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Range-wide population structure of European sea bass *Dicentrarchus labrax*

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The euryhaline European sea bass *Dicentrarchus labrax* L., inhabiting the coasts of the eastern Atlantic Ocean and Mediterranean Sea, has had many opportunities for differentiation throughout its large natural range. However, evidence for this has been incompletely documented geographically and with an insufficient number of markers. Therefore, its full range was sampled at 22 sites and individuals were genotyped with a suite of mapped markers, including 14 microsatellite loci (N = 536) and 46 neutral or gene-linked single nucleotide polymorphisms (SNPs; N = 644). We confirm that the Atlantic and Mediterranean basins harbour two distinct lineages. Within the Atlantic Ocean no pattern was obvious based on the microsatellite and SNP genotypes, except for a subtle difference between South-eastern and North-eastern Atlantic sea bass attributed to limited introgression of alleles of Mediterranean origin. SNP genotypes of the Mediterranean lineage differentiated into three groups, probably under the influence of geographical isolation. The Western Mediterranean group showed genetic homogeneity without evidence for outlier loci. The Adriatic group appeared as a distinct unit. The Eastern Mediterranean group showed a longitudinal gradient of genotypes and most interestingly an outlier locus linked to the somatolactin gene. Overall, the spatial pattern fits those observed with other taxa of between-basin segregation and within-basin connectivity, which concurs well with the swimming capabilities of European sea bass. Evidence from a few outlier loci in this and other studies encourages further exploration of its regional connectivity and adaptive evolution.

ADDITIONAL KEYWORDS: adaptation – DNA microsatellite – marine fish – SNP – somatolactin.

INTRODUCTION

As mutations, gene flow and selection leave distinct imprints in the genome, the patterns and dynamics

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of allelic variability and genomic architecture trace the demographic (neutral) and adaptive (non-neutral) changes that have shaped the evolutionary history of organisms. Marine fishes offer a challenging opportunity to partition neutral and adaptive patterns as most of them have high gene diversity (DeWoody & Avise, 2000) and large effective population sizes (reviewed by Hauser & Carvalho, 2008). The accompanying high fecundity provides a substrate for selection and local adaptation to the variable marine and coastal environment, despite potentially high gene flow (Hauser & Carvalho, 2008; Hellberg, 2009; Oleksiak, 2010). Better understanding of the patterns of local adaptation would lead to better identification of marine population units, and could be useful for management decisions (Funk *et al.*, 2012). Local patterns can be highly structured in time and space (Dannewitz *et al.*, 2005; Riccioni *et al.*, 2013). A consequence of this observation is that the population delineation of marine organisms as inferred by genetic data may be more complex than assumed previously (Hauser & Carvalho, 2008). However, temperate marine fishes do demonstrate shallow population histories, as a consequence of the impact of the successive glacial cycles during the Quaternary (Grant & Bowen, 1998; Hauser & Carvalho, 2008). Hence, their population history has been affected by drift, non-equilibrium demographic patterns and vicariant events that also promoted the creation of well-defined marine contact zones. The latter occurs when genetically differentiated populations come into secondary contact. Moreover, patterns of population structure are complicated by variable survival rates of larval and post-larval stages, and hence large fluctuations in cohort size (Hjort, 1914; Cushing, 1990). This sweepstake recruitment *sensu* Hedgecock (1994) may substantially influence how marine populations evolve, the structure of their coalescence, and the way neutral and non-neutral processes are inferred (see Hedgecock & Pudovkin, 2011). Overall, the marine realm requires more careful examination of the observed patterns of gene diversity, demanding an approach with the right balance between the number of loci and sample size. This should allow for a better estimate of the (outlier) markers involved in adaptive patterns (Nielsen *et al.*, 2012; Teacher *et al.*, 2013) by enhancing 'neutral parametrization' (i.e. low probability to detect false positive outliers; Lotterhos & Whitlock, 2014).

