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ELECTROPHORETIC CHARACTERIZATION OF A HYBRID BETWEEN *ERETMOCHELYS IMBRICATA* AND *CARETTA CARETTA* (CHELONIIDAE)

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Abstract—1. An intermediate morphotype between *Eretmochelys imbricata* and *Caretta caretta* was studied in Praia do Forte, Bahia, Brazil.

2. Three enzymatic systems were successfully analyzed: SOD, lactate dehydrogenase (LDH) and esterase (EST). Isoelectric focusing of total soluble proteins of muscle and transferrin were shown.

3. Esterase exhibited nine phenotype patterns, seven in *C. caretta* and one in the others morphotypes. SOD phenotypes were identical in the three morphotypes. Lactate dehydrogenase and transferrins were characteristic for each species.

4. Jaccard's measure of similarity was calculated and a phenogram with the three morphotypes were constructed using isoelectric focusing of total soluble proteins.

INTRODUCTION

Off the northeast Brazilian coast the sea turtles *Eretmochelys imbricata* and *Caretta caretta* spawn. The species are identified according to the following characteristics: *E. imbricata* has a depressed oval carapace, medium-sized head, scutes on shell imbricated, and four pairs of lateral scutes on the carapace with the anterior pair not touching the pre-central scute, called "Hawksbill" due to a strong horny beak. *Caretta caretta* has a heart-shaped carapace, head rather long and very broad and five pairs of lateral scutes with the anterior pair touching the pre-central scute.

The spawning season of these species is summer, although peaks of spawning occur in different moments for each species. The occurrence of spawns was observed in Praia do Forte (Bahia) with the following intermediate characteristics: heart-shaped carapace and head similar to *C. caretta*; four pairs of lateral scutes as described for *E. imbricata*. This set of characteristics suggested that these individuals could be hybrids between *C. caretta* and *E. imbricata* (Marcovaldi, 1987) (Fig. 1).

Lewis (1940) reported (from the Cayman Islands) the existence of a hybrid between *E. imbricata* and *C. caretta* called "McQueggie". Fishermen have confirmed that such a hybrid exists as they had often seen and captured *Eretmochelys* males which were copulating with *Caretta* females. Hendrickson (1980) has considered the results of a hybrid cross between *Chelonia mydas* and *Eretmochelys imbricata* in a Cayman turtle farm. Kamezaki (1983) reported hybrids from an *Eretmochelys* and *Caretta* cross from eggs deposited on the Chita Peninsula, Japan.

Isozyme expression is a valuable taxonomic tool to characterize hybrids between different species and it is used by many authors, e.g. Danzmann and Down (1982), Avise and Sauders (1984) and Vonwyl (1983). The aim of this work is to characterize the particular pair of species involved in the hybridization event and identify the possible hybrid.

MATERIALS AND METHODS

Fifteen juvenile specimens of each turtle species were sampled in February 1989 during the spawning season of these species on the Sea Turtle Project (TAMAR) in Bahia (Brazil). They were immediately identified as *Caretta caretta*, *Eretmochelys imbricata* or as an intermediate morphotype between them, according to morphological characters such as the number of lateral plates, presence of nape plate, form of the carapace and size of the head.

Blood samples were obtained and serum was separated and usually frozen until used for electrophoretic transferrin runs. Staining procedures for transferrin suggested by Mueller *et al.* (1962) were used.

Liver and skeletal muscle extracts were prepared by mixing each sample with an equal vol of 0.02 M Tris–HCl pH 7.0 buffer and mechanically homogenized for 30 sec at 0°C. The extracts were centrifuged at 10,000 rpm, 0°C for 10 min and stored at −20°C for no longer than 1 month. The supernatant fractions were subjected to vertical electrophoresis using 10% polyacrylamide gels according to Shaklee and Keenan (1986). The enzyme staining procedures employed in this study were similar to those described by Brewer (1970).

Isoelectric focusing of total soluble proteins of muscle and serum transferrin was performed using 7% polyacrylamide gels containing Ampholine (Pharmacia) pH 3.5–10.0. Gels were prepared as described by Levy *et al.* (1983) and then run at 4°C for 6 hr at a maximum power of 3 W. Fixation, staining and preservation of gels were performed according to Lundstron (1980) and Levy *et al.* (1983).

Jaccard's measure of similarity using presence and absence of isoelectric focusing bands was used to construct a phenogram (UPGMA) with the turtle samples.

