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Genetic population structure of *Epinephelus marginatus* (Pisces, Serranidae) revealed by two molecular markers

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Abstract
In this paper, data are presented regarding the population structure of dusky grouper (*Epinephelus marginatus*) which was sampled in the Mediterranean Sea and Atlantic Ocean using two different molecular markers, ND2 RFLP and cytochrome b (cyt b) sequence analysis. The main objective of the study is to analyse the genetic variability of *E. marginatus* in the Mediterranean Sea to define how the biological characteristics of the species, such as the dispersal capability of pelagic larvae and the benthonic-sedentary life style, can affect the genetic population structure or maintaining the gene flow or determining genetic differences. Furthermore, we considered an Atlantic sample to establish the presence of genetic differences between the two biogeographical areas under consideration. Analysis of variation based on ND2 RFLP analysis revealed a genetic difference between the Atlantic and Mediterranean samples, whilst cyt b sequence analysis did not. There exists evidence for genetic differentiation for both molecular markers in the Mediterranean region. The majority of the variation was due to the Ustica Island sample and this could be due to differences in fisheries effort since the island is a protected area where recreational and professional fisheries are almost completely forbidden. The pattern of genetic diversity, haplotype networks and mismatch distribution suggest a historical influence on the genetic structure of *E. marginatus* and recent population expansion. An analysis of the genetic structure of the dusky grouper will be the prerequisite for decisions regarding conservation and fisheries management.

Keywords: *Dusky grouper, cyt b, ND2 RFLP, genetic population structure*

Introduction

The dusky grouper (*Epinephelus marginatus*, Lowe 1834) is one of the most important littoral fish along Mediterranean coasts, and it is variously considered the *flag species* of Mediterranean marine protected areas. It occurs on both sides of the Atlantic Ocean, throughout the Mediterranean Sea and on African coasts close to Mozambique and Madagascar. Adults are known to be associated with rocky reef bottoms and caverns in coastal reefs from shallow waters out to a depth of 50 m.

Considerable information has been published relating to the age, growth, reproduction and behaviour of the dusky grouper and it is reputed to be sedentary and territorial. It is a progynous hermaphrodite in which sex reversal seems to occur when individuals are 14–17 years old and about 80–90 cm long (total length) (Bruslé 1985). The dusky grouper displays a long reproductive period (from June to August), when the dominant male establishes territories where it displays its aggressive behaviour towards neighbouring males (Barnabè 1974; Zabala et al. 1997). In these areas the fish form a large spawning aggregation and much of the southern Mediterranean commercial catch is taken during the summer at specific sites which are well known by local fishermen.

The social behaviour particularly concerning polygyny and the social structure of the dusky grouper is common to other groupers, even if the dusky grouper does not seasonally migrate towards a definite site, as happens with tropical and subtropical groupers. Dusky groupers seem to be distributed patchily over relatively large areas and they do not participate in mass movements towards reproductive sites like other tropical and subtropical groupers. Furthermore, strong homing and site fidelity in

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adults and juveniles has been demonstrated (Lembo et al. 1999, 2002) and only the relatively long-lived pelagic larvae of the dusky grouper may contribute along the wide distribution gene flow. Indeed, in the marine environment, a high potential for dispersal and the absence of strong barriers to migration are believed to ensure genetic homogeneity even between distant populations. Conversely, there is increasing evidence that effective dispersal in the sea might not be as high as expected (Palumbi 2000). With different scientific approaches, Swearer et al. (1999) and Jones et al. (1999) have demonstrated that the larvae of reef fish are often retained by ocean currents in the same location where they are spawned. The dominance of very large Mediterranean dusky grouper individuals and the lack of juveniles might suggest that dusky grouper populations are not self-recruiting and that the populations are renewed by the movement of adult fishes.

The dusky grouper is characterised by slow growth, late reproduction, a large size and a long life span, all of which make it vulnerable to over-exploitation. Although specific fishery statistics for this species are not available, *E. marginatus* is believed to be over-exploited along Mediterranean coasts. In recent years, a constant decrease in dusky groupers along Mediterranean coasts has been noted and in past decades there was a dramatic reduction of north western Mediterranean stocks (Chauvet & Francour 1989). For example, the annual commercial catches along the Spanish coast decreased from over 200 tonnes in the 1970s to almost nothing in the 1990s (Gracia 1996). A high density of dusky groupers can be encountered only in marine protected areas where commercial and recreational fishery effort is very low or completely absent. This has led to the listing of the dusky grouper as an endangered species (ECNC 1998).

