Journal of Fish Biology (2005) **67,** 1746–1752
doi:10.1111/j.1095-8649.2005.00869.x, available online at http://www.blackwell-synergy.com

Genetic differentiation between close eastern Mediterranean *Dicentrarchus labrax* (L.) populations

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(Received 24 September 2004, Accepted 9 June 2005)

Differentiation at nine microsatellite loci revealed that a Levantine Basin sea bass *Dicentrarchus labrax* population probably represents a further subdivision of this species in the eastern Mediterranean. © 2005 The Fisheries Society of the British Isles

Key words: Mediterranean Sea; microsatellites; population genetics; sea bass.

Population genetic studies on sea bass *Dicentrarchus labrax* (L.) using a variety of molecular markers have shown that there is fragmentation of breeding populations not only between the Atlantic and Mediterranean (Benharrat *et al.*, 1983; Naciri *et al.*, 1999) but also within the Atlantic (Benharrat *et al.*, 1983; Child, 1992; Castilho & McAndrew, 1998*a*) and within the Mediterranean (Benharrat *et al.*, 1983; Allegrucci *et al.*, 1997; Caccone *et al.*, 1997; Cesaroni *et al.*, 1997; García de León *et al.*, 1997) populations.

Within the eastern Mediterranean populations in particular, Bahri-Sfar *et al.* (2000) showed significant divergence between samples from the Adriatic, Ionian and North Aegean Seas and the Libyian-Tunisian Gulf. The present work investigated sea bass from the Levantine Basin, part of the Mediterranean Sea from where genetic data on marine teleosts are scarce.

New markers developed by Ciftci *et al.* (2002) were combined with previously published loci to determine the smaller geographic scale population structure of sea bass in this area.

Two samples were collected from the eastern Mediterranean basin, a sample of wild adults from multiple year classes from the Beymelek Lagoon Turkey (36° 15′ N; 30° 02′ E), collected in 1998 ($n = 57$) and a sample of 1 year old fry (F1) produced from wild adults collected in Patras, Greece (38° 14′ N; 21° 44′ E) in the Ionian Sea in 1996 ($n = 48$). One Atlantic sample from Óbidos, Portugal (39° 22′ N; 09° 15′ E) ($n = 60$), represented by a cross-section of multiple year classes, was used as a 'yardstick' of genetic differentiation

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(Fig. 1). Caudal fin clips were taken from live fish (*c.* 0·5 cm^2) from the Greek and Turkish samples and were stored in 1·5 ml Eppendorf tubes in 95% ethanol for up to 2 years in a light proof box held in a refrigerator (4° C). Muscle tissue from the Atlantic individuals were collected and stored at −20° C before DNA extraction. The present study used the microsatellites *Dla-6* and *Dla-11* loci developed by Castilho & McAndrew (1998*b*) and the same frozen library of 60 clones was used to identify another five highly polymorphic loci: *Dla*-12, *Dla-14*, *Dla-20*, *Dla-22* and *Dla-47* (Ciftci *et al.*, 2002). The remaining loci *Labrax-3* and *Labrax-8* were developed by Garcia de Léon *et al.* (1997). Procedures for PCR amplification and genotyping of samples followed Castilho & McAndrew (1998*b*) and Ciftci *et al.* (2002).

Micro-checker version 2.2.1 (van Oosterhout *et al.*, 2004) was used to identify scoring errors due to stuttering, PCR amplification bias against large alleles (large allele dropout) and genotyping errors due to non-amplified alleles (null alleles). Estimations of unbiased gene diversity (Nei, 1987), allelic richness and statistical tests for H_o differences between samples were computed by FSTAT version 2.9.3.2 (Goudet, 1995). Average individual observed and expected heterozygosity at loci (H_o and H_e respectively) and *F*-statistics (Wright, 1951) to summarize population structure comparisons and its statistical significance were computed by GENEPOP 3.4 (Raymond & Rousset, 1995). Adjustment of rejection probabilities for multiple simultaneous tests was done by the sequential Bonferroni technique (Rice, 1989). The genetic structure was also investigated by using a Bayesian approach implemented in the programme STRUCTURE (Pritchard *et al.*, 2000) under varying assumptions (admixture model, correlated frequencies, and identity of population of origin option set to off) and the

