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Biochemical genetic differentiation between *Pomatoschistus marmoratus* and *P. tortonesei*

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Several diagnostic genetic markers were identified in *Pomatoschistus marmoratus* and *P. tortonesei* using polyacrylamide gel electrophoresis (PAGE) of allozymes. Twenty-one loci were resolved, including the electrophoretic pattern of muscle proteins. The *MDH**, *PGM-1,2**, *EST-1,2**, *FUM** and *PGI-2** loci exhibited different alleles which were fixed for the two species being analysed. Genetic distance, as calculated by Nei's index, showed a value of 0·413. Environmental hypersalinity, could have influenced the geographical distribution of *P. tortonesei*.

Key words: allozymes; species differentiation; genetic distance; *Pomatoschistus*; Mediterranean Sea.

INTRODUCTION

In the Mediterranean region the Gobiidae includes over 50 species, most of which are marine or euryhaline forms. The *Pomatoschistus* genus is represented by eight euryhaline species which are small in body size (*c.* 10 cm L_T) and are distributed along the coasts, particularly in estuaries and lagoons (Tortonese, 1975). Systematic relationships among them are somewhat unclear. Small size and morphological similarities among the members of this genus have given rise to considerable taxonomic confusion (Gandolfi *et al.*, 1991; Wallis & Beardmore, 1984). This is particulary true for two Mediterranean species: *P. marmoratus* and *P. tortonesei*.

Pomatoschistus marmoratus is common on the Mediterranean and Atlantic coasts from the Gulf of Gascony to Morocco, while *P. tortonesei*, reported by Miller (1968) in the collection which is to be found in the Museo Civico di Storia Naturale in Genoa, is present in the Marsala Lagoon only (Western Sicily, Italy). Later, Miller (1981) reported *P. tortonesei* to be abundant in the Farwah Lagoon (Libya) near the Tunisian border.

The intraspecific variation in coloration, especially during the reproductive period (Mirto *et al.*, 1996), makes the two species morphologically very similar. Contrasting observations of colour variation during the reproductive period have also been reported (Bini, 1969; Miller, 1981). Mirto *et al.* (1996) observed an overlap in coloration between immature *P. tortonesei* females and *P. marmoratus* males from the Marsala Lagoon, whereas *P. tortonesei* showed a typical nuptial livery during the reproductive period.

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TABLE I. Enzymes stained with E.C. no. abbreviations and loci scored

Enzyme	E.C. no.	Abbreviation	Loci scored	Tissue
Sorbitol deydrogenase	1.1.1.14	SDH	*SDH**	L
Lactate dehydrogenase	1.1.1.27	LDH	*LDH-1,2,3**	E
Malate deydrogenase	1.1.1.37	MDH	*MDH**	E
Isocitrate deydrogenase	1.1.1.42	IDHP	*IDHP**	L
Glucose deydrogenase	1.1.1.47	GLDH	*GLDH**	L
Glucose-6-phosphate dehydrogenase	1.1.1.49	G6PD	*G6PD**	L, M
Xanthine dehydrogenase	1.2.1.37	XDH	*XDH**	L
Superoxide dismutase	1.15.1.1	SOD	*SOD**	L
Phosphoglucomutase	2.7.5.1.	PGM	*PGM-1,2**	M
Esterase	3.1.1.1.	EST	*EST-1,2**	L
Fructose-1,6-diphosphatase	3.1.3.11	FDP	*FDP**	L
Fumarate hydratase	4.2.1.2	FUM	*FUM**	E
Glucose phosphate isomerase	5.3.1.9	PGI	*PGI-1,2**	M
General muscle proteins	NA	PMMs	*PMMs-1,2,3**	M

NA, Not applicable, E, eye; L, liver; M, muscle.

So far, specific differentiation between *P. marmoratus* and *P. tortonesei* has been based only on morphological characters (sensory papillae, canal pores, number of scales, etc.) and has never been investigated from any genetic point of view.

Since genetic differences among species can be evaluated by allozymes (Altukhov, 1982; Carvalho *et al.*, 1991; Arculeo *et al.*, 1996), here, we attempt the genetic approach in order to describe specific differentiation between *P. marmoratus* and *P. tortonesei* from the Marsala Lagoon.

MATERIALS AND METHODS

Specimens (3–7 cm in length) of *P. marmoratus* ($n=18$) and *P. tortonesei* ($n=15$) were collected in the Marsala Lagoon (western Sicily, Italy) (37°48′ N, 12°27′ E) identified according to Tortonese (1975) and Miller (1968, 1981) and kept at −20° C until the removal of the organs.

Eye, liver and muscle were removed from the specimens, homogenized in NaCl 1% on ice, centrifuged at 25 000 ***g*** for 30 min at 4° C and the supernatants, which had been paper-filtered to remove the lipid layer, were stored immediately at −80° C.

Polyacrylamide gel electrophoresis (PAGE) was run according to Davis (1964) and performed in gel slabs. The sample, 1–5 µl in a 100-µl sample buffer, was layered into each well of the spacer gel and run vertically at a constant current of 40 mA. When examined for myogen patterns (*PMMs*), the gel was stained with Coomassie brilliant blue (Merril, 1990). The run buffers and staining procedure were in accordance with Cammarata *et al.* (1991) and Richardson *et al.* (1986). After electrophoresis, the gel slabs were incubated in the appropriate reagent mixture at 37° C until the enzyme bands were visualized; the reaction was stopped by rinsing with water and adding a preservative solution (7% acetic acid).