Among the biologically well-studied marine fish taxa is the European sea bass *Dicentrarchus labrax* L. (Moronidae, Teleostei), which inhabits the continental shelves of the North-east Atlantic Ocean and Mediterranean Sea (Pérez-Ruzafa & Marco, 2014). Wild catches in the Atlantic Ocean and Mediterranean Sea reach about 9000 tonnes annually (FAO, 2014) while aquaculture production reaches 153 182 tonnes (FAO, 2014). Catches are not regulated, apart from a few national plans, because of incomplete data compilation, especially from recreational fishing mortality (ICES, 2014). Lately, biomass of wild Atlantic stocks has been declining rapidly while fishing mortality has been increasing leading to a recommendation for a 80% reduced effort by ICES (2014) and closure of the pelagic trawl fishery in January 2015.

There is clear evidence for the presence of an Atlantic and a Mediterranean lineage (Naciri *et al.*, 1999; Lemaire, Versini & Bonhomme, 2005; Coscia *et al.*, 2012; Quéré *et al.*, 2012; Tine *et al.*, 2014), with a contact zone at the Almeria–Oran front (AOF). Unlike evidence from a short cyt *b* fragment (Lemaire *et al.*, 2005) mitochondrial diversity based on a 6383-bp fragment is higher in the Atlantic Ocean than the Mediterranean Sea ($\pi = 0.00878$ and 0.00352, respectively) (Rondon, 2011). Divergence time between the Western Mediterranean and Atlantic lineages has been estimated at *c.* 270 000 years BP with secondary contact at the beginning of the Holocene *c.* 11 500 years BP (Tine *et al.*, 2014). Populations of the Mediterranean basin are differentiated in an Eastern and a Western group separated along the Siculo-Tunisian Strait (Cesaroni *et al.*, 1997; Bahri-Sfar *et al.*, 2000; Quéré *et al.*, 2012). Except for a large mitochondrial genetic differentiation at the AOF ($F_{ST} > 0.70$; Coscia *et al.*, 2012; Lemaire *et al.*, 2005), overall genetic differentiation is low. Genome-wide estimated nuclear differentiation was estimated at ~2.8% between the Atlantic and Western Mediterranean lineage (Tine *et al.*, 2014), and – on the basis of 20 markers – estimated to be ~2% between the two Mediterranean basins (Quéré *et al.*, 2012). Differentiation within each basin is fairly limited based on both mtDNA and nuclear markers. The Atlantic lineage shows no evidence for population expansion (Coscia *et al.*, 2012) and weak evidence for genetic structure. Samples collected along the Atlantic coasts north of Portugal (Fritsch *et al.*, 2007; Quéré *et al.*, 2010; Coscia & Mariani, 2011; Coscia *et al.*, 2012) do not show any structure, except for the British Isles (Child, 1992) where a genetic difference was identified at the *PGM* locus between juvenile populations from the Irish Sea and coastal UK. The western Mediterranean population is not genetically structured (Naciri *et al.*, 1999; Quéré *et al.*, 2012), while the Eastern Mediterranean contains more patchy populations (Bahri-Sfar *et al.*, 2000; Castilho & Ciftci, 2005; Quéré *et al.*, 2012), with very limited differentiation (< 2%; Bahri-Sfar *et al.*, 2000; Quéré *et al.*, 2012). Hence, similar to many other marine fishes (e.g. Milano *et al.*, 2014; Ruzzante *et al.*, 2006; Teacher *et al.*, 2013), sea bass

has a shallow nuclear genetic structure that can be largely explained by neutral demographic processes (see Patarnello, Volckaert & Castilho, 2007, for a review). Nevertheless, the study of genome-wide variation in sea bass has recently demonstrated the presence of so-called genomic islands of differentiation (Tine *et al.*, 2014), while Quéré *et al.* (2012) demonstrated that markers associated with genes have larger estimates of genetic differentiation both among and within basins. Such findings suggest that adaptive determinisms of genetic variation are also present in sea bass, but hidden within the shallow structure observed for most marine fishes.