More data regarding population structure and levels of genetic variation are important for formulating programmes for biological conservation and resource management. To date, there exist two studies describing the genetic population structure of *E. marginatus*. De Innocentiis et al. (2001) have compared two molecular markers, allozymes and microsatellites, revealing that Mediterranean dusky grouper populations are non panmittic, additionally Gilles et al. (2000), analysing cytochrome b sequences, have suggested the presence of a cryptic species along the Algerian coasts.

New data on the genetic variation of *E. marginatus* which were analysed with two different molecular markers, ND2 RFLP analysis and cytochrome b sequence analysis, will be presented in this paper. The main objective was to study the genetic population structure of dusky grouper in the Mediterranean Sea, identifying discrete populations or groups with a more or less restricted gene flow and to determine whether this species can disperse over large distances or whether larval dispersion is limited, thereby determining genetic differences in the Mediterranean Sea. Furthermore, an Atlantic sample was also considered in order to determine if there exists genetic differences between these two biogeographical areas. Data relating to genetic variations provided information on demographical processes, population history and selection; this information is very important in exploited species where interactions between exploitation and natural processes may intensify the vulnerability of the species under consideration. Moreover, results on genetic variation will provide information pointing to practical guidelines for the design of an appropriate programme of management and conservation along Mediterranean coasts, especially in marine protected areas.

Materials and methods

Sampling and DNA extraction

Specimens of dusky grouper were collected from four Mediterranean locations and one Atlantic. These sites were Annaba (Algeria), Ustica and Lampedusa (Italy), Thessaloniki (Greece) and the Azores (Figure 1). Fish were purchased locally from markets or taken directly from reefs. A small piece of caudal fin from each specimen was preserved in ethanol (70–90%) or frozen at -20°C and the total genomic DNA was extracted from small section of tissue (caudal fin or muscle) using a DNeasy Tissue Kit (QIAGEN). The extracted DNA was suspended in distilled water and stored at -20°C until required.

RFLP analysis

A part of the mtDNA gene, NADH dehydrogenase subunit 2 (ND2), was amplified using primers L4440 5′-AAAGCTTTCGGGCCCATACCCCG-3′ and ND2 interH 5′-GTRAGTRYGGGGGY-TTTTGCYCA-3′ Y being Pyrimidine (T or C) and R Purine (A or G) (Yagishita et al. 2001). PCR was carried out in a Perkin Elmer Cetus Thermal cycler in a 100 µl solution containing 1 ng genomic DNA, 0.2 µM each dNTPs, 0.1 µM each primer, 10 mM Tris–HCl (pH 9), 50 mM KCl, 1.5 mM $MgCl_2$ and 2.5 units of Perkin Elmer Ampli*Taq* Gold polymerase. The thermal cycling profile began at 94°C for 10 min as a hot start,

