.118	0.006 ± 0.017
Total	1.37	0	0.368	0.006 ± 0.009

Table 3. Allele frequencies at 19 loci in *S. longiceps* from three localities
n, number of individuals; χ^2 , difference between observed and expected phenotypic
distributions for each polymorphic enzyme

Locus	Allele	Panaji	Malpe	Mangalore
<i>ADH</i> *	100	1.0	1.0	1.0
	<i>n</i>	20	20	20
<i>G3PDH-1</i> *	100	0.995	0.975	1.0
	50	0.005	0.025	0
	<i>n</i>	94	20	20
	χ^2	0.003	0.013	0
<i>G3PDH-2</i> *	100	1.0	0.975	1.0
	50	0	0.025	0
	<i>n</i>	84	20	20
	χ^2	0	0.013	0
<i>HB</i> *	100	1.0	1.0	1.0
	<i>n</i>	40	20	20
<i>IDHP-1</i> *	150	0.067	0	0.025
	100	0.983	1.0	0.975
	<i>n</i>	60	20	20
	χ^2	0.017	0	0.05
<i>IDHP-2</i> *	100	1.0	1.0	1.0
	<i>n</i>	44	20	20
<i>LDH-A</i> *	100	1.0	1.0	1.0
	<i>n</i>	94	20	20
<i>LDH-B</i> *	100	1.0	0.975	1.0
	50	0	0.025	0
	<i>n</i>	94	20	20
	χ^2	0	0.013	0
<i>LDH-C</i> *	100	1.0	1.0	1.0
	<i>n</i>	40	20	20
<i>MDH-1</i> *	100	1.0	1.0	1.0
	<i>n</i>	94	20	20
<i>MDH-2</i> *	100	1.0	1.0	1.0
	<i>n</i>	94	20	20
<i>MDH-3</i> *	100	1.0	1.0	1.0
	<i>n</i>	94	20	20
<i>MEP</i> *	125	0.008	0	0
	100	0.992	1.0	1.0
	<i>n</i>	60	20	20
	χ^2	0.004	0	0
<i>PGDH</i> *	100	0.984	1.0	0.975
	50	0.016	0	0.025
	<i>n</i>	64	20	20
	χ^2	0.016	0	0.013
<i>PROT-1</i> *	100	1.0	1.0	1.0
	<i>n</i>	94	20	20
<i>PROT-2</i> *	100	1.0	1.0	1.0
	<i>n</i>	94	20	20
<i>CK</i> *	100	1.0	1.0	1.0
	<i>n</i>	54	20	20
<i>AAT</i> *	100	0.991	—	—
	50	0.009	—	—
	<i>n</i>	54	—	—
	χ^2	0.005	—	—
<i>PGM</i> *	100	1.0	—	—
	<i>n</i>	54	—	—

each polymorphic enzyme are given in Table 3. Genotypes for the samples from the three localities conformed to Hardy-Weinberg proportions ($P > 0.05$) at all loci. This suggests that all the *S. longiceps* samples were collected from a single Mendelian population.

The allele frequencies were similar at all loci (Table 3). The genetic distance was zero.

Discussion

Genetic studies of most epipelagic marine fishes generally show little genetic subdivision within the range of a given species. In addition, sardines have a number of behavioural characteristics that tend to reduce their speciation rates (Parrish *et al.* 1989).

The best estimate of genetic variation in natural populations is the average heterozygosity (H) per locus (Allendorf and Utter 1979). The values of P and H vary non-randomly between loci, populations and species. To avoid serious error in the estimation of average heterozygosity, a large number and wide range of isozyme loci should be examined (Allendorf and Utter 1979). On the basis of 19 loci, the average heterozygosity per locus was 0.006 ± 0.011 (Panaji), 0.009 ± 0.020 (Malpe) and 0.006 ± 0.017 (Mangalore), compared with $H = 0.066 \pm 0.11$ for the Indian mackerel, *Rastrelliger kanagurta* (Menezes *et al.* 1990b), $H = 0.051 \pm 0.034$ calculated by Nevo (1978) as the mean for 51 species of fishes and $H = 0.048 \pm 0.039$ as the mean for 82 species of fishes calculated by Winans (1980). High degrees of genetic variation ($H = 0.056$ to 0.106) are also found in marine species of the order Clupeiformes (see Smith and Fujio 1982). The estimate of $H = 0.106$ for the small clupeid fish *Chupea harengus* is above average (Altukhov *et al.* 1974). High heterozygosities might be a feature of the pelagic clupeids or of small fish in general (Selander and Kaufman 1973; Valentine 1976). Pacific sardines, however, showed little genetic variation both within and between populations (Hedgecock *et al.* 1989); in a study of 32 allozyme-coding loci the average heterozygosity ranged from 0.5% to 1.6%, results similar to those of the present study. Slightly greater heterozygosities were found in Spanish sardines off Florida and Brazil, ranging between 2.0 and 2.7% (Wilson and Alberdi 1991). It is probably reasonable to assume that the degree of isozyme variation reflects the relative degree of genetic variation found at other loci in the genome (McAndrew and Majumdar 1983; Menezes *et al.* 1993). Smith (1986) reported evidence of comparatively low degrees of electrophoretically detectable allelic variation in shark species representing several different orders. He postulated that bottlenecks in previous populations (i.e. reductions in the small numbers of breeding individuals) might reduce allelic variation in species.