Several aspects of the genetic structure of European sea bass remain unanswered. The historical Atlantic–Mediterranean pattern is unambiguously supported by several studies (e.g. Tine *et al.*, 2014), but (1) the seemingly homogenous distribution of the Atlantic and Western Mediterranean lineages has not been analysed basin-wide with a sufficiently large number of markers [Atlantic Ocean: max. $N = 13$ microsatellite loci (Coscia & Mariani, 2011); Western Mediterranean Sea: max. $N = 20$ nuclear markers (Quéré *et al.*, 2012)] to conveniently partition stochastic and adaptive processes. In contrast, (2) the observed population structure within the Eastern Mediterranean could have been overestimated because of too few markers. (3) Some basins remain underexplored, such as the Adriatic Sea which is known to be differentiated from the Mediterranean (Garoia *et al.*, 2007; Mejri *et al.*, 2011; Milana *et al.*, 2012). (4) Finally, the information content of the nuclear microsatellite and single nucleotide polymorphism (SNP) markers to detect patterns has not been fully explored (but see Quéré *et al.* (2012). Our revisit of the genetic structure of European sea bass is based on a geographically extensive sampling effort and takes advantage of a combination of nuclear putatively neutral genetic markers (existing microsatellites and newly developed SNPs) and markers putatively influenced by selection (newly developed SNPs).

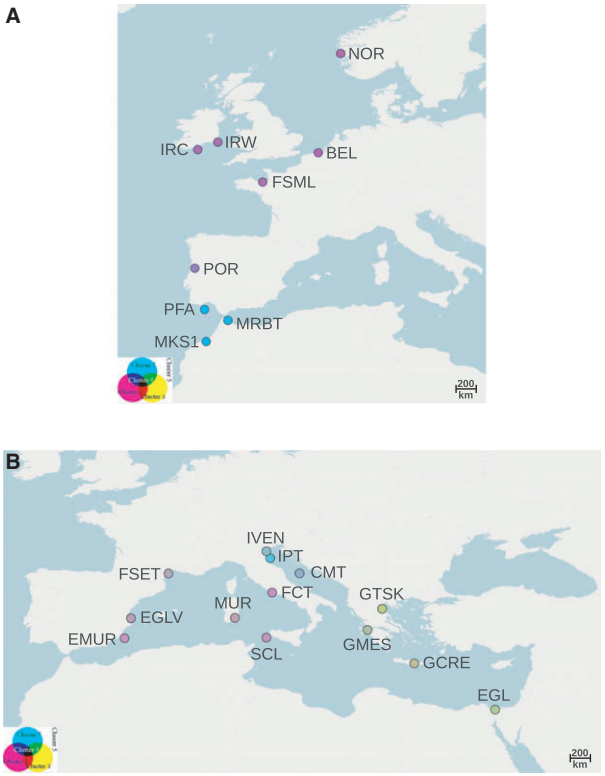

Figure 1. Location of samples and sample-specific genetic differentiation by basin of *Dicentrarchus labrax* based on assignment analysis in STRUCTURE with SNP markers. A, Atlantic Ocean; B, Mediterranean Sea. The colours used to represent the frequencies are derived from the CMYK colour model. One of each of the colours cyan, magenta, yellow and black are assigned to each of the clusters represented. Colours are mixed according to the proportions of the representative clusters at each geographical location. The graph in the bottom left-hand corner of the display shows the precise proportion of each cluster at the represented location. For site locations see Table 1. For full colour representation see the online version of the paper.

MATERIAL AND METHODS

LOCALITIES AND DNA EXTRACTION

The spatial sampling design covered almost the full range of European sea bass (Fig. 1, Table 1). Samples from Aveiro (P), Bardawil (Eg), Fiumicino (I), Marsala (I), Muravera (I), Rabat (Mo), Tanger – Ksar es-Seghir (Mo), Thessaloniki (Gr) and Valencia (Es) have been included in one or several previous studies: Allegrucci, Caccone & Sbordoni (1999), Allegrucci, Fortunato & Sbordoni (1997), Bahri-Sfar *et al.* (2000), Bonhomme *et al.* (2002), Caccone *et al.* (1997), García de León, Chikhi & Bonhomme (1997), Lemaire *et al.* (2000, 2005), Naciri *et al.* (1999), Quéré *et al.* (2012). Samples were collected offshore except for the lagoon samples from Aveiro, Bardawil, Muravera and Venice. Our sampling strategy did not explicitly aim at a temporal analysis of population structure. A small sample of either pectoral fin or muscle tissue, or scales were stored in 80% ethanol and kept at room temperature until DNA extraction. Total genomic DNA was isolated either using the Invisorb DNA Universal Clinical HTS 96 kit or the NucleoSpin Tissue Extraction kit (Machery-Nagel). DNA concentration was measured using a NanoDrop 1000 spectrophotometer (Thermo Scientific; Table 1).

