Mediterranean Marine Science

Vol 1, No 1 (2000)

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doi: 10.12681/mms.284

To cite this article:

DOBROVOLOV, I. (2000). Genetic divergence between the scad subspecies Trachurus Mediterraneus (Carangidae, pisces) from the Black Sea and the Mediterranean. *Mediterranean Marine Science*, *1*(1), 133–141. https://doi.org/10.12681/mms.284

Mediterranean Marine Science
Vol. 1/1, 2000, 133-139

Genetic divergence between the scad subspecies *Trachurus Mediterraneus* (*Carangidae, Pisces*) from the Black Sea and the Mediterranean

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Manucript received: 13 July 1999; accepted in revised form: 27 April 2000

Abstract

Analysis of myogen and eleven enzymes (AAT, ADH, EST, GPI, IDHP, LDH, MDH, MEP, PGDH, PGM and SOD) were carried out by using starch gel and isoelectric focusing electrophoresis on thinlayer and ultrathin polyacrylamide ampholine and servalite gels in scad species from the Black Sea, the Sea of Marmara, the Aegean Sea, and the Mediterranean. Data from electrophoretic investigations was used to clarify the population structure of the scad migrating in the Bulgarian aquatory of the Black Sea and consisting of two main subpopulations: a) a "Black Sea" one hibernating in this sea only, and b) a "Sea of Marmara" one hibernating in that sea and contiguous Black Sea regions around the Bosporus. Non-specific muscular esterases were used, because they were appropriate for identification of both subspecies: Trachurus mediterraneus ponticus Aleev and Tr.m.mediterraneus Steindachner. Nei's genetic distance (D=0,0113) was used as an indication of their isolation dated back to the Carangate Period. An attempt was made to check the hypothesis of the origin of "large" ("giant") scad in the Black Sea as a result of the heterozygotic interbreeding between Tr.m.ponticus Aleev and Tr.m.mediterraneus Steindachner. A new allele Est-1E as well as the previously known alleles Est-1A and Est-1-B, were found in Mediterranean scad Tr.m.mediterraneus caught off Nice (France). It's proposed that the presence of the large form of large scat in this area is also a consequence of the heterozytic efect from the interbreeding between a population marked by Est-1-A and Est-1-B and a population marked by Est-1-E, probably inhabiting the waters around Gibraltar in the Atlantic Ocean.

Keywords: Electrophoresis, Isoelectricfocusing, Giant scad, Myogens, Enzymes, Heterozygosis.

Introduction

There exists a commonly accepted opinion that the scad *Trachurus mediterraneus ponticus* Aleev has entered in the Black Sea about 5000-7000 years ago, i.e. after the last connection of the Black Sea with the Sea of Marmara and the Mediterranean through the Bosporus and the Dardanelles (ALEEV, 1957). NEVESKI (cited after MISHEV, POPOV, 1978) assumed that this connection took place 10.000 years ago. However, we can not accept this hypothesis. Biochemical genetic investigations of the scad from the Black Sea, the Sea of Marmara, and the Mediterranean (DOBROVOLOV, 1977; DOBROVOLOV, 1978; DOBROVOLOV, 1979; DOBROVOLOV, 1988; DOBROVOLOV, DOBROVOLOVA, 1983; SALEM, KTARI, 1992) revealed patterns of variation, that suggest an interrelation between the populations from the Black Sea and the Mediterranean.

The present paper contains data supporting the concept of genetic divergence of the scad that now inhabits the Black Sea and the Mediterranean Seas from the now extinct "large" ("giant") Black Sea scad, which according to TIKHONOV *et al.* (1955) reached of 52-55 cm in length and of 1200 g in weight.

