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Marine biogeography of the Mediterranean Sea: patterns and dynamics of biodiversity

Molecular markers and geographic variation in Mediterranean fish

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SUMMARY

Mediterranean Sea shows high level of species richness especially if we consider its relative small area, indeed it is only 0.82 % in surface area of the world ocean. Fish fauna also is represented by a consistent number of species, being constituted by 540 species, 52 of them being endemic. Several population genetic studies have been carried out on different species distributed in Mediterranean Sea and in adjacent Atlantic coasts. Analysis of available genetic data from 19 fish species revealed on one hand high genetic homogeneity and on the other hand, the existence of species subdivided in discrete groups. Among geographically structured species consistent genetic structure and low levels of gene flow are found between populations inhabiting the east and the west side of the Gibraltar Strait. In other cases, genetic discontinuity was observed between the eastern and western basins within Mediterranean Sea. Geographic distance between populations seems to have an important role in determining such differentiation, even if, in some cases, natural selection is the evolutionary force determining the level of genetic population structure. Genetic data provide evidence to consider the Mediterranean basin as a definite biogeographic province, different from the Northeast Atlantic and subdivided in eastern and western regions, even if a clear phylogenetic break has rarely been observed.

INTRODUCTION

Mediterranean Sea shows high level of species richness especially if we consider its relative small area, indeed it is only 0.82 % in surface area of the world ocean (Defant, 1961). Endemic species are also significantly present (Tortonese, 1985). In particular, in Mediterranean Sea are present 6.3% of marine world species and endemic species represent 25% (Bianchi and Morri, 2000). The causes of this high biodiversity have to be looked for in the complex geological history of the Mediterranean.

During the most part of the Tertiary, Mediterranean Sea represented a part of the Tethys Sea that was connected both to Atlantic and to Indopacific regions and typically inhabited also by tropical fauna (Mars, 1963; Peres et Picard, 1964). Subsequently, communication with the Indopacific region was

interrupted and most of tropical fauna disappeared. Approximately 5.5 million years ago, at beginning of Messinian, communication with Atlantic Ocean was closed and the Mediterranean Sea was converted into a series of saline lakes. This event caused the extinction of the most species inhabiting Mediterranean (Por, 1989; Por and Dimentman, 1985; Selli, 1985). Communication with Atlantic Ocean was re-established several hundred thousand years later, with the opening of the Strait of Gibraltar, in the early Pliocene (about 5 million years ago). This allowed migration of Atlantic species in Mediterranean basin (Sarà, 1985; Por, 1989). At the end of Pliocene with the opening of Dardanelles and Bosporus Straits, a connection between Black and Aegean Seas was established (Tortonese, 1985). Since this period, several glaciations' events occurred which caused the interruption of communication between the two seas that were connected again during interglacial periods (Sarà, 1985).

The alternation of the ice ages with the warm interglacial during the whole Quaternary resulted in different immigration waves of Atlantic fauna of boreal or subtropical origin. Classically, we can distinguish several groups of species populating the Mediterranean and belonging to different biogeographic categories: glacial migrants, tropical migrants, temperate migrants, Red Sea migrants and endemic species, probably representing Tethys fauna relicts (Quignard, 1978).

The Mediterranean Sea is divided into two great basins, the west basin and the east one, separated by a shallow sill running from Sicily to Tunisia. West basin appears to be richer in floral and fauna diversity than the east one and species abundance also decreases from west to east. This is probably due to the fact that warmer and more saline waters are found in eastern Mediterranean. This feature is uncongenial to the majority of Atlantic derived species. On the other hand, some of the recent Lessepsian immigrants from the Red Sea find the warm saline waters of the East basin a suitable environment and are slowly spreading westwards. There are also some differences in species numbers and abundance from north to south, being the northern waters richer than the southern ones. Such differences can be explained, again, with the increasing of water temperature, proceeding from north to south.

Keeping in mind this picture and considering that Mediterranean fauna is a mix of tropical and boreal fauna, could the Mediterranean Sea be considered as a defined biogeographic province, or is it an arm of the Northeast Atlantic? How many biogeographic provinces can we distinguish within the Mediterranean Sea?