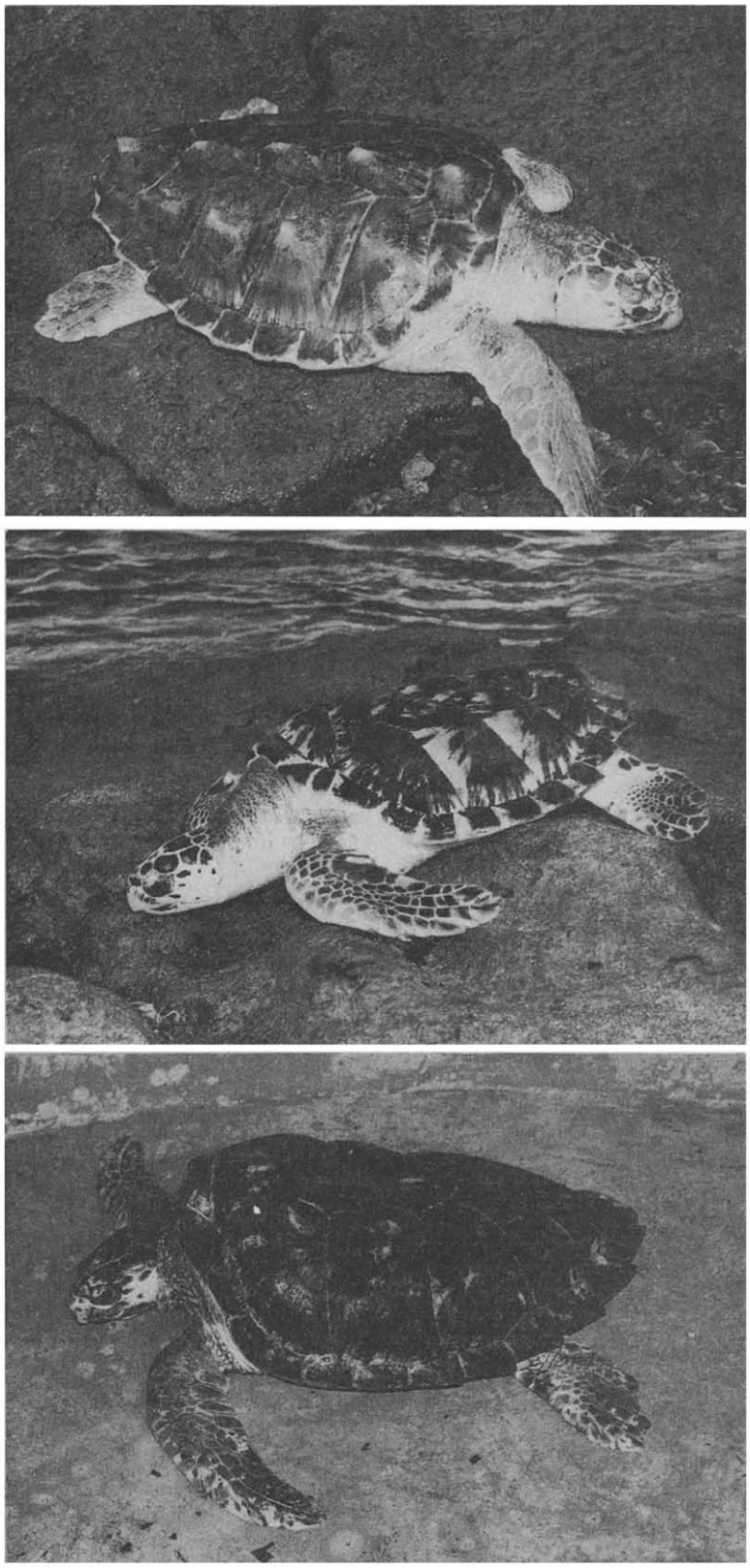

Fig. 1. Specimens observed in Praia do Forte (Bahia), Brazil, (a) *Caretta caretta*, (b) *Eretmochelys imbricata* and (c) hybrid.

RESULTS

Several enzymatic systems were tested but only SOD, LDH and Est were successfully analyzed for the sea turtles studied (Fig. 2). SOD phenotypes were identical between the morphotypes analyzed, showing a single band between them. Esterase showed a high variation making a satisfactory interpretation of patterns difficult. Thus, frequencies of phenotypes rather than those of alleles were used in analyses. Esterase exhibited nine phenotype patterns among which seven were present in *C. caretta*, one in the hybrid and another in *E. imbricata*. The patterns of LDH were characteristic for each species and the hybrid pattern was equal to that of *C. caretta*.

Transferrin patterns showed two alleles, Tf1 and Tf2. Tf1 was present only in *C. caretta* and Tf2 only in *E. imbricata*, while the hybrid pattern showed these two alleles having a codominant pattern.