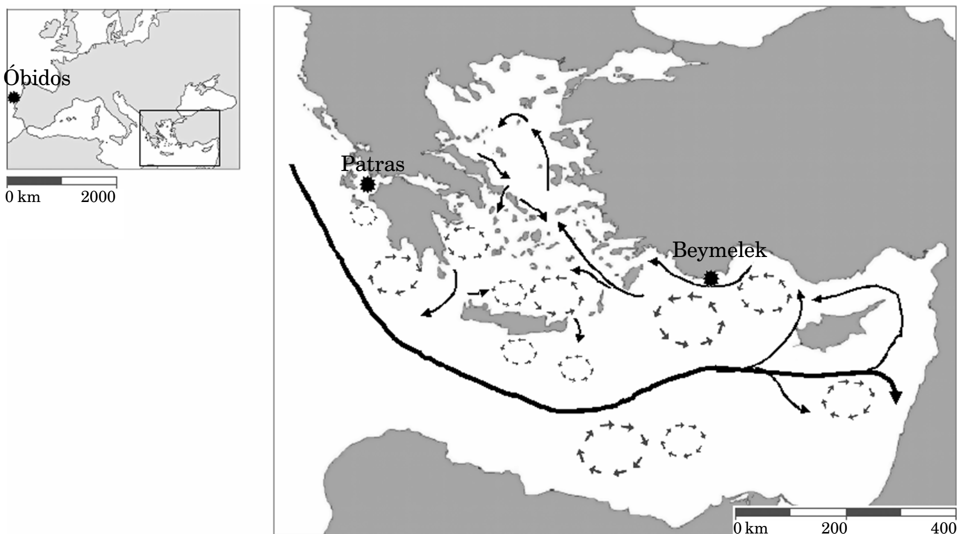

FIG. 1. Map of the sampling locations (●) in the Mediterranean Sea (inset) and Atlantic Ocean. ←, the predominant direction of current (modified from Lykousis *et al.*, 2002; Robinson *et al.*, 2001) and indicate a possible route for the passive drift of eggs and larvae.

number of putative clusters or populations (K). This procedure was used to assess the number of families in the hatchery produced Greek sample. The likelihood of the data was calculated and posterior probabilities for different values of clusters STRUCTURE was run setting the number of populations from one to six. For each run, burn-in time and replication number were set to 50 000 and 200 000. The run with the maximum likelihood was used to assign individuals to clusters. The factorial correspondence analysis (FCA) (Benzécri, 1973), which displays in a multivariate dimensional graphical space the genetic similarity among samples and an assessment of the genetic variability in each population, was undertaken using the software Genetix v. 4.02 (Belkhir *et al.*, 2004).

High numbers of alleles were found in all samples (>91), a common feature in many teleostean microsatellite studies. This is the first report assessing the sea bass genetic diversity between a Levantine Basin and Ionian Sea populations. The Greek population had 91 alleles in 47 individuals (average allelic richness = 10·0) and the Turkish population 95 alleles in 57 individuals (average allelic richness = 10·3). The Portuguese population had the highest number of different alleles with 151 in an average of 54 individual fish (average allelic richness = 16·2). In total there were 192 different alleles observed in the three populations. A total of 29 private alleles were observed, 15·1% of the total number. The Portuguese population had 23 private alleles and the Mediterranean populations contained three alleles each. All composite population samples at a single locus showed significant departure from the Hardy-Weinberg equilibrium (HWE), except for *Dla-14* (Table I). The multilocus test across populations also indicated there were also significant variations from HWE. *Labrax-3* in the Turkish and Greek populations, *Dla-12*, *Labrax-8* in the Greek population and *Dla-6* and *Dla-11* in the Portuguese population remained significant after Bonferroni correction. Possible scoring errors due to stuttering were detected in *Labrax-3* (Turkey) and *Labrax-8* (Turkey and Greece). The possible presence of null alleles was detected for genotypes at *Dla-12* (Greece and Portugal), *Dla-20*, *Labrax-8* (Turkey and Greece), and *Labrax-3* (Turkey, Greece and Portugal), *Dla-6* in Portugal. The Greek hatchery sample was analysed to assess whether it represented a natural Greek population. Results from STRUCTURE showed that the mean highest log-likelihood was achieved when the number of clusters was set to one revealing no indication of the existence of family groups. As three out of nine HWE tests produced statistically significant deviations in the Greek population, this might support the alternative hypothesis (presence of family groups).