A way to answer to these questions could be found in studying the genetic and phylogeographic structure of species, using molecular markers.

Molecular markers, allowing direct comparisons of relative levels of genetic differentiation, are powerful tool to study genetic diversity in any groups of

organisms. They may provide useful information at different levels: patterns of historical biogeography, phylogenetic relationships, population structure and levels of gene flow. In particular, by partitioning genetic diversity among populations it is possible to obtain information on the factors shaping their evolution, i.e., founder effect, gene flow, genetic drift and selection. By estimating the levels and patterns of genetic differentiation, we can infer phylogeny and therefore establish the direction of colonization and the geographic origin of the ancestral population. In this paper, I attempt to review results from different studies carried out on the genetic structure of Mediterranean fish species.

GENETIC MARKERS

Since, to some extent the patterns of genetic structuring are depending on the kind of genetic marker used, it seems advisable to summarize some of the most frequently used genetic markers.

Allozymes. They are defined as one of several forms of an enzyme, which are the products of alternative alleles segregating at a locus within a species. The method used to detect allozymes takes advantage of the fact that proteins with different net charge migrate, in an electric field, at different rates through a supporting media such as starch and acrylamide gels or cellulose acetate strips. Allozymes are codominant markers, inherited in Mendelian way. Heterozygotes are distinguishable from homozygotes and the calculation of gene frequencies is rather easy. They have been used extensively to describe the genetic structure of populations, are particularly useful at the level of conspecific populations, and closely related species. The main disadvantages are the low abundance and the low level of polymorphism, it is also clear that the allozymes do not represent a random sample of genomes and thus may bias some population genetic inferences, they, indeed, reflect variability in protein coding regions and may thus be selectively constrained.

Restriction fragment length polymorphism (RFLP). RFLPs are the identification of specific restriction enzymes that reveal a pattern difference between the DNA fragment sizes in individual organisms. A number of enzymes have been isolated that recognize specific, short (4-6 base pairs) DNA sequences and cut the DNA within these regions. Resulting fragments are separated according to their molecular size using gel electrophoresis. The size of each fragment is measured by its mobility relative to known standards and the sum of fragment sizes equals the size of the cut sequence. Differences in fragment length result from base substitutions, additions, deletions or sequence rearrangements within restriction enzyme recognition sequences. As allozymes, RFLPs have been extensively used at the level of conspecific populations and closely related species.

Polymerase chain reaction (PCR). Discover of PCR technique (Saiki et al., 1988) induced a methodological revolution in population biology. This method allows producing large quantities of a particular DNA sequence for study. The target sequence is identified by a specific pair of DNA primers (oligonucleotides usually about 20 nucleotides in length). The quantities of produced DNA are sufficient to be directly visualised on a gel by fluorescence after coloration with stains such as ethidium bromide. The main interest for population biology is that it is now possible to work with a very small initial amount of DNA. It is possible to work on museum samples and even on relatively young fossils. Amplified DNA may then be manipulated with other techniques for several applications. It is, now, relatively easy to sequence the target DNA region or analyse polymorphisms at different levels of genome DNA.

Microsatellites. Microsatellites are short sequences, arranged in tandem arrays of repeated sequences of no more than six/eight-base long. They are especially found in non-coding DNA regions and can vary both at level of nucleotide sequence due to mutation and at level of the number of repeats, due to unequal crossing over and slippage replication. They are highly polymorphic, having high mutation rates. Because their intrinsic nature, microsatellite loci are increasingly replacing or complementing other molecular markers for numerous applications in evolutionary biology, from population genetic to the parentage and pedigree analyses.

Random Amplified Polymorphic DNA (RAPD). RAPD markers are fragment of DNA, distributed everywhere in the genome, amplified by short arbitrary sequences of nucleotides. Large number of fragments is produced by this technique (William et al., 1990). They are inherited in Mendelian way and have been increasingly employed for population studies in recent years, especially in plant biology, where it is simple to verify inheritance of amplified fragments and the reproducibility of technique. The main disadvantage is the fact that, generally, RAPDs are dominant markers, this characteristic limits inferences from RAPDs and may bias some population genetic parameters when compared with codominant, multiallelic markers (Lynch and Milligan, 1994).