Table 1. Description of the samples of *Dicentrarchus labrax* collected, including geographical origin, sampling site, code, sampling date, sample size (*N*) of fish genotyped for SNPs, microsatellites, and SNPs and microsatellites combined

Geographical origin	Sampling site	Code	Longitude	Latitude	Sampling date	Sampling contact – source publication	No. of SNPs	No. of micro-satellites	No. of SNPs & micro satellites
Atlantic Ocean	Masfjord, Norway	NOR	5°20′E	60°49′N	2005	J. Holmen	40	34	34
	Wexford, Ireland	IRW	6°26′W	52°20′N	2007	S. Mariani	21	16	16
	Cork, Ireland	IRC	8°15′W	51°50′N	2006–2007	S. Mariani	25	23	22
	Zeebrugge, Belgium	BEL	3°11′E	51°21′N	2007	E. Cuveliers	42	40	40
	Saint-Malo, France	FSML	2°08′W	48°46′N	2002	B. Chatain	41	23	23
	Aveiro, Portugal	POR	8°42′W	40°39′N	1993	Allegrucci *et al.* (1997); Caccone *et al.* (1997); Naciri *et al.* (1999); Allegrucci *et al.* (1999)	21	19	19
	Faro, Portugal	PFA	7°51′W	36°53′N	2008	A. Canario	12	12	12
	Rabat, Morocco	MRBT	7°36′W	33°53′N	1996	Naciri *et al.* (1999); Bonhomme *et al.* (2002); Lemaire *et al.* (2005)	31	24	24
	Ksar es-Seghir, Morocco	MKS1	5°33′W	35°51′N	1997	Naciri *et al.* (1999); Lemaire *et al.* (2005)	23	23	23
Western Mediterranean Sea	Murcia, Spain	EMUR	0°47′W	37°44′N	2004	Quéré *et al.* (2012)	40	40	40
	Valencia, Spain	EGLV	0°10′E	39°22′N	1994	García de León *et al.* (1997); Naciri *et al.* (1999); Bahri-Sfar *et al.* (2000)	36	18	17
	Sète, France	FSET	3°45′E	43°22′N	2006	Quéré *et al.* (2012)	40	36	36
	Muravera, Italy	MUR	9°37′E	39°26′N	1992	Allegrucci *et al.* (1997); Caccone *et al.* (1997); Cesaroni *et al.* (1997); Allegrucci *et al.* (1999); Lemaire *et al.* (2000)	33	29	29
	Fiumicino, Italy	FCT	12°70′E	41°51′N	1990	Allegrucci *et al.* (1997); Caccone *et al.* (1997); Cesaroni *et al.* (1997); Allegrucci *et al.* (1999); Lemaire *et al.* (2000); Quéré *et al.* (2012)	21	16	15
	Marsala, Italy	SCL	12°18′E	37°50′N	1991	Allegrucci *et al.* (1997); Caccone *et al.* (1997); Cesaroni *et al.* (1997); Naciri *et al.* (1999); Allegrucci *et al.* (1999); Lemaire *et al.* (2000); Bahri-Sfar *et al.* (2000); Quéré *et al.* (2012)	21	19	18

Table 1. *Continued*

Geographical origin	Sampling site	Code	Longitude	Latitude	Sampling date	Sampling contact – source publication	No. of SNPs	No. of micro-satellites	No. of SNPs & micro satellites
Eastern Mediterranean Sea	Venice, Italy	IVEN	12°18′E	45°20′N	2005	L. Bargelloni	39	43	39
	Porto Tolle, Italy	IPT	12°52′E	44°59′N	2000–2005	L. Bargelloni	41	40	40
	Murter, Croatia	CMT	15°14′E	43°23′N	2006	T. Patarnello	11	12	11
	Messolonghi, Greece	GMES	21°20′E	38°18′N	2005	F. Bonhomme	45	39	39
	Crete, Greece	GCRE	25°36′E	35°20′N	2006	F. Bonhomme	6	9	6
	Thessaloniki, Greece	GTSK	22°50′E	40°06′N	1997	Bahri-Sfar *et al.* (2000); Bonhomme *et al.* (2002)	31	–	–
	Bardawil, Egypt	EGL	32°58′E	31°07′N	1991	Allegrucci *et al.* (1997); Caccone *et al.* (1997); Cesaroni *et al.* (1997); Allegrucci *et al.* (1999); Lemaire *et al.* (2000); Bahri-Sfar *et al.* (2000)	24	21	21