Materials and Methods

The electrophoretic analysis of enzymatic and nonenzymic proteins included a total of 4.500 scad specimens from the Black Sea, which were collected through summer-autumn from the west part of the distribution and in the winter from east part of the Black and Marmara Seas. Also, 107 samples from the Sea of Marmara, 66 from Thessaloniki, 49 from Lesbos island, 18 from Alexandria (Egypt), 11 from Piraeus, 18 from Venice, and 59 from Nice (France) were examined. The collection and analysis of the samples covered a long period of investigations (1973-1997). Homogenization and extraction of white dorsal muscle or other organs, separation on horizontal starch-gel electrophoresis, isoelectric focusing (IEF) on thinlayer polyacrylamide ampholine gel with pH - gradient between 3,5 and 9,5 and on servalyt-precotes PAG layer with pH-gradient between 3 and 10 visualization of myogens (water-soluble proteins), and enzymes had been described earlier (DOBROVOLOV, 1973; DOBROVOLOV, 1976; DOBROVOLOV, 1988; DOBROVOLOV, 1994). Gene frequencies of the polymorphic loci were calculated after Hardy-Weinberg equilibrium. Calculation of indexes for genetic similarity and genetic distance was performed after NEI (1972,1982).

Results

ASPARTATAMINOTRASFERASES (EC 2.6.11-AAT). Two *s*-AAT*(GOT 1)* and one *m*-AAT*(GOT 2)* were clearly observed in the catode zone during starch gel electrophoresis. No polymorphism was found. No differences between the fractions' electrophoretic activities of Black Sea and Mediterranean scad were established.

ALCOHOL DEHYDROGENASE (EC 1.1.1.1-ADH). The observed polymorphism was controlled by at least two alleles of one autosome locus. The allelic frequencies, are presented in Table 1.

ESTERASE (EC 3.1.1.1-EST). There were 2 zones of strongly expressed esterase activity out 7 zones of less expressed ones. We postulated a four-allele (A, B, C, and D) codominant system for the first polymorphic zone for the scad from the Black and Marmara Seas (of 10 phenotypes) and a diallelic system (A, B) for the scad from Mediterranean Sea (DOBROVOLOV, DOBROVOLOVA, 1983). The only exception was observed in the sample from the Aegean Sea (close to Thessaloniki). *Est-1*^{C} (0,015), in the Aegean Sea is rare to find, while it typical for Black Sea scad.

A new allele *Est-1*^{E} was found only in the scad from the Mediterranean Sea (Nice, France) (Fig. 1).

In the second polymorphic zone in Black

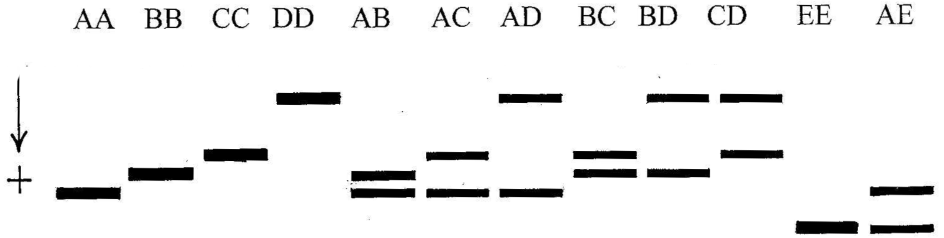

Fig. 1: Schematic presentation of the various EST-1 isoenzyme phenotypes observed in T*rachurus mediterraneus ponticus* and *Trachurus m. mediterraneus.*

Table 1
Genetic similarity index I (Nei)* by *Trachurus mediterraneus ponticus* (*Tmp*) and *Tr.m.mediterraneus* (*Tmm*).