Amplified fragment length polymorphism (AFLP). This approach includes both the restriction digestion and PCR (Vos et al., 1995). A large number of polymorphic DNA markers are detected, rapidly and in a reproducible manner. AFLP reveals presence or absence of restriction fragments rather than differences in their length.

DNA sequencing. Target DNA region, amplified by PCR, may be directly sequenced. By this technique, it is possible to obtain the most comprehensive

information, as we analyse polymorphism at the source level of variation. In many taxa several DNA coding regions are conserved and flanked by non-conserved spacer regions. On the basis of this characteristic, it is possible to use "universal" primers (i.e., primers annealing the same region in different taxa; Kocher et al., 1989) that make possible the sequencing of several genes in different phyla.

GENETIC DIFFERENTIATION IN MEDITERRANEAN FISH

As with other taxa, fish species diversity appears to be high in the Mediterranean Sea. Cartilaginous and bony fish species occur at a percentage equal to 9.5% and 4.1%, of the world marine species, respectively (Bianchi and Morri, 2000).

Generally speaking, the sea is a suitable environment for gene flow, and marine organisms are expected to show little genetic subdivision between geographically separated populations. Gene flow in such environment occurs at different developmental stages, by the passive transport of larvae at larval stage and/or by active adult migration at adult stage. These movements contribute to maintaining the genetic homogeneity of populations also at a large geographic scale or to generate clines, where genetic differentiation increases with geographic distance.

On the other hand, gene flow can be insufficient to counteract differentiation caused by genetic drift and/or local selective pressures. In these cases, significant heterogeneity among populations geographically close can be found.

To compare different patterns of species' subdivision in Mediterranean fish three different biogeographic windows (i.e., Northeast Atlantic Ocean, West Mediterranean Basin and East Mediterranean Basin) and the F_{ST} index (Wrigth, 1943, 1951, 1965) were considered (see also Borsa et al., 1997). This index measures genetic heterogeneity among populations within species, expressing the proportion of genetic variation due to differences among populations. It is inversely correlated to gene flow, i.e., high F_{ST} values correspond to low level of gene flow.

Table I summarizes results from several studies, carried out using different molecular markers. F_{ST} values are dependent both on the degree of detected polymorphism and on the genetic marker employed; in particular, the number of genetic variants expressed by each marker is fundamental in determining the order of magnitude of the value itself. Observing the F_{ST} values reported in Table I, with respect to the marker used, differences are evident especially between microsatellites and allozymes. Generally, microsatellite loci have a number of alleles much higher than allozymes resulting in lower F_{ST} values (Hedrick, 1999). On the other hand, allozymes may be selectively constrained, providing higher F_{ST} values. Although F_{ST} values obtained by using this two markers differ by one order of magnitude, results are relatively comparable (Gratton et al., 2003) .

Genetic differentiation within and between biogeographic regions appears to be correlated to the life habits of the species considered; as expected, demersal species are more differentiated than the pelagic ones, the former showing higher F_{ST} values.

Tab. I - F_{ST} index for the analyzed fish species, by considering different geographic windows.