Genotyping of microsatellite markers

Fourteen mapped microsatellite loci were amplified and analysed: *DLA0008*, *DLA0119*, *DLA0016*, *DLA0020*, *DLA0105*, *DLA0228E*, *DLA0244*, *DLA0237*, *DLA0248*, *DLA0146*, *DLA0142*, *DLA0110*, *DLA0145* and *DLA0140* (Chistiakov *et al.*, 2005; Supporting Information, Table S1). All were selected based on their position on the linkage map to provide maximal coverage and level of polymorphism. Amplification of the loci was performed in a 20-μL polymerase chain reaction (PCR) cocktail containing 1× *Taq* buffer (50 mM KCl, 10 mM Tris–HCl, pH 9, at 25 °C, 0.1% Triton X-100; Promega), 1 mM $MgCl_2$, 150 nM of each primer, 70 μM dNTPs, 0.8 U of *Taq* and 50 ng of genomic DNA. Samples were amplified on a DNA Thermal Cycler (One-Advanced Euroclone) with the following thermal profile: (1) predenaturation at 94 °C for 2 min; (2) 30 cycles of denaturation at 94 °C for 45 s, annealing at 48 °C for 45 s and extension at 72 °C for 45 s each; and (3) additional extension at 72 °C for 10 min. The forward primers were labelled with different fluorescent dyes allowing fragment detection on an ABI PRISM 3100 or 3700 automated sequencer (with size standard ROX-400). They were combined in two multiplex assays and the fragment analysis was run at BMR Genomics (www.bmr-genomics.it). Allele scoring was performed with the software Genotyper v3.7 (Applied Biosystems). To minimize scoring errors two operators independently read and edited the program output. Only consensus genotypes (in total 98%) were retained.

Genotyping SNP markers

A total of 51 SNPs from a large data set of sequenced and *in silico* detected unique SNP candidates (Chistiakov *et al.*, 2008; Kuhl *et al.*, 2010; Arias *et al.*, 2012; L. Bargelloni, pers. comm.) were successfully genotyped with the MassARRAY system (Sequenom). PCR primers and extension primers were designed and optimized at the Genetic Service Facility of the Flanders Institute of Biotechnology (Antwerp, Belgium). Two SNPs gave no reaction in more than 25% of the individuals and were discarded from the analysis. Similarly, 26 individuals for which more than 25% of the SNPs gave no reaction were discarded and excluded from Table 1.

Of the 49 successfully genotyped SNPs, 37 were developed from expressed sequence tags (ESTs; Supporting Information, Table S2). Ten of them were developed by resequencing several genes in ten individuals of Mediterranean origin (Chistiakov *et al.*, 2008; D. Chistiakov, pers. comm.). Twenty-seven were developed by resequencing several ESTs in four

individuals from the Mediterranean Sea and four individuals from the Atlantic Ocean (Arias *et al.*, 2012). However 12 of these SNPs were first detected in Atlantic ESTs before being validated on Mediterranean and Atlantic individuals and 15 were discovered while resequencing the ESTs on Mediterranean and Atlantic individuals. Finally, 12 SNPs were developed from BAC end sequences and validated using Mediterranean individuals (L. Bargelloni, pers. comm.). All sequences were annotated using BLASTN and BLASTX against the GenBank database (Supporting Information, Table S2). To infer distribution across the sea bass genome, sequences were mapped to the genome of European sea bass (Kuhl *et al.*, 2010; Tine *et al.*, 2014).