The soluble proteins analyzed by isoelectric focusing showed evident differences between the patterns of the three marine turtles, *C. caretta*, *E. imbricata* and the hybrid (Fig. 3). Forty-one bands were resolved for the species and with them Jaccard's coefficient of similarity was calculated and a phenogram (UPGMA) was constructed (Fig. 4). The phenogram showed higher similarity between *E. imbricata* and the hybrid than between *C. caretta* and the hybrid.

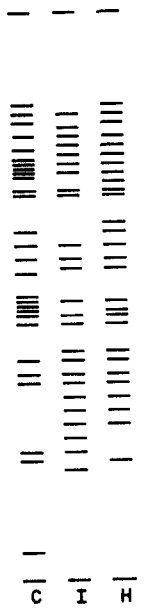

Fig. 3. Isoelectric focusing patterns of total soluble protein in (C) *Caretta caretta*, (H) hybrid and (I) *Eretmochelys imbricata*.

DISCUSSION

According to the biological species concept of Mayr (1970), a species is reproductively isolated, i.e. there is no gene flow between two different species. Hybrids have always been the object of intense interest particularly because they are sometimes seen as antithetical to the biological species concept (Ferguson, 1980). In these cases, hybrids are morphologically intermediate between the parental species, and there is no difficulty in recognizing them as such. Other indicators often used are chromosome structure and number (Moran *et al.*, 1980) or behaviour (Littlejohn *et al.*, 1971). Bickham and Carr (1983) studying a karyology of the Cheloniidae family, showed it to possess $2n = 56$, but other diploid numbers were reported by Nakamura (1937, 1949) for *C. caretta*.

Enzymes were analyzed through frequencies of phenotypes because the individuals sampled were juveniles and we did not do an ontogenetic analysis.

In SOD both hybridizing species and the hybrid had identical mobilities. When the hybridizing species had particular electrophoretic mobilities and individual polymorphism the hybrid had a codominant phenotype. This was observed in Est and transferrin phenotypes. Lucotte and Dubouch (1980) obtained similar results with experimental hybrids between *Papio anubis* and *P. cynocephalus*. Despite our small sample size we found high polymorphism in Est for *C. caretta*. New studies with more enzyme systems should be made for discussing it.

Despite the difficulties in establishing the taxonomic significance of general protein patterns, isoelectric focusing of total protein was shown to be characteristic for each group.

Frair (1982) showed through serum electrophoresis and immunoelectrophoresis that all sea turtles had similar migrating lines and that some intraspecific variability occurred. He postulated that sea turtles

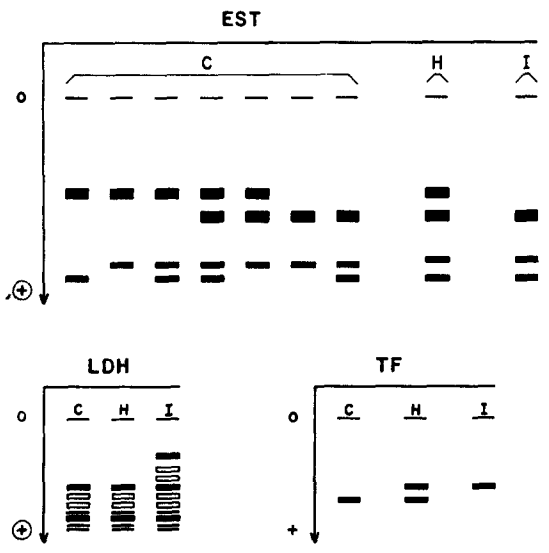

Fig. 2. Phenotypic patterns observed of lactate dehydrogenase (LDH), esterase (EST) and transferrin (Tf) in (C) *Caretta caretta*, (H) hybrid and (I) *Eretmochelys imbricata*.

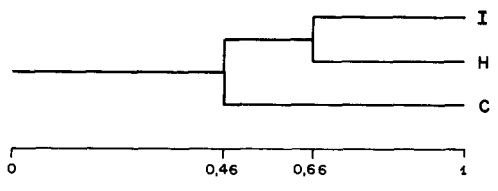

Fig. 4. Phenogram (UPGMA) with Jaccard's similarity of isoelectric focusing pattern in (C) *Caretta caretta*, (H) hybrid and (I) *Eretmochelys imbricata*.

constitute a natural group which should be included together in a rank no higher than family.

Our data contribute to this postulate by confirming the presence of a natural hybrid between *C. caretta* and *E. imbricata*. We do not have concrete data on the frequency of hybrid occurrence but its presence shows the high genetic similarity between these species. According to Philipp *et al.* (1983) and Frankel (1987) morphological abnormalities and aberrant expression of allelic isozymes increase concomitantly with greater genetic distance from parental species.

These data are congruent with the reports of Lewis (1940), Hendrickson (1980) and Kamezaki (1983) and agree with the idea of existence of hybridization between these species.

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