TAXON	Genetic marker	NE Atl.	West Med.	East Med.	NE Atl. West Med	West/ Med East Med	References
PERCIFORMES							
Dicentrarchus labrax	allozymes (G_{st})	–	–	–	0.145	0.079	Benharrat et al., 1983
Dicentrarchus labrax	allozymes (θ)	–	0.231	0.382	0.401	0.339	Allegrucci et al., 1997
Dicentrarchus labrax	microsatellites (θ)	0.003	0.008	–	0.023	0.013	Naciri et al., 1999
Dicentrarchus labrax	microsatellites (θ)	0.012	NS	0.017	–	0.020	Bahri-Sfar et al., 2000
Dicentrarchus punctatus	microsatellites (θ)	0.081	–	0.014	0.122	0.131	Bonhomme et al., 2002
Epinephelus marginatus	microsatellites (θ)	–	–	–	–	0.018	De Innocentiis et al., 2001
Epinephelus marginatus	allozymes (θ)	–	–	–	–	0.060 0.214	De Innocentiis et al., 2001
Mugil cephalus	mtDNA (G_{st})	–	–	–	0.249	0.005	Crosetti et al., 1994
Mugil cephalus	allozymes (G_{st})	–	–	–	0.163	0.024	Rossi et al., 1998
Mullus surmuletus	allozymes (G_{st})	–	–	0.020	–	0.035	Mamuris et al., 1999
Mullus surmuletus	RAPD (G_{st})	–	–	0.024	–	0.053	De Metrio, 1995
Thunnus thynnus	allozymes (G_{st})	–	0.029	–	0.033	0.002	Bargelloni et al., 2003
Dentex dentex	allozymes (θ)	–	–	0.005	0.952	0.011	Bargelloni et al., 2003
Lithognathus mormyrus	allozymes (θ)	–	–	0.007	0.817	–0.006	Bargelloni et al., 2003
Pagrus pagrus	allozymes (θ)	–	–	–	0.135	–	Bargelloni et al., 2003
Pagellus bogaraveo	allozymes (θ)	–	–	0.005	–0.006	0.068 0.011	Bargelloni et al., 2003
Xiphias gladius	mtDNA (G_{st})	–	–	–	0.247	–0.004	Kotoulas et al., 1995b
Xiphias gladius	allozymes (G_{st})	–	0.007	0.007	0.020	0.013	Pujolar et al., 2002
GADIFORMES							
Merluccius merluccius	allozymes (G_{st})	0.001	–0.014	–	0.035	0.012	Pla et al., 1989; Roldan, 1995
Merluccius merluccius	microsatellites (θ)	0.013	–	–	0.026	0.003	Lundy et al., 1999
Trisopterus minutus	allozymes (G_{st})	–0.051	–	–	0.304	–	Tirard et al., 1992
Trisopterus minutus	allozymes (θ)	0.014	NS	–	NS	NS	Mattiangeli et al., 2000
PLEURONECTIFORMES							
Pleuronectes flesus	allozymes (G_{st})	0.047	–0.164	0.047	0.152	0.528	Borsa et al., 1997
Psetta maxima	allozymes (G_{st})	–0.101	–	–	0.063	0.031	Blanquer et al., 1992
Scophthalmus rhombus	allozymes (G_{st})	–0.328	0.105	–	0.099	–	Blanquer et al., 1992
Solea vulgaris	allozymes (θ)	–0.001	–0.000	–	0.020	0.045	Kotoulas et al., 1995a
CLUPEIFORMES							
Engraulis encrasicolus	allozymes (G_{st})	–	0.003	–	–	–	Tudela et al., 1999
Sardinella aurita	mtDNA (θ)	0	–0.091	–	0.490	–	Chikhi et al., 1997

Within Mediterranean basin, among the demersal species, sea bass (*Dicentrarchus labrax* and *D. punctatus*, Benharrat et al., 1983; Allegrucci et al., 1997; Bonhomme et al., 2002), dusky grouper (*Epinephelus marginatus*, De Innocentiis et al., 2001) and flounder (*Platichtys flesus*, Borsa et al., 1997) show significant high values of F_{ST}, suggesting consistent differentiation between eastern and western populations. Other species such as *Mullus surmuletus* (Mamuris et al., 1999) and *Xiphias gladius* (Pujolar et al., 2002) show, generally, low genetic differentiation, but with higher F_{ST} values in comparisons including eastern and western populations.

The highest F_{ST} values are found when comparing Northeast Atlantic and West Mediterranean, revealing high genetic discontinuity between the two seas. This is the case of *Mugil cephalus* (Crosetti et al., 1994; Rossi et al., 1998), *Dentex dentex*, *Lithognathus mormyrus* (Bargelloni et al., 2003) that show high genetic differentiation both at mtDNA and allozyme level. Also, populations belonging to *Dicentrarchus labrax* and *D. punctatus* are highly genetically differentiated, showing, in all cases, significant values of F_{ST} (Benharrat et al. 1983; Allegrucci et al., 1997; Naciri et al., 1999; Bonhomme et al. 2002).

