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ISOZYMIC DIFFERENTIATION OF TWO SIBLING SPECIES OF *SQUATINA* (CHONDRICHTHYES) IN SOUTH BRAZIL

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Abstract—1. Two sympatric morphotypes of the nominal species *Squatina argentina* from South Brazil are compared by the frequencies of 25 isozymes distributed over 14 presumptive loci.

2. The Nei's Genetic Identity index obtained in a comparison of the populations is of 0.71.

3. The loci EST-2 and EST-4 are fixed at different isozymes for each population, indicating a reproductive isolation between them.

4. The most significant morphological and meristical differences between the morphotypes are the presence or absence of dorsal spines and the dental formula.

5. The agreement between these characteristics and the genetical data indicates that the two morphotypes belong to different species.

INTRODUCTION

The genus *Squatina*, the only one of the Order Squatinomorpha, comprises eleven recent species (Bigelow & Schroeder, 1948). It is a conservative group, with little morphological variation since the first fossil records from Upper Jurassic (Schaeffer, 1967), and is characterized by a great amount of DNA/nucleus in relation to other elasmobranchs and teleosts (Stingo, 1979; Stingo *et al.*, 1980). Based on morphological criteria, it has been considered that only one species (*Squatina argentina*, Marini, 1930) occurs in the Southern part of South America (Bigelow & Schroeder, 1948; Cousseau, 1973; Roux, 1976; Figueiredo, 1977) in opposition to the existence of two species claimed by Marini (1930, 1936). Population dynamics studies (Vooren, 1982) have shown the existence of two morphologically identifiable populations in South Brazil.

Biochemical systematics, based on genetic frequencies of allozymes (Avise, 1975, 1976; Thorpe, 1979) has been widely used in studies of populations (Wisemann *et al.*, 1978; Buth *et al.*, 1980) and in the detection and comparison of sibling species of teleosts (Gorman & Kim, 1977; Comparini & Rodino, 1980; Smith & Robertson, 1981) but has never been used for elasmobranch populations, for which estimates of mean heterozygosity are also lacking.

The aim of this work is to estimate the mean heterozygosity in *Squatina* populations and to verify the level of genetical identity between them in order to determine whether they actually belong to different species or not.

MATERIALS AND METHODS

Squatina argentina fishes of both sexes and all lengths were collected in 1981 during cruises of the O.V. "Atlantico Sul", between 30 and 34°S, from 20 to 120 m deep. They were immediately classified in morphotypes I and II according to the presence or absence of a row of 20–30 spines between the head and the first dorsal fin. Samples of brain, eye, axial white muscle, heart, kidney, liver, spleen and testis were taken on board from freshly killed mature fishes of both morphotypes and immediately frozen at −20°C. For classification purposes, the teeth of both jaws of all fishes were counted, the shape of the nasal curtains recorded and 24 body proportions measured after Cousseau (1973). The differences between the body proportions of the two morphotypes were subjected to a Student's *t*-test. Enzymes were extracted at the laboratory by homogenizing the tissues in one volume of 0.02 M Tris–HCl buffer (pH 7.5) for 30 sec at 0°C with a Polytron homogenizer. The extracts were centrifuged for 20 min at 0°C and 20,000 rev/min and stored at −20°C. Horizontal electrophoresis was carried out at 4°C either on Cellulose Acetate plates (Cellogel–Chemetron) or on 7% polyacrylamide gels. Four buffer systems, after Brewer (1970) and Toledo & Magalhães (1973) were tested for each enzyme on each tissue. Visualization of the isozymes was achieved after Brewer (1970) and Shaw & Prasad (1970) but using Thiazolyl Blue Tetrazolium (MTT) instead of Nitroblue Tetrazolium (NBT). Enzymes nomenclature and abbreviations follow Toledo & Magalhães (1973). Identification of each allele was made by assigning the value 100 to the isozyme of greatest mobility, and relative values for the slower ones.

RESULTS AND DISCUSSION

The extracts of liver, heart and white yield the best resolution, and were selected for the observation of the enzymes activities (Table 1).

The number of teeth of the upper jaw was predominantly 20–22 in morphotype I (84 analysed specimens) and 18 in morphotype II (58 analysed specimens). The lower jaw had 20–22 teeth in both morphotypes. The length of the pectoral fin and the size of the head were significantly greater in morphotype II, all other body proportions were similar in both morphotypes. The observed differences alone seemed to be not sufficient for identification purposes, and all obtained values were within the usually accepted range of variation for *Squatina argentina* (Cousseau, 1973; Bigelow &

Table 1. Selected electrophoretical conditions for the analysed enzymes

Enzyme	E.C.	Support	Buffer*	Tissues
ADH	1.1.1.1	Cellulose acetate	I	Liver
AAT	2.6.1.1	Cellulose acetate	I	Liver
AlPH	3.1.3.1	Polyacrylamide	II	Heart
EST	—	Polyacrylamide	III	Heart, liver
GDH	1.4.1.3	Cellulose acetate	I	Liver
LDH	1.1.1.27	Polyacrylamide	II	Heart, muscle
MDH	1.1.1.37	Polyacrylamide	II	Heart, muscle
ME	1.1.1.40	Polyacrylamide	II	Heart
SOD	1.15.1.1	Polyacrylamide	IV	Heart, liver

* I, Veronal pH 8.7 (Brewer, 1970); II, Hist–Citrate pH 8.0 (Brewer, 1970); III, Tris–citrate pH 8.7 (gel) and NaOH–borate pH 8.1 (bridge) (Toledo & Magalhães, 1975); IV, Tris–borate pH 9.0 (Toledo & Magalhães, 1973).