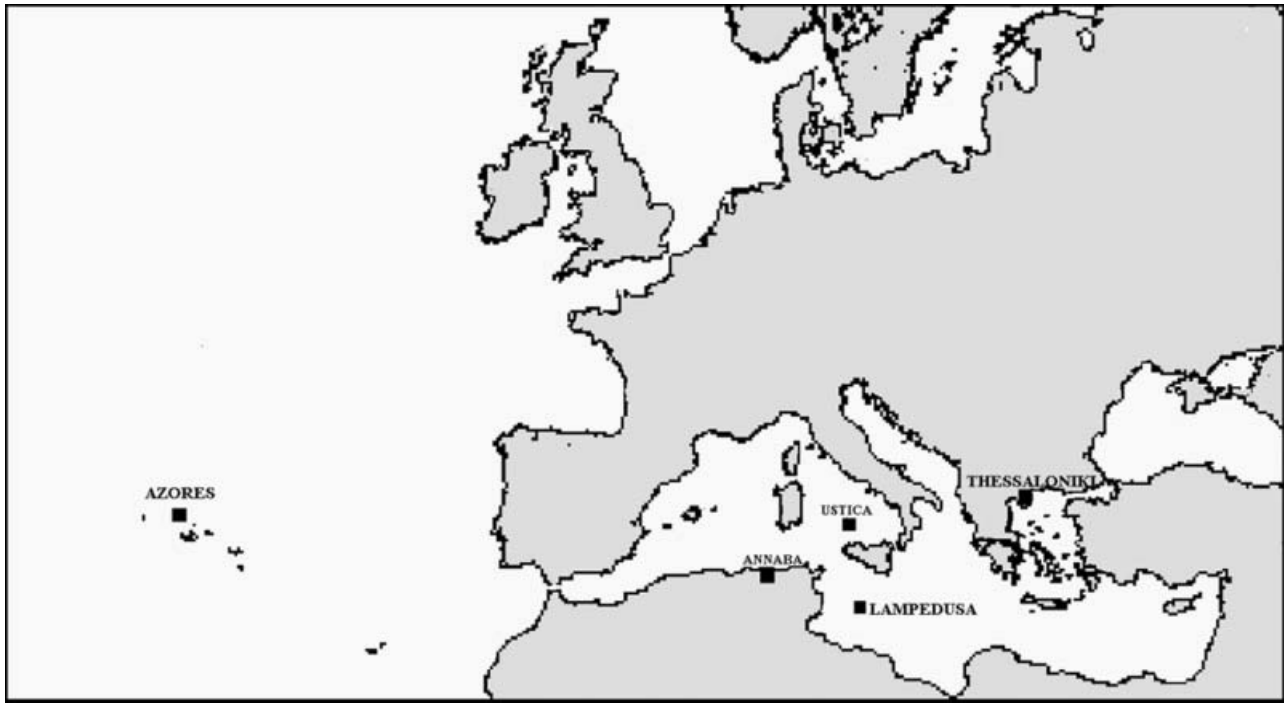

Figure 1. Collection locations for *Epinephelus marginatus* in the Mediterranean Sea and in the Atlantic Ocean (Azores).

followed by 35 cycles of 94°C (60 s), 52°C (45 s) and 72°C (60 s), with a final step of 10 min at 72°C for the termination of PCR. PCR products were visualised on a 2% agarose gel which had been stained with ethidium bromide. Thereafter they were purified using a Qiaquick PCR purification Kit (QIAGEN). After purification, the amplified products of the ND2 gene were digested with 20 restriction endonucleases, nine 4-base cutters (*Alu*I, *Cfo*I, *Hae*III, *Hpa*II, *Msp*I, *Nde*II, *Taq*I, *Rsa*I, *Sau*3A), three 5-base cutters (*Dde*I, *Ava*II, *Hinf*I) and eight 6-base cutters (*Bam*HI, *Eco*RV, *Eco*RI, *Hind*III, *Pst*I, *Pvu*II *Xba*I, *Xho*I) according to the manufacturer's instructions (Boehringher Mannheim). Restriction digests were performed in a 20 µl reaction volume containing 5 µl amplified DNA, 1.5 µl of restriction enzymes (5 units/µl), 2 µl of 10 × buffer (commercially provided) and H_2O to final volume. The DNA was digested over night for 20 h and reactions were stopped at 65°C for 10 min and on ice. The resulting restriction fragments were separated in 2% agarose gel in TBE for 120 min at 50 V. Fragment size was estimated by running a ladder standard 50 bp and 20 bp.

The DSE program in REAP package (McElroy et al. 1992) was used to estimate the nucleotide sequence divergence value among haplotypes (Nei & Tajima 1981; Nei & Miller 1990). Within-sample variation was estimated as nucleotide diversity, the average number of nucleotide substitutions per site for the sequences sampled (Nei & Tajima 1981), and as haplotype diversity (Nei 1987) using the DA program in REAP package. The DA program in REAP package was also used to measure nucleotide divergence among populations. The spatial distribution of haplotypes among the five analysed populations was statistically analysed using a Monte Carlo simulation (Roff & Bentzen 1989). The probability of heterogeneity in the haplotypic distribution was determined by comparison between observed χ^2 and those obtained from 10,000 random permutations of original data. To assess genetic differentiation among populations, we carried out an Analysis of MOlecular VAriance (AMOVA) as implemented in ARLEQUIN (Excoffier et al. 1992); the hierarchical analysis was performed by considering samples belonging to Mediterranean and Atlantic groups. Φ statistics were calculated from haplotypic frequencies partitioning the molecular variance among groups (F_{ct}), among populations within groups (F_{sc}) and within populations (F_{st}). Deviation from equilibrium expectations were tested with the Tajima D (Tajima 1989) neutrality test based on an infinite-site model.