Genetic diversity, expressed either as average number of alleles or as average heterozygosity, is usually higher in species with wider geographic ranges, higher fecundity, greater longevity, and larger population sizes (Nevo *et al.* 1984). Soule (1976) identified population size as the most important variable in population genetics theory and long-term maintenance of a large population size as a necessary condition for greater variability.

The abundance of oil sardines in the fishery off India, as revealed by landings, is subject to wide fluctuations that were first noticed by Day (1865). Scarcity of oil sardines has been attributed to indiscriminate capture of immature fish during years of abundance (Raj 1934-40), overfishing (Devanesan 1943), capture of spawning fish during the breeding season (Chidambaram 1950), unfavourable surface temperatures (Chidambaram 1950; Nair 1953), and low availability of *Fragillaria oceanica* (Nair 1953; Nair and Subrahmanyam 1955), which is the preferred food of the oil sardine.

A greatly reduced population size for a single generation can have a dramatic effect on genetic variation (Crow and Kimura 1970). With the loss of genetic variability, the population may no longer be able to exploit its former ecological niche (Ryman 1981). The low genetic variation in Pacific sardines was attributed to a small number of founders rather than to a subsequent bottleneck (Hedgecock *et al.* 1989). Seeb *et al.* (1990) indicated that there was no measurable loss of genetic variability in the spiny lobster, *Panulirus marginatus*, despite fishing pressure over seven years. Early records of oil sardine landings

are not available; however, data given by Nair and Chidambaram (1951) on sardine products such as cured fish, oil, fertilizers and fish-meal indicate the extreme annual fluctuations seen in the fishery. A reduction in population size in the past, due to commercial exploitation, might thus explain the present low genetic variation in the Indian oil sardine, *Sardinella longiceps*.

The need for conservation and management of genetic resources has been recognized by fishery scientists for some time, especially in relation to the overfishing of natural stocks and the effects of large-scale changes to aquatic systems (Anon. 1981; Gross 1991). As Ryman (1991) has pointed out, genetic variability should be conserved, so that a population may maintain its potential to respond to environmental perturbations.

