GENETIC STRUCTURE OF FOUR MARINE SPECIES FROM THE GULF OF PAGASITIKOS (GREECE) BASED ON ALLOZYMES, RAPD AND MTDNA MARKERS

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Abstract

In the present work we used three molecular techniques (allozymes, RAPDs and mtDNA RFLPs) in order to study the genetic structure of four marine species (*Mullus surmuletus*, *Mullus barbatus*, *Merluccius merluccius* and *Pagellus erythrinus*). Each species was sampled from three regions of the Gulf of Pagasitikos as well from two neighbour regions outside the Gulf (Trikeri and Allonissos). Values of genetic heterozygosity and genetic diversity found for all populations studied were above the mean values observed in marine fishes. None of the three methods used reveal diagnostic patterns which could allow the identification of individuals to one of the populations. The results revealed that the three populations within Pagasitikos were homogenous representing thus a panmictic stock. However, there were evidences of genetic population subdivision between localities from inside and outside of the Pagasitikos Gulf.

Keywords : Aegean Sea, Fishes, Genetics.

Introduction

Gene flow through larval exchange is assumed to be the major mechanism in homogenizing spatially discrete marine populations. However, several studies of marine species have illustrated examples where apparently panmictic or neighbor populations demonstrate surprisingly high levels of genetic structuring. Genetic structuring in marine populations might be caused by several factors such as by differences in local selection pressures, or by local hydrographic conditions 1. The Gulf of Pagasitikos (west central Aegean Sea) presents particular interests and advantages for such kind of analyses due to local topographical and managerial peculiarities of the area i.e. it is a shallow, semi-enclosed basin where trawling is by low prohibited. The work presented here is part of a larger research project aiming on understanding the structure, function and dynamics of the Pagasitikos Gulf ecosystem, with a final target to achieve its sustainable management. It is well established that for a proper fishery management knowledge of the genetic population structure is important [2]. In the present study we examined both nuclear (allozymes and RAPDs) and mitochondrial DNA (mtDNA) variation in collections of four commercial marine species made in five different regions within and outside the Gulf of Pagasitikos with the goal of describing their genetic population structure.

Materials and Methods

The four studied species were red mullet (*Mullus barbatus* L.) striped red mullet (*Mullus surmuletus* L), European hake (*Merluccius merluccius*) and common pandora (*Pagellus erythrinus*) . An average of 200 individuals of each species were collected at five locations (average 40 individuals per location), three from the Pagasitikos Gulf and two from neighbour areas that is from Trikeri and Allonissos. Samples of white muscle, liver and eye were taken from each individual and stored at -40^{0}C until further treatment. Allozyme analysis was carried out employing standard horizontal starch-gel electrophoresis. Fourteen enzymic systems coding for a total of 20 (16 in *P. erythrinus*) putative loci were analysed for each species except for hake where 12 loci were analysed. The electrophoretic data were analyzed using BIOSYS-1 [3]. For the purposes of DNA analyses, DNA was extracted from muscle tissue following protocols reported in Mamouris et al [4]. RAPD analyses were performed using initially 40 decamer primers, 5-10 of which were used for intraspecific analyses. Experimental conditions as well as data analyses for the RAPD method are documented in Mamuris et al [4]. Finally, mitochondrial DNA variation was analysed by restriction fragment length polymorphisms (RFLPs) performed on four PCR-amplified mtDNA regions: control region (D-loop), COI, 12S-16S rRNA and cytochrome b. The amplified segments from each specimen were subsequently screened for polymorphism with 15 restriction endonucleases. The restriction site pattern data was analysed using the REAP [5] computer packages. N_{ST} [6] was used to estimate the degree of population subdivision at the nucleotide level within each species.

Results and Discussion

Mean heterozygosity is considered the most important way of measuring genetic variation. The results of the present study revealed that the values of heterozygosities and nucleotide diversities found from allozyme/RAPD and mtDNA analyses, respectively, were above the average values found for other marine teleosts using the same methods. For example the values of the observed heterozygosity found from allozyme analyses range from 0.062 to 0.137 while the average for all marine teleosts has been estimated as 0.064 [7]. The large values of heterozygosities imply that the populations studied have had a long unbroken history in the area without population bottlenecks.

None of the three methods used for each of the four species studied succeed to yield any diagnostic marker that could lead to unambiguous identification of the various geographical populations. Regardless of the method used, genetic population subdivision was not evident for any species within the Pagasitikos Gulf, suggesting homogeneity within the Gulf. On the other hand, there was some evidence of genetic population subdivision between localities from within and outside of the Pagasitikos Gulf. The estimated values (through allozyme and RAPD analyses) of effective migration rates (N_em) for the populations sampled range from 6.2 to 9.7 depending of the method used. These values were much smaller than the mean value for marine fishes (N_em=22.76) but they were close to those estimated for other marine species in the Mediterranean Sea [1]. These values of effective migration rates were small enough to allow significant divergence of gene frequencies among samples, although large enough to counteract the effect of genetic drift if one assumes an infinite island model.

References

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