The detection of isoenzymes and the nomenclature of locus designation followed Shaklee *et al.* (1990) (Table I). Alleles were designated by their electrophoretic mobilities relative to the anodal mobility of the most common allele which was designated as *100*.

TABLE II. Allele frequencies in *Pomatoschistus tortonesei* and *P. marmoratus*

Loci	Alleles	*P. tortonesei*	P. marmoratus
*SDH**	*100	1	1
*LDH-1**	*100	1	1
*LDH-2**	*100	1	1
*LDH-3**	*100	1	1
*MDH**	*100	0	1
	*118	1	0
*IDHP**	*100	1	1
*GLDH**	*100	1	1
*G6PD**	*100	1	1
*XDH**	*100	1	1
*SOD**	*100	1	1
*PGM-1**	*98	0	1
	*100	1	0
*PGM-2**	*96	0	1
	*100	1	0
*EST-1**	*97	1	0
	*100	0	1
*EST-2**	*98	1	0
	*100	0	1
*FDP**	*100	1	1
*FUM**	*94	0	1
	*100	1	0
*PGI-1**	*45	0·07	0·20
	*100	0·86	0·80
	*121	0·07	0
*PGI-2**	*90	0	1
	*100	1	0
*PMM-1**	*100	1	1
*PMM-2**	*100	1	1
*PMM-3**	*100	1	1

A locus was considered as polymorphic within species when the frequency of the most common allele was <0·95. The genetic distance (*D*) between the species were calculated according to Nei (1978) using version 1·7 of the BIOSYS-1 package (Swofford & Selander, 1989).

RESULTS AND DISCUSSION

Eighteen loci were resolved by enzyme analysis and three from myogen proteins (Table I) in both species; the electrophoretic pattern of the myogenic loci (*PMMs**) did not show any differences between the species. *MDH**, *PGM-1,2**, *EST-1,2**, *FUM** and *PGI-2** loci were monomorphic and they exhibited different alleles fixed for the two species (Table II) (Fig. 1). Only one of the enzymatic loci (*PGI-1**) was polymorphic (Table II). *PGI-1** showed two alleles in *P. marmoratus* and three in *P. tortonesei*. The most common allele was the same in both species (Table II).

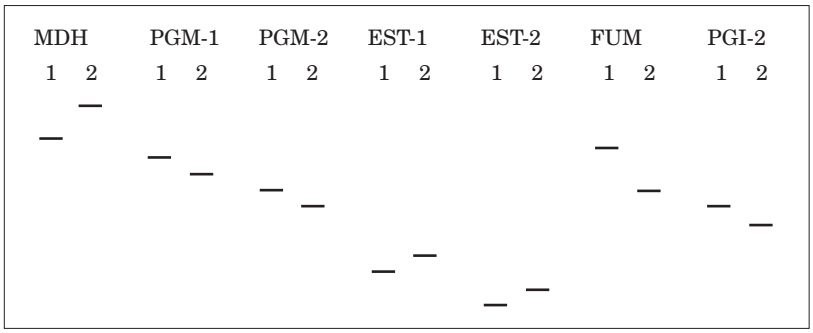

FIG. 1. Species-specific markers of monomorphic loci between *Pomatoschistus tortonesei* (1) and *P. marmoratus* (2).

Pomatoschistus marmoratus was among the species analysed electrophoretically by Wallis & Beardmore (1984) and Miller *et al.* (1994). However, differences in electrophoretic conditions and tissues used, and small sample sizes in those studies and this study [Adriatic (n=16), Veneto, Po Delta (n=6), and Sicily (n=18), respectively], impede the establishment of corresponding loci and alleles for those enzymes which were analysed in all three studies.

Thorpe (1982) and Shaklee *et al.* (1982) reported genetic distances between different marine fishes using electrophoretic data. The genetic distance (D of Nei, 1978) found here between *P. marmoratus* and *P. tortonesei* (D=0.413) corresponds to species level ($0.025<D<0{\cdot}609$). This taxonomic level is in accordance with the morphological and biochemical data and the presence of *P. tortonesei* in the Marsala Lagoon is confirmed.

Pomatoschistus tortonesei is known to live in the Marsala Lagoon (Sicily) and the Farwah Lagoon (Libya) (Miller, 1981). This species has not been reported as present in other western lagoons (Quignard *et al.*, 1984), in the eastern Mediterranean or along the coast of Israel (Ben-Tuvia, 1971), whereas the congeneric species, *P. marmoratus*, is common along the coasts of the Mediterranean Sea (Tortonese, 1975).

It is noteworthy that the Marsala and Farwah Lagoons share similar environmental conditions (Miller, 1981; Riggio & Chemello, 1992) and probably have a common geological origin (Incandela, 1995). Both are typical marine lagoons in the South Mediterranean and along the North African coasts (Riggio & Chemello, 1992). Salinity in the Marsala and Farwah Lagoons is 37–43‰ and exceeds that of the Mediterranean open sea water.

Miller *et al.* (1994) proposed that the separation of the monophyletic line of Gobiidae may have coincided with the Messinian salinity crisis which occurred in the late Miocene. *Pomatoschistus tortonesei* could have had its origin somewhat later during the Lago Mare phase of the early Pliocene when a hypersaline condition might have arisen. At present, the Marsala and Farwah populations could be relict populations confined to marine lagoons.

As suggested by Miller (1981), it is important to search for *P. tortonesei* in different areas of the Mediterranean coasts in order to clarify its precise distribution and degree of genetic variation.

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