Even when genetic differentiation is low, the species genetic structure is more defined between Atlantic and Mediterranean comparisons than within the whole Mediterranean (see, for example, *Psetta maxima*, Blanquer et al., 1992 or *Merluccius merluccius*, Pla et al., 1989; Roldan, 1995; Lundy et al., 1999). However, some exceptions are represented, among sparid species, by *Pagrus pagrus* and *Pagellus bogaraveo* (Bargelloni et al., 2003) and, among soleid species, by *Solea vulgaris* (Kotoulas et al., 1995a).

Allozyme and mtDNA data from sparid species reveal moderate differentiation for *P. pagrus* and no differentiation for *P. bogaraveo* (Bargelloni et al. 2003). The lack of genetic differentiation between atlantic and mediterranean populations of *P. pagrus* could be explained as a consequence of a strong bottleneck and a subsequent expansion, in accordance with the hypothesis of extinction-recolonization. On the other hand, the distribution range of *P. bogaraveo*, occurring only in the western-most part of the Mediterranean basin, might indicate that the presence of this species in the Mediterranean has been established only recently (Bargelloni et al., 2003).

As far as *Solea vulgaris* is concerned, this is a demersal species with a pelagic larval stage, which shows more differentiation within the entire Mediterranean basin (F_{ST} = 0.045) than between Northeast Atlantic and West Mediterranean (F_{ST} = 0.020). Regression test between genetic and geographic population distance suggests that isolation by distance could explain the observed pattern of geographic variation (Kotoulas et al., 1995a). Genetic differentiation, in this case, is mostly attributable to historical factors and has been interpreted as a long-term equilibrium between gene flow and genetic drift (Slatkin, 1993).

On the other hand, the low genetic structure revealed by *T. thynnus* does not seem to follow a geographical gradient, but to reflect the biological habits of this species, adapted to migrate over large geographic distances (De Metrio, 1995). On the contrary, swordfish, *Xiphias gladius*, another pelagic species, shows high differentiation between Atlantic and Mediterranean populations, as revealed by a study analysing RFLPs at mtDNA level (F_{ST} = 0.247, Kotoulas et al., 1995b). However, this outcome contrasts with results obtained by Pujolar et al. (2002), where allozyme analysis highlights no significant differentiation between Atlantic and Mediterranean populations (F_{ST} = 0.020).

Molecular markers may be informative also in revealing morphologically indistinguishable, cryptic species. This is for example the case of the anchovy, *Engraulis encrasicolus*, and of the dusky grouper *Epinephelus marginatus*. These two species have been studied in some detail, using different genetic markers that revealed unexpected aspects of their biology.

The European anchovy, *Engraulis encrasicolus*, is a small pelagic fish distributed in the Mediterranean and Black Seas, along the Atlantic coasts of North Africa and Europe up to the British Isles. It is a euryhaline species able to tolerate high salinity ranges. Different population genetic studies carried out on this species (Pasteur and Berrebi, 1985; Spanakis et al., 1989; Bembo et al., 1996; Magoulas et al., 1996; Tudela, 1999; Tudela et al., 1999) evidenced unusually high levels of genetic heterogeneity. Such heterogeneity was revealed by morphology, allozymes and mitochondrial DNA. In a review paper, Borsa (2002) highlighted the presence of two distinct forms, one constituted by open-sea or oceanic populations and one by inshore-water populations that are both present in the Gulf of Lion and in the Adriatic Sea. One species inhabits the inshore waters of the two regions, the other one is found offshore in the oceanic waters of the Biscay Gulf, the western Mediterranean, the central and southern Adriatic Sea and the Ionian Sea. At allozyme level, open sea anchovy populations are genetically different from inshore-water ones within the same region (F_{ST} = 0.035-0.067; Borsa, 2002), while genetic differentiation between geographically distant populations belonging to the same form is rather weak (F_{ST} = 0.005-0.006; Borsa, 2002). These results are confirmed by mitochondrial DNA haplotype frequencies distribution. Moreover, the existence of a third distinct form in Aegean Sea and in Sicily may be hypothesized on the basis of preliminary data from morphometry, allozymes and mtDNA haplotypes (Spanakis et al., 1989; Borsa, 2002).