Cytochrome b

A portion of mtDNA gene, cytochrome b was amplified using 28FOR (5′-CGAACGTTGATA-TGAAAAACCATCGTTG-3′, Meyer et al. 1990)

and 34REV (5′-AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3′, Cantatore et al. 1994) primers. PCR was carried out in a Perkin Elmer Cetus Thermal cycler in a 100 µl solution containing 1 ng genomic DNA, 0.2 µM each dNTPs, 0.1 µM each primer, 10 mM buffer 10 ×, 1.5 mM $MgCl_2$ and 2.5 units of Perkin Elmer *Taq* polymerase. The thermal cycling profile began at 94°C for 3 min as a hot start, followed by 5 cycles of 94°C (60 s), 48°C (45 s) and 72°C (60 s), 35 subsequent cycles of 94°C (60 s), 52°C (45 s) and 72°C (60 s), with a final step of 10 min at 72°C for the termination of PCR. PCR products were separated on a 2% agarose gel which had been stained with ethidium bromide. Thereafter they were purified using a Qiaquick PCR purification Kit and sequenced on an ABI Prism 310 automated sequencer (Applied Biosystem).

Sequences were aligned using the alignment software Clustal × (Thompson et al. 1994) using default setting. Data were analysed for estimating intra population diversity as nucleotide and haplotype diversity; we computed total number of haplotypes per population, shared and singleton haplotypes, number of conserved, variable and informative sites. The overall genetic differentiation among samples was computed by AMOVA considering the samples belonging to two major groups, the Mediterranean Sea and the Atlantic Ocean. Φ statistics were calculated from haplotypic frequencies partitioning the molecular variance among groups (F_{ct}), among populations within groups (F_{sc}) and within populations (F_{st}). The genetic differentiation between each sample population was tested using pairwise *F*-statistics and genetic distance (Tamura–Nei).

Exact test of population differentiation of haplotypes and exact test of random distribution of individuals among pairs of populations were done using Monte Carlo simulation of Fisher's exact test (Raymond & Rousset 1995). Demographic history was investigated by analysing mismatch distributions of pairwise differences between all individuals of each population (Rogers & Harpening 1992) and by departure from mutation-drift equilibrium with Tajima D test (Tajima 1989). The time of possible population expansion (t) was calculated through the relationship $\tau = 2\mu t$ where τ is the mode of the mismatch distribution, μ is the mutation rate of the sequences considering that $\mu = 2\mu k$ (μ is mutation rate per nucleotide and k is number of nucleotides). In the case of cyt b, a mutation rate of 2% per nucleotide per million years (Myr) was used as the mean rate for vertebrate mtDNA (Brown et al. 1979). The generation time for *E. marginatus* was taken as approximately 35 years (Chauvet 1988). All statistical data were conducted using DNAsp (Rozas & Rozas 1999), MEGA (Kumar et al. 2004) and Arlequin (Excoffier et al. 1992) software.

Results

RFLP analysis

The primers successfully amplified the 750 base pairs fragment of the ND2 gene. No differences were found in fragment size of the samples. Regarding the ND2 fragment, 10 enzymes had no restriction sites in any of the specimens, nine enzymes had restriction sites in all samples without being polymorphic and only one enzyme (*Dde I*) revealed a polymorphic restriction pattern for all samples. The two haplotypes differed for the gain or loss of a restriction site; the haplotype A showed a pattern with two bands of 400 bp and 350 bp, while the haplotype B showed no restriction pattern.

Nucleotide sequence divergence between two haplotypes was 0.626 (Nei & Tajima 1981). The mean haplotype diversity within samples was 0.246 ± 0.011 SE; the maximum value was 0.476 ± 0.092 in Algerian sample and the minimum value was 0 for the samples from Ustica and the Azores (Table I). These two samples presented only one haplotype for all the specimens analysed, haplotype A in the sample from Ustica and haplotype B for the samples from the Azores. Haplotype A is the most common in Mediterranean samples with a minimum frequency of 66% (Algeria). Table I shows the nucleotide diversity within population; the mean value was 0.003 ± 0.00001, the minimum value reported is from Ustica and the Azores, meanwhile the other populations displayed similar values.

The nucleotide divergence among populations ranged from 0.0107, between Ustica and Azores, to 0.0001, between Algeria and Lampedusa. Heterogeneity in haplotypes frequency distribution

Table I. Intrapopulation nucleotide (π) and haplotype diversities (h) for *E. marginatus* ND2 RFLP and cyt b sequence analysis. *n*(A) and *n*(B) number of individuals with pattern A and B, respectively; *n*=number of scored specimens.