Locus	Subspecies	Allelic frequence				Similarity index
		A	B	C	D	I
sAat 1	Tmp	1	0			1
	Tmm	1	0			
sAat 2	Tmp	1	0			1
	Tmm	1	0			
MAat	Tmp	1	0			1
	Tmm	1	0			
Adh 1	Tmp	1	0			1
	Tmm	1	0			
Adh 2	Tmp	0.989	0.011			0.9979
	Tmm	0.929	0.071			
Est 1	Tmp	0.528	0.031	0.428	0.013	0.7772
	Tmm	0.948	0.052	0	0	
Est 2	Tmp	1	0			1
	Tmm	1	0			
Est 3	Tmp	1	0			1
	Tmm	1	0			
Est 4	Tmp	1	0			1
	Tmm	1	0			
Est 5	Tmp	1	0			1
	Tmm	1	0			
Est 6	Tmp	1	0			1
	Tmm	1	0			
Est 7	Tmp	1	0			1
	Tmm	1	0			
Est 8	Tmp	1	0			1
	Tmm	1	0			
Est 9	Tmp	1	0			1
	Tmm	1	0			
Gpt 1	Tmp	1	0			1
	Tmm	1	0			
Gpi 2	Tmp	1	0			1
	Tmm	1	0			
Ldh A	Tmp	0.968	0.032			1
	Tmm	0.982	0.018			
Ldh B	Tmp	1	0			1
	Tmm	1	0			
Ldh C	Tmp	0.675	0.325			0.9809
	Tmm	0.571	0.429			
sMdh 1	Tmp	0.955	0.045			0.9989
	Tmm	1	0			
sMdh 2	Tmp	1	0			1
	Tmm	1	0			
mMdh	Tmp	1	0			1
	Tmm	1	0			
sMep	Tmp	0.941	0.059			0.999
	Tmm	0.910	0.090			
Pgdh	Tmp	1	0			1
	Tmm	1	0			
Pgm 1	Tmp	1	0			1
	Tmm	1	0			
Pgm 2	Tmp	1	0			1
	Tmm	1	0			
Pt 1	Tmp	0.869	0.131			0.9913
	Tmm	0.982	0.018			
Pt 2	Tmp	0.154	0.297	0.549		0.9091
	Tmm	0.020	0.167	0.813		
Sod 1	Tmp	1	0			1
	Tmm	1	0			
Sod 2	Tmp	1	0			1
	Tmm	1	0			

Total value **0.0113**

* Loci with rare allele we assumed like monomorphic.

Sea scad we observed a rare allele *Est-2*^{B} (0,006-0,013) and *Est-2*^{C} (0,003). Similar electrophoretic variants were found also in Mediterranean scad.

GLUCOSE-6-PHOSPHATE ISOMERASE (EC 5.3.1.9-GPI). Two fractions with this enzyme activity, in samples from all investigated Black and Mediterranean Sea areas were vizualized. In our opinion these fractions were determined by two monomorphic loci.

ISOCITRATE DEHUDROGENASE (EC 1.1.1.42-mIDHP). A polymorphism with unclarified genetic control was detected.

LACTATE DEHYDROGENASE (EC 1.1.1.27-LDH). Both *Ldh-A* locus and *Ldh-C* loci were polymorphic (DOBROVOLOV, 1978; DOBROVOLOV & DOBROVOLOVA, 1983). The gene frequencies of *Ldh-A, Ldh-B* and *Ldh-C* of the Black Sea and Mediterranean Sea scad are presented in Table 1. An extremely rare allele *Ldh-B'* (0,005) was found in Black Sea scad.

MALATE DEHYDROGENASE (EC 1.1.1.37-MDH). Three fractions were visualized by starch gel electrophoresis. The results suggested the existence of two loci, one of which is polymorphic (*Mdh-1*^{B} allele frequency is 0,045) in Black Sea scad. The middle fraction was considered as a hybrid between loci. One fraction of mMDH with slow electrophoretical mobility was also observed.

MALIC ENZYME (EC 1.1.1.40-MEP). It was controlled by a single polymorphous locus (*sMep*). The allele frequency is presented in Table 1. We also observed one fraction (*mMEP*) which represents a rare allele in the two mentioned subspecies.

MUSCLE PROTEINS (PT) (Water-soluble proteins). Using horizontal starch-gel elecrophoresis a total of 21 fractions (4 polymorphic and 17 monomorphic zones) could be detected. IEF revealed 2 additional polymorphic zones of unclarified genetic control as well as three times more fractions, i.e., a total of 56 fractions. The gene frequencies of the polymorphic loci were in Hardy-Weinber equilibrium (DOBROVOLOV, 1977; DOBROVOLOV, 1988; DOBROVOLOV, DOBROVOLOVA, 1983). The allelic frequencies are presented in Table 1.

The genetic analysis demonstrated that two scad shoal groups migrate in the Bulgarian aquatory sector of the Black Sea: 1. *Pt-1*^{B} with mean allelic incidence rate of 0,070, and 2. *Pt-1*^{B} with mean rate of 0,131. These variations were more significant than the expected ones from subpopulation.