The dusky grouper, *Epinephelus marginatus*, is a rocky-bottom associated species inhabiting coastal reefs from shallow water out to a depth of 50 m along all Mediterranean coasts and on both sides of the Atlantic Ocean (Heemstra and Randall, 1993). The dusky grouper was considered to be fairly common in the Mediterranean but its abundance has appreciably decreased so that it is now classified as a "vulnerable" species and recently listed as an endangered species

(ECNC, 1998). Genetic studies have been carried out on this species to assess phylogenetic relationships between west Mediterranean populations (Gilles et al., 2000) and to analyse the genetic population structure in the west-central Mediterranean (De Innocentis et al., 2001).

Partial sequencing of *cytochrome b* from mtDNA genome revealed the existence of two distinct forms within *E. marginatus*, one including French, Algerian and Tunisian populations and one another Algerian population. The two forms are highly genetically differentiated in spite of their close morphological similarity (Gilles et al., 2000).

Genetic variation analysed both with seven microsatellites and 28 allozyme loci in populations coming from west-central Mediterranean revealed significant genetic differentiation among all populations, suggesting strong population genetic structure. No evidence of isolation by distance was found. These results suggest that dusky groupers are not panmictic in the Mediterranean Sea and they should be managed on local geographic scale (De Innocentiis et al., 2001).

The case of European Sea Bass, *Dicentrarchus labrax*

The European sea bass, *Dicentrarchus labrax* (L., 1758) (Teleost; Perciforms, Moronidae) is a widespread euryhaline and eurythermic fish species distributed in the northeastern Atlantic Ocean and in the Mediterranean Sea (Tortonese, 1986a, 1986b). Its life cycle is rather complex. Spawning occurs in the open sea, fertilized eggs hatch between 4 and 9 days, depending on the water temperature (Pickett and Pawson, 1994). Within one month of hatching, bass larvae move inshore (Jennings and Pawson, 1992), where they stay until the post-larval stage is reached. At this age (generally 2-3 months), they begin to migrate actively into juvenile nursery habitats in brackish waters (Barnabé, 1980). Here, developmental accomplishment occurs. Juvenile and adolescent sea bass make seasonal movements, which are restricted in relation to the geographical location of their nursery area (Pickett and Pawson, 1994). Sexual maturity occurs at age 4-5 years. During the autumn adults move between summer feeding areas and winter pre-spawning areas and in the spring back to the summer areas.

D. labrax is the object of several genetic studies carried out in our laboratory, aimed at the comprehension of evolutionary mechanisms underlying a huge array of genetic variation as seldom observed in a finfish (Allegrucci et al., 1997). For the past ten years we have been analyzing the distribution of genetic variability in reared and wild populations of sea bass *D. labrax* using different genetic markers, such as allozymes (Allegrucci et al., 1994, 1997), RAPDs (Allegrucci et al., 1995; Caccone et al., 1997) and mitochondrial DNA (mtDNA; Cecconi et al., 1993, 1995; Venanzetti et al., 1994; Cesaroni et al., 1997; Allegrucci et al., 1998, 1999).

Over two subsequent years (1989, 1990) we monitored the changes in genetic variation in reared samples before and after acclimation to freshwater. Both allozyme and RAPD markers showed that survival to acclimation to freshwater was not random. Survival rates and relative fitnesses per genotype per locus were estimated and in both years the same genotypes at certain loci showed the highest probability of survival (Allegrucci et al., 1994; 1995). Subsequently, in order to evaluate whether the genetic variation in sea bass populations could account for adaptation, we studied allozyme and RAPD variation in wild populations from the Mediterranean Sea, sampled in either open sea or coastal lagoons. Results suggested that, at least for those loci, which showed shifts in allele frequencies between starting and acclimated samples, the levels of genetic differentiation were more correlated to ecological differences among populations rather than to their geographic distance. In addition, neutral markers, revealed from both techniques, suggested that the Mediterranean populations are clustered according to a northwest-southeast transect (Allegrucci et al., 1997, Caccone et al., 1997). Similar results were obtained with microsatellite studies where a genetic transition in the Siculo-Tunisian strait was observed (Bahri-Sfar et al., 2000). Based on both the results, the hypothesis was made that the whole western Mediterranean is likely to harbour a single panmictic population (Garcia de Leon et al., 1997; Naciri et al., 1999).