	RFLP				Cytochrome b		
	h	π	*n*(A)	*n*(B)	h	π	*n*
Algeria	0.476	0.005	12	6	0.889	0.014	17
Grecia	0.428	0.004	12	4	0.934	0.013	15
Lampedusa	0.327	0.003	18	4	0.743	0.004	22
Ustica	0.000	0.000	20	0	0.889	0.007	20
Azzorre	0.000	0.000	0	15	0.833	0.004	15

among samples was tested Monte Carlo χ^2 analysis with 1000 randomizations of the data set. Significant geographical heterogeneity ($\chi^2=27.9$, $P<0.0001$) was identified among the samples. The analysis was also carried out considering the Mediterranean samples; where no geographical heterogeneity could be detected among the samples ($\chi^2=5.21$, $P=0.167$). This suggests that the significant heterogeneity observed in populations detected in the first Monte Carlo analysis was primarily attributable to the Atlantic sample. Therefore, these analyses indicated that the Atlantic sample was significantly different from the Mediterranean samples, while the Mediterranean samples did not differ between themselves. Much of this heterogeneity was due to the B haplotype which was characterised by a 100% frequency in the Atlantic sample and 19% frequency in the pooled Mediterranean sample. AMOVA was performed, partitioning molecular variance into three levels: among groups, among populations within group and within populations. The analysis of molecular variance on two groups, Mediterranean and Atlantic, indicated that high proportion of the total variance could be attributed to differences between groups and within population (Table II) with significant values ($P<0.05$); only 6% of the variation could be attributed to difference between populations within groups but this value was also significant, thereby suggesting that there were significant differences within the Mediterranean group.

Table II. Analysis of molecular variance (AMOVA) of *Epinephelus marginatus* for both molecular markers, ND2 RFLP and cytochrome b sequence analysis.

Molecular marker	Source of variation	Total variance (%)	Fixation indices	*P*
RFLP	Among groups	63.16	$F_{ct}=0.631$	<0.05
	Among population within groups	6.00	$F_{sc}=0.163$	<0.05
	Within populations	30.84	$F_{st}=0.691$	<0.05
Cyt b	Among groups	12.90	$F_{ct}=0.129$	0.191
	Among population within groups	7.00	$F_{sc}=0.080$	<0.05
	Within populations	80.09	$F_{st}=0.199$	<0.05

Estimates of genetic differentiation among five samples, using *F*-statistics, is reported in Table III. The Azores sample displayed a high level of genetic differentiation as compared with all the other samples. Pairwise F_{st} among Mediterranean samples were also significantly high, showing genetic heterogeneity within Mediterranean group, relating to Ustica–Greece and Ustica–Algeria comparisons.

Cytochrome b

The primers successfully amplified the 440 base pairs fragment of cytochrome b gene. Of a total of 440 aligned base pairs, 381 were conserved and 59 were variable, of which 17 were informative. The greater part of nucleotide variation was due to transitions with a transition/transversion ratio of 2.3. The nucleotide frequencies were C=0.286, T=0.302, A=0.253 and G=0.159. Haplotype diversity within populations was high, ranging from 0.743 in Lampedusa to 0.934 in Greece. Nucleotide diversity was low ranging from 0.004 in Lampedusa and the Azores to 0.014 in Algeria (Table I).

The 440 base pairs revealed 42 different haplotypes, of which only seven were shared among different individuals and the remaining 35 were unique (singleton). The 42 haplotypes are available on request. Three of the shared haplotypes were represented at more than one site, while the others were shared among individuals originating from the same sample site. The most common haplotype was found in all sample sites except for Greece. The Minimum Spanning Network which is reported in Figure 2 was constructed for all populations and the most common haplotype represents specimens from Ustica, Lampedusa, Algeria and the Azores. The star-shaped MSN of cyt b haplotypes is consistent with a recent population expansion.

Pairwise mismatch distributions and the results of Tajima's D test, as performed on each population, are reported in Figure 3. The parameters of the sudden expansion model (Rogers & Harpening 1992) and the 'goodness of fit' test to the model are reported in Table IV. All five populations showed negative Tajima's D test values although two of

Table III. Population pairwise F_{st} value for cyt b sequence analysis below the diagonal and for ND2 RFLP analysis above the diagonal;* indicates significant at 0.05%.