Sample analysis from the Sea of Marmara taken in winter demonstrated an allelic *Pt-1*^{B} with frequency 0,070, while the scad sample from the Caucasian seacoast was 0,125. Allelic frequency on the polymorphic loci *Pt-1* and *Pt-2* is given in Talbe 1.

PHOSPHOGLUCONATE DEHYDROGENASE (EC 1.1.1.44-PGDH). One fraction with common electrophoretic mobility of scad from all investigated areas of Black and Mediterranean Sea is observed. We assumed that this enzyme is determined by one monomorphic locus.

PHOSPHOGLUCOMUTASE (EC 5.4.2.2-PGM). Two fractions, which probably were determined by two loci, were visualized. These fractions were identical to the scad from all regions. *Pgm-1*^{B} (0,015) is a rare allele, which is found in Black Sea scad.

SUPEROXIDE DISMUTASE (EC 1.15.1.1-SOD). Two fraction of this enzyme activity were established. The presence of a rare allele *Sod 1*^{B} with frequency of 0,01 was found in Black Sea scad.

Discussion

ALEEV (1956) suggested that the Black Sea scad is a subspecies *Trachurus mediterraneus ponticus* Aleev, distinct from the Mediterranean one, i.e., *Tr.m.mediterraneus* Steindachner (1868). However, this intraspecies divergence required a sufficiently long-lasting isolation in the geological past and a sufficient time interval for independent evolution. The required time exceeds the period since the last connection of the

Black Sea through Bosporus and Dardanelles with the Mediterranean that happened 5000-10000 years ago. In our opinion, this period is extremely short for formation of two subspecies under natural conditions. Genetic distance after NEI (1972) between the Black Sea and the Mediterranean scad calculated on the basis of 32 genetic loci was 0,0109. If a proportionality coefficient of $c=5.10^6$ (after NEI, 1981) is used, a divergence time of $t=56500$ years is obtained. Even considering Sarich's coefficient (1977) of rapidly evolving proteins of $c=2{,}4.10^6$ it would result in $t=27120$ years. Taking into consideration the infix of genes from the Mediterranean to the Black Sea scad, the genetic distance should be larger. These facts suggest that *Tr.mediterraneus* entered during the carangate stage of the development of the Black Sea (with salinity rate of about 28 per thousand) terminated 26000-27000 years ago (DEGENS and ROSS, SEMENKO and KOVALYUKH -cited after GRIGORIEV & GOZHIK, 1976) not 70000-100000 years ago, as previously assumed.

Concerning the salinity reduction after Bosporus "closure", according to GRIGORIEV & GOZHIK (1976), it had not dropped below 8,5-9,0 per thousand in some regions at all. The waters in the north-western and northern parts of the Black Sea were obviously more desalinated due to flows from the Danube, the Dnepar, the Dnestar and other rivers while, however, the waters near the Bosporus and against Anatolia and Caucasus should have been, in our opinion of higher salinity.

Freezing during the last Glacial Period did not reach the Black Sea shores (EREMEEVA, 1965). Water temperature was probably and north-western ones. This allowed us to accept that the isolation of the Black Sea scad had happened during the aforementioned Carangate Period.

We suppose that both subspecies originated from common polymorphic population. As a consequence of long-lasting isolation and adaptation to the environment, there has been an elevation of the concentration of *Est-1*^{C} in the Black Sea scad and of the *Est-1*^{A} in the Mediterranean. On the other hand, the frequencies of *Est-1*^{D} and *Est-1*^{B} have remained lower in the Black Sea and the Mediterranean scad.

In present time, the frequent appearance of *Est-1*^{A} and *Est-1*^{B} in the Black Sea scad proves the influx of genes from the Mediterranean. Probably, this influx has started a long time ago as the frequency of *Est-1*^{A} of the Black Sea scad is rather high-of 0,528, while *Est-1*^{C} could be detected in any Mediterranean samples (except for these in the Aegean Sea). The influx of genes from the Mediterranean scad to the Black Sea one can be proved by the existence of the large scad. For the presence of the large scad in the Black Sea there has been reported in 1913 by S. A. ZERNOV (ALEEV, 1956). However, after that time this scad has disappeared, but it appeared again in the territorial waters of the USSR at Georgian shores in 1947 being intensively fished for 10 years. In 1954, draughts in the eastern parts of the Black Sea amount to 8601,7 tons (TIKHONOV *et al.*, 1955). Since 1958, only single specimens are found in the nets some of which are used in our electrophoretic analysis.