In parallel with the analysis of genetic variation expressed by nuclear markers, we studied genetic variation in mtDNA. The same populations were analyzed both for D-loop length variation (Allegrucci et al., 1998, Cesaroni et al., 1997) and for cytochrome *b* sequencing variation (Allegrucci et al., 1999).

Length variation of the mtDNA control region revealed high gene diversity, especially among individuals within the same population, and high levels of heteroplasmy, due to variation in number of repeats of two repeats arrays. Although the diversity among populations was rather low, it was still possible to identify a phylogeographical component to the genetic divergence between populations. Also in this case, Mediterranean sea bass populations clustered according to a northwest-southeast transect (Cesaroni et al., 1997).

The cytochrome *b* gene has been completely sequenced in eight Mediterranean populations of *D. labrax* and in one of *D. punctatus* (Allegrucci et al., 1999). Despite overall low levels of sequence variation, these sequence data revealed a substructuring of the Mediterranean populations, which suggests a clear geographical partitioning of the genetic variants.

In short, these studies revealed two types of genetic markers: "neutral" markers which are capable to trace the phylogeography of sea bass populations and markers "under selection" which indicate strong genetic structuring among populations, with a percentage of overall diversity sorted according to ecological criteria.

How could this diversity be maintained? This species is able to migrate over large geographic distance and shows a complex life cycle, with larvae actively

migrating into the lagoons, where they complete their development, and young sub-adults and adults returning to their nursery area. On this basis, gene flow should play a remarkable role and we should observe a higher homogeneity than that resulted. The observed differentiation between marine and lagoon samples might be a simple by-product of differential survival of genotypes in lagoons, but could also result from differential migration between lagoons and mating areas and/or assortative mating.

To elucidate on the role of the different evolution forces in maintaining the observed polymorphism, fish at different age were analyzed using allozyme, RAPD and D-loop markers. Fish were captured in subsequent years from larval stage to adult one and collections were made so that it was possible to analyze fish belonging to the same cohort. Results from all the three molecular markers indicated greater genotype diversity in juvenile stages than in the adolescent or adult ones, suggesting that some open sea-like genotypes may occur in the lagoon as post-larvae and/or juveniles. Actually, adolescents or adults always show lagoon-like genotypes. We suggest that both habitat choice and natural selection are involved in shaping the complex genetic structure of sea bass populations (Allegrucci et al., in prep.).

CONCLUSIONS

Biogeographic relationships of Northeast Atlantic and West Mediterranean marine fauna are rather complex, as revealed by genetic studies on several fish species, reported in this paper. From the morphological point of view, very similar biotas populate the adjacent coasts of the two basins, so Tortonese (1964) and Ekman (1968) concluded that the two regions form together the Atlantic-Mediterranean province. On the other hand, Quignard (1978) proposed that the Strait of Gibraltar could be considered the borderline between two marine biogeographical "regions", the North-East Atlantic and the Mediterranean Sea. These hypotheses have been investigated by studying several marine species, occurring in both regions, and analysed by means of molecular markers. Evidences for both hypotheses are not so clear; a variety of organism show intraspecific genetic variation, but many other species show no genetic differentiation between Atlantic and Mediterranean populations. It seems, therefore, that only a portion of species, presently occurring in both Atlantic and Mediterranean, experienced an interruption in gene flow between adjacent populations. These discordant phylogeographical results across different taxa could be explained by historical causes that could have determined differences in dispersal capability, in sensitivity to selective factors or to geographic barriers, or in other ecological/demographical factors such as effective population size (Avise, 1998). Interestingly, a similar situation is observed among populations of fish species inhabiting the east and west side of Mediterranean basin. Also in this case,

the genetic interruption coincides with the shallow sill running from Sicily to Tunisia, which, as the Gibraltar Strait, could be considered a geographic barrier.

In conclusion, on the basis of available genetic data, it is not possible to define clearly two marine biogeographical regions, the North-East Atlantic and the Mediterranean Sea, also, it is not so clear if the Mediterranean basin can be biogeographically subdivided in eastern and western regions, even if strong evidence for these hypotheses have been provided. I hope, however, that genetic data reviewed in this paper provide relevant information to be considered in the general context of this conference.

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