Sample	Algeria	Greece	Lampedusa	Ustica	Azores
Algeria		−0.047	0.043	0.368*	0.579*
Greece	−0.015		0.005	0.316*	0.631*
Lampedusa	0.091*	0.035		0.157	0.789*
Ustica	0.151*	0.099*	0.059		1.000*
Azores	0.224*	0.168*	0.253*	0.261*	

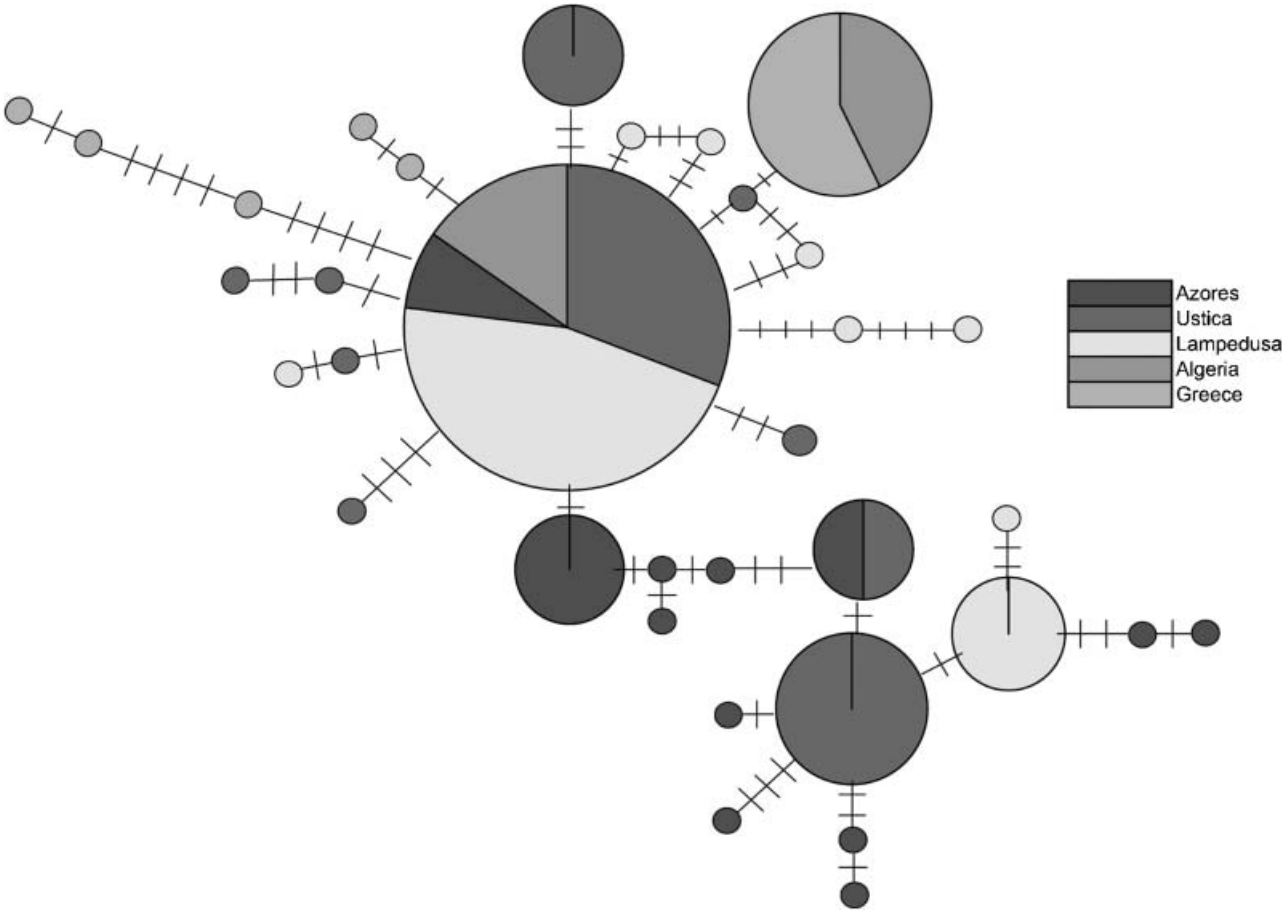

Figure 2. Minimum Spanning Network constructed by *E. marginatus* cyt b sequences analysis; each colour represents a sample site.

these were significant (Greece and Algeria). The Algerian population could not be fitted to an expansion model while the sample from Greece presents an unimodal curve, which indicates a sudden expansion in population size. The time of this expansion was estimated to be approximately 4.04 Myr.