There are several hypotheses about the presence of the large scad in the Black Sea: a) the large scad is a new immigrant from the Mediterranean (ALEEV, 1956); b) it is the same small scad with accelerated growth under extremely favorable conditions (TIKHONOV *et al.*, 1955; SHAVERDOV, 1964); c) it is an ecological breed that hibernates in the warmest areas (ALEEV, 1957), or it is an ecotype (SHAVERDOV, 1964); d) it belongs to another species present in the Mediterranean or even in the Atlantic Ocean and in case of extremely high species numbers some shoals enter the Black Sea enlarging their nutritive territory (ALTUKHOV & SALMENKOVA, 1981); e) it is a polyploid form of the small scad originating in the Black Sea (GEORGIEV & KOLAROV, 1962); f) the "giant" scad is a new species *Trachurus gigas,* n.sp (BANARESCU, NALBANT, 1979).

According to SHAVERDOV (1964), both "large" and "small" forms of the Black Sea scad belong to one and the same subspecies established by ALEEV (1957). GOLOVKO (1964) studied the electrophoretic spectra of the serum proteins of these two scad forms. Based on that, SHULMAN & KULIKOVA (1966) reconsidered their own earlier assumption about the belonging of small and large scads to taxonomically close but different species. TKACHEVA (1957) performs crosses between small and large scad under field conditions on board a research motor boat, which showed the possibility to obtain hybrids. Until now, there does not exist any information confirming the polyploidity of the large form of scad. Chromosome numbers of the Black Sea scad are 2n=48 (VASILEV, 1978).

Our hypothesis (DOBROVOLOV, MANOLOV, 1983; DOBROVOLOV, DOBROVOLOVA, 1983; DOBROVOLOV, 1988) is based on biochemical genetic evidence. According to it, the large scad in the Black Sea is a case of heterosis resulting from crossing between two subspecies, *Tr.m.ponticus* and *Th.m.mediterraneus*. In our opinion, hybridization could have happened both in the Sea of Marmara and in the Black Sea.

Clarifying the natural phenomenon of the "large" scad is of theoretical and applied importance. Restoration of the population of the "large" scad in the Black Sea and the Mediterranean is quite possible. SHULMAN (1994, personal communication) has caught some large-scad specimens in the Aegean Sea. We tried to find biochemical genetic evidence. Two of a total of 66 specimens provided from the Aegean Sea (Thessaloniki) were heterozygous - $Est\text{-}1^A/Est\text{-}1^C$. The allelic incidence rate of $Est\text{-}1^C$ of 0,015 is a sufficient proof of gene influx from the Black Sea scad to the Aegean Sea one, while this allele does not occur in any other regions of the Mediterranean.

Specimens of size to 55 cm were not caught in the Eastern Mediterranean Sea. Nevertheless the "giant" scad is found in the Western Mediterranean Sea. In the samples, collected nearly Nice (France), a new allele -$Est\text{-}1^E$ was found (Fig. 1), characteristic only in this area. The presence of heterozygous individuals $Est\text{-}1^A/Est\text{-}1^E$ is the probable reason for the heterosis effect, similar to that in the Black Sea.

It is assumed that the presence of the large forms of scad in this area is a consequence also of the heterosis from the interbreeding of the population marked by $Est\text{-}1^A$ or $Est\text{-}1^B$ and the one marked by $Est\text{-}1^E$, inhabiting probably the area of Gibraltar-Atlantic Ocean (along the Spanish, Portuguese and French coast). We suppose that the scad (*Tr. mediterraneus)* from this region has diverged in distant geological times as a result of the isolation after the closing of the Gibraltar.

Acknowledgments

The author thanks G. Manolov and S. Volodin for kindly presented samples from different Mediterranean Sea waters.

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