Multilocus pair-wise F_{ST} estimates showed a smaller F_{ST} value for the Turkey and Greece comparison ($F_{ST} = 0{\cdot}041$) than between the Turkey and Portugal ($F_{ST} = 0{\cdot}119$) and the Greece and Portugal ($F_{ST} = 0{\cdot}126$) pairs. The three estimates were all statistically significant even after Bonferroni correction. Pair-wise comparisons between populations showed significant differences between all loci except for *Dla-6* in the Turkey and Greece and *Dla-14* in the Greece and Portugal tests. These findings indicate a heterogeneous distribution of alleles across populations. The estimates of gene flow between the Turkish and Greek populations using F_{ST} yielded an effective number of migrants of $N_em = 5{\cdot}31$. STRUCTURE results based on 163 individuals indicated that a K (number of

TABLE I. Measurements of genetic variation of the three samples and deviation from Hardy-Weinberg expectations

Sample	Turkey			Greece			Portugal			Average
N	57			48			58			54·3
N_A	10·6			10·1			16·8			21·3
H_e	0·7304			0·7305			0·8355			0·834
H_{obs}	0·6902			0·6603			0·7626			0·7058
Loci	*Fis*	AR	G	*Fis*	AR	G	*Fis*	AR	G	*Fis*
Dla12	−0·001	11·5	0·771	**0·040**	12·8	0·847	0·107	15·8	0·927	**0·053**
Dla14	0·006	4·0	0·565	0·071	4·0	0·718	0·052	7·8	0·728	0·045
Dla20	0·113	16·8	0·910	0·140	14·9	0·897	0·065	17·4	0·939	**0·104**
Dla22	0·117	13·3	0·874	−0·087	15·9	0·880	0·051	17·3	0·908	**0·034**
Dla47	0·007	2·0	0·460	0·176	4·0	0·620	0·070	6·8	0·649	**0·080**
Dla6	0·040	7·6	0·670	0·072	7·0	0·646	**0·263**	12·8	0·679	**0·117**
Dla11	−0·107	7·8	0·713	0·088	5·9	0·365	**0·104**	19·2	0·923	**0·021**
Labrax3	**0·125**	19·9	0·931	**0·254**	17·6	0·866	0·107	26·2	0·934	**0·156**
Labrax8	0·197	9·6	0·743	**0·205**	8·0	0·813	0·084	23·0	0·921	**0·158**
Multilocus	**0·064**	–	–	0·107	–	–	**0·098**	–	–	**0·088**

N, sample size; N_A, number of alleles; H_e, expected heterozygosity; H_{obs}, observed heterozygosity; AR, allelic richness; G, unbiased gene diversity (Nei, 1987). Tests for deviation from Hardy-Weinberg expectations in each population and in the whole sample at nine microsatellite loci based on exact probability test (Guo & Thompson, 1992). F_{is} values are shown. The test was performed using the Markov chain method, based on 1000 dememorizations, 50 batches and 1000 iterations per batch. Significant values (the probability of rejecting HWE) after Bonferroni correction are indicated in bold. Indicative adjusted nominal level for 27 tests at 5% significance level is 0·00185.

populations) = 3 was found to converge well and gave comparable or higher likelihoods than $K = 1$–2 and 4–6 among runs of the programme. The run with highest log-likelihood at $K = 3$ for the observed data was used to define the model based groups. It is remarkable that >96·9% of the individuals were rightly assigned to their original population.

The FCA produced three obvious clusters of compound microsatellite frequencies for the *D. labrax* samples, from Turkey, Greece and Portugal (Fig. 2), with a few intermediate individuals between Turkey and Greece.

The interpretation of the results presented here depend on how closely the Greek (F1) sample analysed represents the adult wild population. The evidence was not conclusive in either direction so the results must be treated with caution.

The present results, although with implicit sampling limitations, suggest that the three samples are not a single panmictic population, but more importantly that both samples from the northern part of the eastern Mediterranean do not belong to the same genetic unit F_{ST} value 0·045. The only published Aegean and Ionian Sea comparisons are between Messolongi and Crete and Messolongi and Thessaloniki and give non-significant F_{ST} values of 0·032 and 0·026 respectively (Bahri-Sfar *et al.*, 2000). No closer comparison is possible, as no other Turkish sample has been analysed to date.