The hierarchical partition of variance showed that the most part of variance was attributed to differences within population with a high significant value of F_{st} (Table II); a little part of the variance (12.9%) was due to difference among groups (Mediterranean and Atlantic) but the corresponding value of Φ-statistics, F_{ct}, was not significantly high. However the variance component which was attributable to differences among populations within groups, was low (7%) but the F_{sc} value was significant; this suggested that, as revealed by the RFLP analysis, there were significant differences within the Mediterranean group. Estimates of genetic differences among all five populations using F-statistics is reported in Table III. Significantly high values of F_{st} resulted for all comparisons except those for Algeria–Greece, Greece–Lampedusa and Ustica–Lampedusa. This confirms the significant value obtained by AMOVA.

Discussion

A high genetic variation, calculated as haplotype diversity, and very low nucleotide diversity were found in all the populations analysed either in RFLP or cytochrome b. Regarding the cytochrome b analysis, haplotype and nucleotide diversity values are comparable to those reported for *Helicolenus dactylopterus* (Aboim et al. 2005) which is a benthonic fish species with a sedentary lifestyle. This low genetic diversity which was found in both markers is consistent with long-distance or jumping events between the Atlantic Ocean and the Mediterranean Sea rather than stepping-stone modes of dispersal. However, this is not consistent with the behavioural characteristics of the dusky grouper, since long-distance or jumping events require adult and juvenile movements across long distances. Moreover, the pattern of genetic diversity

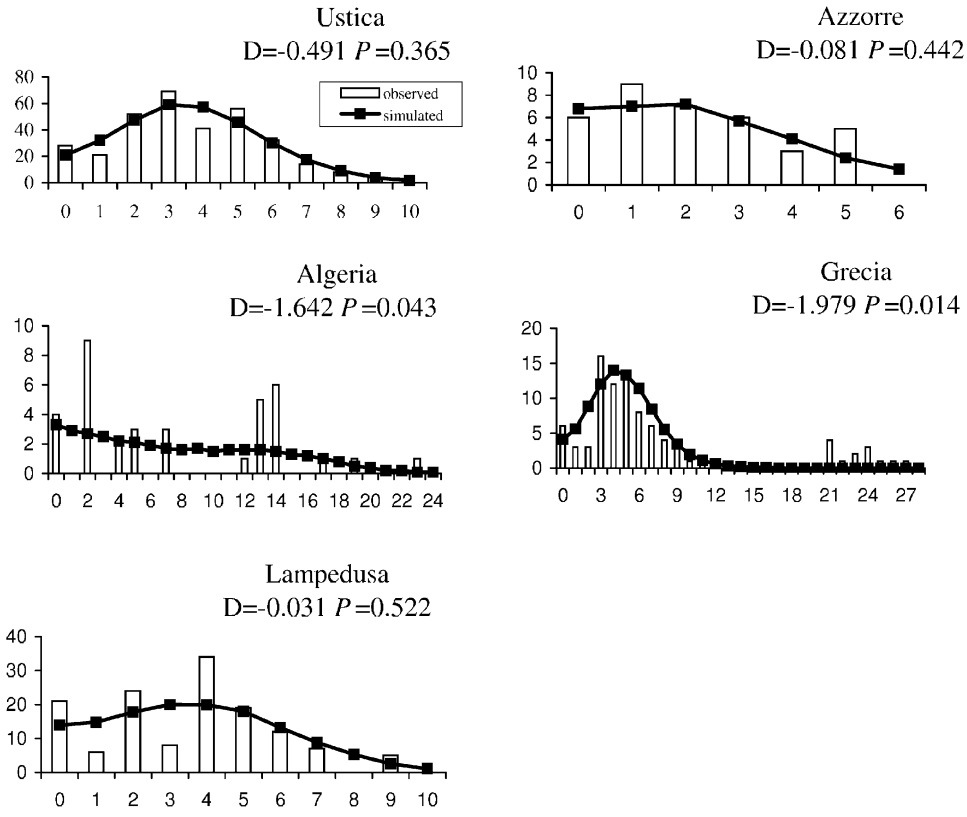

Figure 3. Pairwise mismatch distribution, simulated model of sudden expansion and results of Tajima's D test with associated probability for each population in cyt b sequence analysis.

can be attributed to a recent population expansion after a low effective population size which has been caused by bottlenecks or founder events (Grant & Bowen 1998). Whilst reviewing mtDNA diversity in marine fishes, Shields & Gust (1995) have underlined a common pattern of a single or a few prevalent haplotypes with numerous singleton haplotypes which differed by one or two mutations from common haplotypes; this pattern gives rise to a star-shaped haplotype network. The causes of this pattern in genetic variation could be a large variance in reproductive success that leads to the presence of only a few haplotypes. Other factors include overexploitation, recent habitat reduction, population bottleneck or founder events. Thus the hypothesis of historical dispersal events among populations followed by population expansion is consistent with the star-shaped haplotype network. In the case of *E. marginatus*, a star-shaped haplotype network which had been constructed on cyt b sequences was obtained and this minimum spanning network was found to be similar to those presented for the historical extinction- recolonization models of other fish taxa (Grant & Bowen 1998). The mismatch distribution and Tajima's D test negative values, calculated on cyt b data, further support a population bottleneck followed by expansion in at least the Greek population (Figure 2). The recent population