Genetic variation

Data quality of microsatellites was checked for null alleles, allele dropout and stuttering by using the software Micro-checker v2.2.3 (van Oosterhout *et al.*, 2004). Data quality of SNPs was assessed by calculating the frequency at which the less common allele occurs in a given population (minor allele frequency – MAF) and by checking Mendelian inheritance of those SNPs that were polymorphic on the Venezia-Fbis family, which has been used for mapping purposes (Chistiakov *et al.*, 2008). Loci with null alleles and non-Mendelian inheritance were discarded.

Genetic diversity was estimated for each population by calculating the mean number of alleles (A), and observed and unbiased expected heterozygosities. Departure from Hardy–Weinberg expectations was assessed using Wright's inbreeding coefficient F_{IS} for each population and lineage. The significance of F_{IS} values was calculated by permutation of alleles and corrected using the sequential Bonferroni method. Significant differences between basins were tested with a non-parametric analysis of variance Kruskal–Wallis test. Linkage disequilibrium was inferred for each pair of loci within each of the 22 samples and for each pair of loci within the Atlantic and Mediterranean lineages.

Visualization of allele and genotype frequencies was done with the web-based interactive geo-visualization software available at https://fishreg.jrc.ec.europa.eu/map/genetics_geobrowser (see Fig. 1). An interesting feature is that allele frequencies are plotted quantitatively. Various population genetic maps are available online for visualization and environmental data can be added as additional layers on the map.

Genetic structure

Genetic differentiation was assessed with three approaches based on all loci to understand overall structure before screening for outlier markers. (1) Divergence among populations was measured using Wright's pair-wise F_{ST} values for each marker type and for samples including at least 15 individuals (but see Kalinowski, 2005; Willing, Dreyer & Oosterhout, 2012). The significance of F_{ST} values was calculated by permutation of individuals and corrected using the sequential Bonferroni method. All calculations were performed using the Genetix software v4.05 (Belkhir *et al.*, 1999). (2) Individual genotypes of each marker type and the combined microsatellite and SNP markers were clustered through discriminant analysis of principal components (DAPC) (Jombart, Devillard & Balloux, 2010) as implemented in R (R Development Core Team 2014). Data were first transformed using principal components analysis (PCA). After retaining an appropriate number of PCs, the K-means algorithm was run and the Bayesian information criterion (BIC) was used to select the most suitable K number of genetic clusters. As the BIC is known to overestimate the number of clusters, several lower K values were tested and the value fitting the data the best was retained. Assignment of individuals to the K clusters and DAPC were then performed. Analyses were conducted using the adegenet package (Jombart & Ahmed, 2011) for the R software (http://www.r-project.org). (3) Population structure was inferred by clustering the genotypes for all markers through running the software Structure v2.3.4 (Pritchard, Stephens & Donnelly, 2000). Unlike DAPC, which maximizes genetic separation among groups and minimizes variation within groups, Structure groups individuals in clusters based on the minimizing of Hardy–Weinberg and linkage disequilibria. This was done for a number of populations (K) ranging from 1 to 10, using the four models available (no admixture and allele frequencies correlated, no admixture and allele frequencies not correlated, admixture and allele frequencies correlated, admixture and allele frequencies not correlated). A burn-in length of 10^3 iterations and subsequently 10^4 additional Monte Carlo Markov chain (MCMC) iterations were performed. Each assessment of K was repeated five times to check the repeatability of the results. The most likely K, selected according to Evanno, Regnaut & Goudet (2005), was then used to assign each individual to its population.

Detection of outliers

The approach used to detect loci influenced by directional selection is based on the expectation that they exhibit lower intrapopulation variability and larger interpopulation differentiation than neutral loci (Shikano, Ramadevi & Merilä, 2010). We investigated