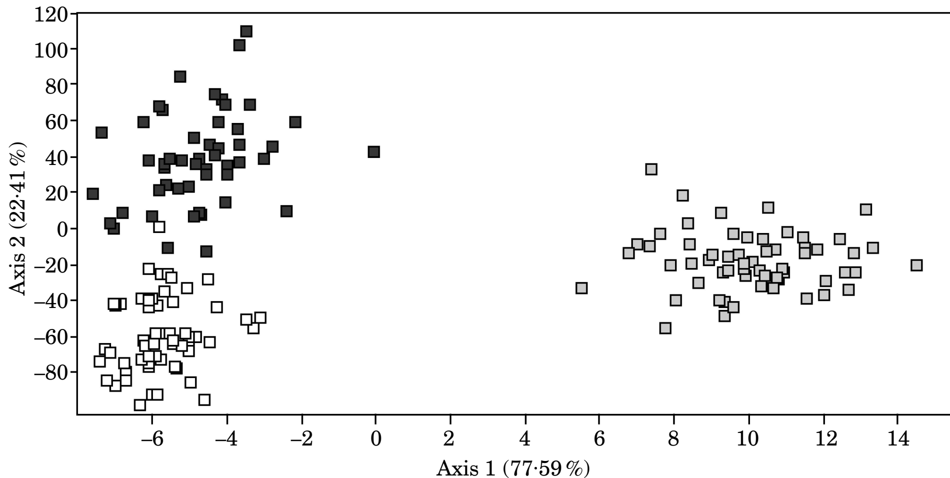

FIG. 2. Factorial correspondence analysis (FCA) plot of 163 *Dicentrarchus labrax* individuals from Turkey (■), Greece (□) and Portugal (▩) (see Fig. 1). Some individuals exhibit identical genotypes, therefore the number of squares does not add up to the total sample size. The first two factorial axes capture 100% of variation in the groupings (percentage of total inertia carried by an axis in parentheses).

Bahri-Sfar *et al.* (2000) previously reported the eastern Mediterranean sea bass sub-divisions based on the main hydrological features of the basin: the Adriatic, Ionian and Aegean Seas, and the Libyan-Tunisian Gulf. The present work warrants a further subdivision, for the populations in the Levantine Basin. Bahri-Sfar *et al.* (2000) linked the patterns of distribution with past and present hydrological conditions in the Mediterranean Sea but could not explain why these patterns had persisted with the known dispersal capabilities of the juveniles and adults of this species. They propose that some mechanism beside the passive retention by currents is acting to maintain the current levels of differentiation (*e.g.* selection or phylopatry). Castilho (1998) reported the non-panmixia along the Portuguese coast, in an apparently continuous water mass with no obvious geological or hydrological barrier (past or present) to the movement of all life stages of this species. Castilho (1998) hypothesized that the localized sea bass spawning grounds, known to occur consistently along the Portuguese coast near estuaries and lagoons (R. Castilho, pers. obs.), give the larval sea bass the best chance of entering the lagoons by active or passive mechanisms and the young sea bass to grow in protected nursery areas. Phylopatry to these localized spawning grounds over many generations would contribute to the sub-division of the species. The currents in the Ionian and Aegean Seas and in the Levantine Basin tend to follow an anti-clockwise direction, with an anticyclonic eddy near the Peloponnese Peninsula (Fig. 1). The main circulation in the Aegean Sea, although difficult to delineate, tends to go north along the western coast of Asia Minor, before turning to flow west and then south-west in the North Aegean. Due to the many islands and channels between them, currents can be diverted, so that in some places water flows in a completely opposite direction. In the Levantine Basin, the mid-Mediterranean jet flows eastwards whereas near the

coast of Asia Minor the currents flow westwards into the Southern Aegean. Although the adult sea bass is a powerful swimmer, known to migrate several hundreds of km (Pickett & Pawson, 1994), larvae and juveniles can be retained in hydrological formations such as eddies or small gyres, preventing the mixing of gene pools. Moreover, the real number of migrant exchanges between populations may be smaller than expected given the species characteristics (Castilho & McAndrew, 1998*a*; Naciri *et al*., 1999; Bahri-Sfar *et al*., 2000) and is probably just a tiny proportion of the population size of this species. It seems probable thus, that although the present current patterns do not prevent intermixing, they do not facilitate the mixing of the two populations in the eastern Mediterranean.

Grant support was through Fundação para a Ciência e Tecnologia (FCT), Portugal, Programa Praxis (BD/5820/95) to RC. We thank V. Bakopoulos for the sampling in Greece and the collaboration of M. J. Brito for the sampling in Óbidos.

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