Schroeder, 1948). The shape of the nasal curtains, claimed by Roux (1976) as the most important character for identifying *Squatina* species, was identical in both morphotypes.

The loci EST-3, AAT-1, AAT-2 and GDH were polymorphic in both morphotypes (Fig. 1 and Table 2), the locus ADH was polymorphic only in morphotype II. The proportion of polymorphic loci and the mean heterozygosities are thus respectively 0.286 and 0.130 for morphotype I and 0.367 and 0.164 for mor-

Table 2. Allozyme frequencies of *Squatina* populations

Locus	Alleles	Morphotype I	Morphotype II
AAT-1	−100*	0.40	0.67
	−82*	0.60	0.33
	n	6	6
AAT-2	100	0.63	0.31
	85	0.37	0.69
	n	13	16
ADH	100	0.00	0.14
	71	1.00	0.14
	57	0.00	0.72
	n	6	10
AlPH	100	1.00	1.00
	n	15	26
EST-1	100	1.00	1.00
	n	15	26
EST-2	100	1.00	0.00
	94	0.00	1.00
	n	15	26
EST-3	100	0.00	0.42
	95	0.46	0.00
	89	0.00	0.58
	84	0.54	0.00
	n	15	26
EST-4	100	0.00	1.00
	92	1.00	0.00
	n	15	26
GDH	100	0.75	0.43
	90	0.25	0.57
	n	18	29
LDH	100	1.00	1.00
	n	36	50
MDH	100	1.00	1.00
	n	15	26
ME	100	1.00	1.00
	n	15	26
SOD-1	100	1.00	1.00
	n	26	47
SOD-2	100	1.00	1.00
	n	26	47

* Migrating toward the anode.
n, number of analysed individuals.

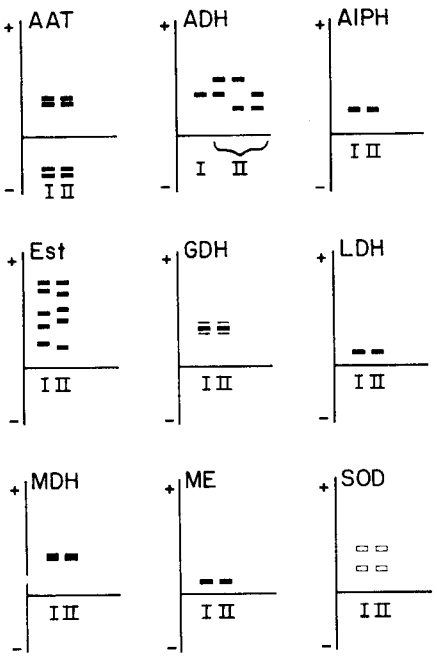

Fig. 1. Observed patterns of the studied enzymes. In polimorphic loci, the heterozygous pattern is presented.

photype II. These values are slightly higher than the ones generally reported for teleosts (Selander, 1976).

The observed genotypic proportions for the polymorphic loci are in accordance to the expected proportions for populations in the Hardy–Weinberg equilibrium (Dobzhansky *et al.*, 1980), showing that each morphotype constitutes a genetical unit, as suggested by Vooren (1982). The enzymes ADH, EST-3, AAT-1 and AAT-2 seem to have a monomeric structure, since that the heterozygotes presented only two bands. GDH presents a dimeric structure, with 3-banded heterozygotes. All polymorphic loci but ADH showed to be diallelic. ADH was triallelic in morphotype II.

The Nei's Identity (Nei, 1972) and the Thorpe's Similarity (Thorpe, 1979) indexes between both morphotypes are 0.71 (genical distance, $D = 0.34$) and 0.66 ($D = 0.34$) respectively. These values are as the ones generally found in the comparison of different species of the same genus (Thorpe, 1979; Avise, 1976). Furthermore, the EST-2 and EST-4 loci are fixed at different alleles in each population, showing that there is a true reproductive isolation between them. It may be concluded that the nominal species *Squatina argentina* of the South-western Atlantic Ocean includes at least two different species, which may be differentiated principally by the dental formula, presence of spines in the dorsal line and Esterases pattern from heart extracts. These features coincide with important differences between parameters of population dynamics (Vooren, 1982).

The low morphological differentiation maintained after a considerable biochemical divergence is in accordance with the great specialization (Compagno, 1977) and conservativeness of the genus (Stingo, 1979), which comprises few species and is antique, following probably the genetical differentiation model I of Avise & Ayala (1975).

As the currently used systematic diagnostic characteristics did not distinguish the studied species, it is suggested that a review of the systematics of the genus be made.

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