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Genetic characterisation of *Epinephelus marginatus* through cytogenetic, allozyme and microsatellite analyses: preliminary results

*Caractérisation génétique d'*Epinephelus marginatus *par analyses cytogénétique, enzymatique et des microsatellites : résultats préliminaires*

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ABSTRACT

Sola L., S. Papalia, A.R. Rossi, E. Gornung, S. De Innocentiis, G. Marino, P. Di Marco, S. Cataudella, 1999 - Genetic characterisation of Epinephelus marginatus *through cytogenetic, allozyme and microsatellite analyses: preliminary results.* Mar. Life*, **9** (1) : 67-68.*

Karyological, allozyme and microsatellite surveys of Mediterranean populations of the dusky grouper, Epinephelus marginatus*, were undertaken, in order to contribute to the genetic characterization of the species, which is of use for the proper planning of restocking programmes. Samples have been so far collected along the coast of Cagliari (Sardinia, Central Thyrrenian Sea), Messina (Sicily, South Thyrrenian Sea), Porto Cesareo (Apulia, Ionian Sea) and Lampedusa (African continental shelf, Mediterranean Sea). All specimens show a 48 acrocentric chromosomes karyotype and Ag- and CMA_3-NORs located in subcentromeric position of the smallest chromosome pair. However, after* in situ *hybridization (FISH,) with a 18S rDNA probe, additional NORs on chromosome pair number 2 were found in specimens from Sardinia. The $(TTAGGG)_n$ telomeric sequences, identified by FISH, were found to be restricted to the telomeres and no interstitial sites were detected. Starch gel electrophoresis conditions for 32 isozyme loci, which encode 22 enzymes, were developed. At least 10 of these enzyme loci show more than one allele. PCR amplifications were carried out on* E. marginatus *total DNA extracts, using primers originally designed to amplify 7 microsatellite loci in* Dicentrarchus labrax *(Moronidae). Five of these primer pairs gave PCR products.*

RÉSUMÉ

*Sola L., S. Papalia, A.R. Rossi, E. Gornung, S. De Innocentiis, G. Marino, P. Di Marco, S. Cataudella, 1999 - [Caractérisation génétique d'*Epinephelus marginatus *par analyses cytogénétique, enzymatique et des microsatellites : résultats préliminaires].* Mar. Life*, **9** (1) : 67-68.*

*Des analyses génétiques (caryotypes, allozymes et microsatellites) ont été réalisées sur des populations méditerranéennes de mérou (*Epinephelus marginatus*) en vue de mettre en place des programmes de repeuplement. Les poissons ont été échantillonnés en mer Thyrrénienne centrale (Cagliari, Sardaigne), et sud (Messine, Sicile), en mer Ionienne (Porto Cesareo, Apulia) et à Lampedouse (Méditerranée sud, plateau continental africain). Tous les individus possèdent un karyotype de 48 chromosomes acrocentriques, les Ag- et CMA_3-NORs étant situés dans la région subcentromérique de la plus petite paire de chromosomes. Cependant, par hybridation* in situ *(FISH) d'une sonde ADNr 18S, des NORs supplémentaires ont été mis en évidence sur la deuxième paire de chromosomes des individus de Sardaigne. Les séquences télomériques $(TTAGGG)_n$, identifiées par FISH, sont limitées aux télomères et aucun site interstitiel n'a été détecté. Les conditions d'électrophorèse enzymatique sur gel d'amidon ont été mises au point pour 32 loci codant pour 22 enzymes. Au moins 10 de ces loci allozymiques montrent plus d'un allèle. Des amplifications PCR ont été réalisées à partir d'ADN total d'*E. marginatus*, à l'aide de 7 paires d'amorces initialement définies chez le loup (*Dicentrarchus labrax*, Moronidae) pour la détection des microsatellites. Cinq de ces paires ont donné des produits d'amplification.*

In order to contribute to the genetic characterisation of the dusky grouper, *Epinephelus marginatus*, karyological, allozyme and microsatellite surveys were undertaken, aimed at analysing the genetic variability and the degree of genetic structuration in the species and at identifying possible population-specific genetic molecular markers.

Specimens of *E. marginatus* were collected along the coast of Cagliari (SEM, Central Thyrrenian Sea), Messina (MEM, South Thyrrenian Sea), Porto Cesareo (BEM, Ionian Sea) and Lampedusa (LEM, Mediterranean Sea). Somatic metaphases were prepared using conventional air-drying techniques. Ag-staining, C-banding and fluorescent staining with chromomycin A_3 (CMA_3) and DAPI were carried out as reported in Sola *et al*. (1992). For FISH, a 2 kb fragment of human 18S rDNA and a $(TTAGGG)_7$ repeat were used as probes (Lawrence *et al*., 1988).

Allozymes were detected by horizontal starch gel electrophoresis and visualised using enzyme specific stains. Alleles at each locus were designated by their anodic mobilities (x 100) relative to the most frequent allele in the LEM population. Nuclear DNA from 93 specimens was extracted from muscle as described in Maniatis *et al*. (1982) and PCR amplifications were carried out using primers originally designed to amplify 7 microsatellite loci in *Dicentrarchus labrax* (Moronidae) (Garcia de Leòn *et al*., 1995).

In the populations investigated (SEM, MEM and LEM), the chromosome complement is composed of 48 acrocentric chromosomes uniformly decreasing in size. Chromosome pair number 24 can be easily identified because of its smallest size and because it is unique in showing differential staining. Indeed, two Ag- and CMA_3-positive NORs can be observed in subcentromeric position of this chromosome pair. At the same location a slightly negative heteropycnosis after DAPI-staining and C-positive signals after C-banding are observed. The constitutive hetero-chromatin shows centromeric distribution. Except for NORs, neither eu- or heterochromatin show diffe-rential increase of AT- or GC-rich DNA. In all populations, the $(TTAGGG)_n$ telomeric sequences are restricted to the telomeres and no additional interstitial sites were detected.

In specimens from MEM and LEM, FISH with 18S rDNA detects a single location of NORs on chromosome pair number 24 whereas, in specimens from SEM, additional, though weaker, hybridization signals on telomeric regions of both homologues of chromosome pair 2 can be observed. Thus, chromosome pair 24 is likely the species-specific NOR-bearing chromosome pair, but additional and variable NORs may exist.

A subset of 60 individuals from SEM, LEM and BEM populations have so far been assayed for genetic variation at 32 gene loci, which encode 22 enzymes. Ten enzyme loci show more than one allele. Levels of genetic variability seem high enough to investigate genetic structure and gene flow among populations.

Five of the *D. labrax* primers pairs used gave PCR products in *E. marginatus*. However, most of these microsatellite loci show a lower level of polymorphism in the dusky grouper than in the source species.

On the basis of these preliminary results, both chromosomes, allozymes and microsatellites seem to be promising genetic markers to investigate dusky grouper population structure.

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