Table IV. Parameters of the sudden expansion model and goodness of fit test to the model with the respective significance for each population. θ_0 pre-expansion population size; θ_1 post expansion population size; τ time in number of generations; SSD sum of squared deviations.

Parameters	Algeria	Greece	Lampedusa	Ustica	Azores
θ_0	0.015	1.119	0.001	0.000	0.923
θ_1	10.216	24.230	9.302	18.053	5.364
τ	15.04	4.073	4.700	4.113	2.086
Goodness of fit test					
SSD	0.085	0.014	0.029	0.006	0.008
P	0.226	0.471	0.257	0.500	0.859

expansion for the Greek population dates back to 4.04 Myr and it suggests a historical influence on the genetic structure of *E. marginatus*.

Regarding population structure, *F*-statistics and AMOVA indicated a genetic structure in the *E. marginatus* populations analysed, even if ND2 RFLP and cyt b produced discordant results for the 'among groups' variation. In the case of ND2 RFLP most of the total variance was due to differences between Atlantic and Mediterranean populations, meanwhile for cyt b most of the total variance was due to differences within populations. RFLP on the ND2 region showed little nucleotide sequence divergence and hence few restriction site polymorphisms. Nevertheless the two unique haplotypes, which were revealed in *Dde*I, distinguished the Mediterranean from the Atlantic samples in this present study. This is due to the spatial distribution of the different haplotypes and it may be more useful for assessing the structure and gene flow among populations; an increased number of polymorphic restriction sites should be necessary to prove this difference. The differences found in the cyt b sequence analysis were primarily within populations and there was no apparent difference among Mediterranean and Atlantic populations. Allendorf & Phelps (1981) have suggested that small amounts of gene flow could homogenise populations genetically, even though geographic samples are demographically discontinuous. Moreover, observed homogeneity may reflect historical events. Analysed populations could be isolated spatially but have had enough contact in the recent past to overshadow haplotype frequencies by historical gene flow. In this case the examination of a different molecular marker may provide discordant data such as the RFLP data. However, gene flow between these populations could be maintained by larval migration as adult and juvenile migration is not consistent with behavioural observations for the dusky grouper. It was demonstrated that neither the egg type nor length of larval stage for pelagic larvae appeared to be a useful predictor of geographical structure in reef associated fishes (Shulman & Berminghan 1995). Therefore, even if the dusky grouper has a long pelagic larval phase, there is no reason to assume *a priori* that gene flow occurs via larvae dispersal. Further research regarding life history, particularly on adult and juvenile movements, are clearly necessary in order to understand how gene flow could occur.

There is some evidence for genetic differentiation in the Mediterranean region for both markers. AMOVA has shown that only a small component of variance was attributable to populations within groups, even if it was significant. There appears to be little or no gene flow between the Mediterranean samples and generally the Ustica population displayed the highest values of genetic distance and significant values for pairwise F_{st}. This could be due to differences in fishery practices; Ustica has been a marine protected area since 1986 and the professional and recreational fishery has been almost totally prohibited. Studies on marine protected areas and other area closures have shown that fishery target species, especially if they are overexploited, have increased in abundance and expanded their age structure within the closed area. Nowadays, a marine protected area is considered to be of great ecological benefit regarding the fishery management of relatively sedentary species with high site fidelity, broad larval dispersal whose recruitment is limited. Marine reserves are predicted to benefit adjacent fisheries through two mechanisms: the net density-dependent emigration of adults and juveniles across borders, termed spillover, and an increase in the export of pelagic eggs and larvae due to an increase in the reproductive potential of the species.

Our data, showing differences between Ustica and other Mediterranean samples, have suggested that the effect of a marine reserve on fishery is not even evident. This could be due to two main reasons: first, no samples situated close to reserve borders, where the effect could be more evident, were analysed; and second, the effect might not be evident because of the spillover and larval export scales; these can vary across species and ecosystems. In the case of sedentary species with high site fidelity, spillover and larvae export will give long-term benefit to the species.

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