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References

- Allendorf, F. W., and Utter, F. M. (1979). Population genetics. In 'Fish Physiology'. (Eds W. S. Hoar, D. S. Randall and J. R. Brett.) Vol. 8, pp. 407-54. (Academic Press: New York.)
- Altukhov, Yu. P., Salmenkova, E. A., Omel'chenko, V. T., Sachko, G. D., and Slyn'ko, V. I. (1974). Number of monomorphic and polymorphic loci in a population of the tetraploid salmon *Oncorhynchus keta* Walb. *Genetika* 8, 181-8.
- Anon. (1981). Conservation of the genetic resources of fish: problems and recommendations. Report of the Expert Consultation on the Genetic Resources of Fish, Rome, 9-13 June 1980. FAO Fisheries Technical Paper No. 217.
- Antony Raja, B. T. (1964). Some aspects of spawning biology of Indian oil-sardine, *Sardinella longiceps* Val. *Indian Journal of Fisheries* 11, 45-120.
- Antony Raja, B. T. (1970). Estimation of age and growth of the Indian oil-sardine, *Sardinella longiceps* Val. *Indian Journal of Fisheries* 17, 26-42.
- Bal, D. V., and Rao, V. R. (1984). 'Marine Fisheries.' (Tata McGraw-Hill: India.) 470 pp.
- Balan, V. (1965). The fecundity and sex composition of *Sardinella longiceps* Val. of the Cochin coast. *Indian Journal of Fisheries* 12, 473-91.
- Bensam, P. (1964). Growth variations in the Indian oil sardine *Sardinella longiceps* Val. *Indian Journal of Fisheries* 11, 699-708.
- Chidambaram, K. (1950). Studies on the length frequency of oil sardine *Sardinella longiceps* Cuv. and Val. and on certain factors influencing their appearance on the Calicut coast of the Madras Presidency. *Proceedings of the Indian Academy of Sciences* 31, 252-86.
- Clayton, J. W., and Tretiak, D. N. (1972). Amine-citrate buffers for pH control in starch gel electrophoresis. *Journal of the Fisheries Research Board of Canada* 29, 1169-72.
- Crow, J. F., and Kimura, M. (1970). 'An Introduction to Population Genetics Theory.' (Harper: New York.)
- Day, F. (1865). 'The Fishes of Malabar.' (Bernard, Quaritch: London.) 293 pp.
- Devanesan, D. W. (1943). A brief investigation into the causes of the fluctuations of the annual fishery of the oil sardine of Malabar, *Sardinella longiceps*, Val., determination of its age and an account of the discovery of its eggs and spawning grounds. *Madras Fishery Bulletin* 28, 1-38.
- Devanesan, D. W., and Chidambaram, K. (1943). On the alleged existence of races in the population of the oil sardine, *Sardinella longiceps* in the Arabian Sea. *Proceedings of the 30th Indian Science Congress III*, 60 (abstract).
- Dhulkhed, M. H. (1962). Observations on the food and feeding habits of the Indian oil sardine, *Sardinella longiceps* Val. *Indian Journal of Fisheries* 9, 37-47.
- Dhulkhed, M. H. (1964). Observations on the spawning behaviour of Indian oil sardine, *Sardinella longiceps* Val. determined by ova diameter studies. *Indian Journal of Fisheries* 11, 371-6.
- Gross, M. R. (1991). Salmon breeding behavior and life history evolution in changing environments. *Ecology* 72, 1180-6.

- Hartl, D. L. (1980). 'Principles of Population Genetics.' (Sinauer Association: Sunderland, Massachusetts.)
- Hedgecock, D., Hutchinson, E. S., Li, G., Sly, F. L., and Nelson, K. (1989). 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*. *Fishery Bulletin (US)* **87**, 653–71.
- Hornell, J., and Nayudu, M. R. (1924). A contribution to the life history of the Indian oil sardine with notes on the plankton of the Malabar coasts. *Madras Fishery Bulletin* **17**, 129–92.
- Ihssen, P. E., Booke, H. E., Casselman, J. M., McGlade, J. M., Payne, N. R., and Utter, F. M. (1981). Stock identification: materials and methods. *Canadian Journal of Fisheries and Aquatic Sciences* **38**, 1838–55.
- Iles, T. D., and Sinclair, H. (1982). Atlantic herring: stock discreteness and abundance. *Science (Washington, DC)* **21**, 627–33.
- McAndrew, B. J., and Majumdar, K. C. (1983). Tilapia stock identification using electrophoretic markers. *Aquaculture* **30**, 249–61.
- McElligott, E. A., and Cross, T. F. (1991). Protein variation in wild Atlantic salmon, with particular reference to southern Ireland. *Journal of Fish Biology* **39** (Supplement A), 35–42.
- Menezes, M. R., and Taniguchi, N. (1988). Interspecific genetic divergence in sciaenids from Japan and its adjacent waters. *Japanese Journal of Ichthyology* **35**, 40–6.
- Menezes, M. R., Taniguchi, N., and Seki, S. (1990a). Degree of intraspecific genetic divergence and variability in three sciaenid species. *Japanese Journal of Ichthyology* **37**, 39–48.
- Menezes, M. R., Naik, S., and Martins, M. (1990b). Genetic and morphological variations in the Indian mackerel *Rastrelliger kanagurta* (Cuvier 1817) from the Goa region. *Proceedings of the Indian Academy of Sciences (Animal Sciences)* **99**, 457–65.
- Menezes, M. R., Naik, S., and Martins, M. (1993). Genetic characterisation in four sciaenid species from the Arabian Sea. *Journal of Fish Biology* **43**, 61–7.
- Nair, R. V. (1953). Studies on the revival of the Indian oil sardine fishery. *Indo-Pacific Fisheries Council Proceedings* **2**, 115–29.
- Nair, R. V., and Chidambaram, K. (1951). A review of the Indian oil sardine fishery. *Proceedings of the National Institute of Sciences in India* **17**, 71–85.
- Nair, R. V., and Subrahmanyam, R. (1955). The diatom *Fragillaria oceanica* Cleve, an indicator of abundance of the Indian oil sardine, *Sardinella longiceps* Cuv. and Val. *Current Science (Bangalore)* **24**, 41–2.
- Nei, M. (1972). Genetic distance between populations. *American Naturalist* **106**, 283–92.
- Nevo, E. (1978). Genetic variation in natural populations: patterns and theory. *Theoretical Population Biology* **13**, 121–77.
- Nevo, E., Belies, A., and Ben-Shlomo, R. (1984). The evolutionary significance of genetic diversity: ecological, demographic and life history correlates. In 'Evolutionary Dynamics of Genetic Diversity'. (Ed. G. S. Mani.) pp. 13–137. (Springer-Verlag: New York.)
- Parrish, R. H., Serra, R., and Grant, W. S. (1989). The monotypic sardines, *Sardina* and *Sardinops*: their taxonomy, distribution, stock structure, and zoogeography. *Canadian Journal of Fisheries and Aquatic Sciences* **46**, 2019–36.
- Raj, B. S. (1934–40). Administrative reports for the years 1932–33 to 1939–40. Madras Fisheries Department, Madras.
- Ryman, N. (1981). Conservation of genetic resources: experiences from the brown trout (*Salmo trutta*). In 'Fish Gene Pools'. (Ed. N. Ryman.) *Ecological Bulletins—NFR* **34**, 61–74.
- Ryman, N. (1991). Conservation genetics considerations in fishery management. *Journal of Fish Biology* **39** (Supplement A), 211–24.
- Ryman, N., and Utter, F. (Eds) (1987). 'Population Genetics and Fishery Management.' (University of Washington Press: Seattle.) 420 pp.
- Seeb, L. W., Seeb, J. E., and Polovina, J. J. (1990). Genetic variation in highly exploited spiny lobster *Panulirus marginatus* populations from the Hawaiian Archipelago. *Fishery Bulletin (US)* **88**, 713–18.
- Selander, R. K., and Kaufman, D. W. (1973). Genic variability and strategies of adaptation in animals. *Proceedings of the National Academy of Sciences of the United States of America* **70**, 1875–7.
- Shaklee, J. B., Allendorf, F. W., Morizot, D. C., and Whitt, G. S. (1990). Gene nomenclature for protein-coding loci in fish. *Transactions of the American Fisheries Society* **119**, 2–15.
- Smith, P. J. (1986). Low genetic variation in sharks (Chondrichthyes). *Copeia* **1986**(1), 202–7.

- Smith, P. J., and Fujio, Y. (1982). Genetic variation in marine teleosts: high variability in habitat specialists and low variability in habitat generalists. *Marine Biology (Berlin)* **69**, 7-20.
- Soule, M. (1976). Allozyme variation: its determinants in time and space. In 'Molecular Evolution'. (Ed. F. J. Ayala.) pp. 60-77. (Sinauer Associates: Sunderland, Massachusetts.)
- Spanakis, E., Tsimenides, N., and Zouros, E. (1989). Genetic differences between populations of sardine, *Sardina pilchardus*, and anchovy *Engraulis encrasicolus*, in the Aegean and Ionian Seas. *Journal of Fish Biology* **35**, 417-37.
- Stahl, G. (1987). Genetic population structure of Atlantic salmon. In 'Population Genetics and Fishery Management'. (Eds N. Ryman and F. Utter.) pp. 121-40. (University of Washington Press: Seattle.)
- Valentine, J. W. (1976). Genetic strategies of adaptation. In 'Molecular Evolution'. (Ed. F. J. Ayala.) pp. 78-94. (Sinauer Associates: Sunderland, Massachusetts.)
- Wilson, R. R., Jr, and Alberdi, P. D., Jr (1991). An electrophoretic study of Spanish sardine suggests a single predominant species in the eastern Gulf of Mexico, *Sardinella aurita*. *Canadian Journal of Fisheries and Aquatic Sciences* **48**, 792-8.
- Winans, G. A. (1980). Geographic variation in milkfish *Chanos chanos*. I. Biochemical evidence. *Evolution* **34**, 558-74.

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