signatures of directional selection based on three conceptually different approaches, each set in the context of an island model, to reduce the number of false positives. (1) To detect increased population differentiation, we adopted the hierarchical Bayesian method of F_{ST} (Foll & Gaggiotti, 2008). This estimates population-specific F_{ST} coefficients accounting for different intensities of genetic drift in the various populations. We used BayeScan v2.1. (http://cmpg.unibe.ch/software/bayescan) to perform the analyses. Ten pilot runs of 5×10^3 iterations was performed after a burn-in of 50×10^3. Loci with a $\log_{10}$ of Bayes Factors between 1.5 and 2 and larger than 2 were considered as outlier loci with a confidence interval of 95 and 99%, respectively, and a *q*-value (i.e. minimum false discovery rate) of 0.05. (2) In a second approach, we screened for reductions in heterozygosity between populations using the LnRH test. Indeed, a reduction in heterozygosity could be caused by the occurrence of a selective sweep. LnRH tests were performed pair-wise on the microsatellite genotypes according to Kauer, Dieringer & Schlötterer (2003). LnRH estimates were standardized with a mean of 0 and a standard deviation of 1; 95 and 99% of all loci are expected to have values ranging from −1.96 to 1.96 and −2.18 to 2.18, respectively. Loci with values outside these boundaries were considered significant. Loci were considered as outliers if they were significant in at least two pair-wise comparisons. With a false positive rate of 0.05, 25, 46, ten and ten false positives are expected in Atlantic, Mediterranean, Western Mediterranean and Eastern Mediterranean basins, respectively. (3) To evaluate F_{ST} and heterozygosity at the same time, we employed a coalescent approach developed by Beaumont & Nichols (1996) as implemented in Lositan (Antao *et al.*, 2008). The parameters of Lositan were as follows: the confidence interval was set to 99 and 99.5% with a false discovery rate set to 0.1 and 0.05, respectively, the number of permutations to 2×10^4 and the population size to 50. The infinite allele model was used for the SNP markers while the stepwise mutation model was used for microsatellite markers. In all cases, the 'neutral' mean F_{ST} was used. All three approaches have their advantages and sensitivities in detecting false negatives and positives, although BayeScan produces a low rate of false positives under a range of demographic scenarios (Narum & Hess, 2011; De Mita *et al.*, 2013). As the detection of outliers through the independent application of multiple methods increases the certainty that these are truly non-neutral, we used the information on outliers from the tests as such (without Bonferroni correction) to minimize the number of false positives. Loci were considered under directional selection when two or three tests were significant for directional selection. Although the concept of balancing selection is well established, there are still methodological limitations for its identification in hitchhiking mapping (Hansen, Meier & Mensberg, 2010). Therefore, we discuss only loci under directional or positive selection.

Patterns of geographical and genetic distance

A matrix of Euclidian distances (in m) was prepared by measuring the shortest distance between two adjacent sampling points over sea within a single basin (Atlantic Ocean and Mediterranean Sea). Geographical and genetic distances were compared through a partial Mantel test by opposing the geographical distance matrix to the matrix of genetic distances ($F_{ST}[F_{ST}/(1 - F_{ST})]$ (Reynolds, Weir & Cockerham, 1983) using the Mantel coefficient *Z* (Rousset, 1997) in the software package IBD (Bohonak, 2002). We tested isolation by geographical distance (IBD) within the Mediterranean and Atlantic basins separately because each represents an evolutionary significant unit (Lemaire *et al.*, 2005). Following Hemmer-Hansen *et al.* (2007) we controlled geographical distance for latitude and longitude to examine if geographical distance had the same effect in each of these dimensions. In that case population structure is best explained by a pattern of isolation by geographical distance. If not, it points to a minor role for geographical distance *per se*. Low sample sizes ($N < 15$) relative to the high allelic diversity of the microsatellite loci were excluded; they may lead to low signal-to-noise ratios when calculating pairwise F_{ST} values (Kalinowski, 2005). The significance of the Mantel test was assessed by 10^6 permutations of the population in the genetic distance matrix.

RESULTS

Genetic variation of the microsatellite and SNP loci

A total of 536 individuals from 21 sites were genotyped at 14 mapped microsatellite loci; genotyping of the samples from Thessaloniki failed (Table 1). Several sample numbers from the Mediterranean Sea were low, but all samples (in the case of DAPC and Structure analysis) and some samples (in the case of F_{ST} analysis; $N > 15$) were retained because of the valuable information. Seven of 25 linkage groups (LGs) are covered, with three loci mapping to LG 10 and 15. The average number of alleles per locus varied from 6.00 (Crete – GCRE) to 16.29 (Zeebrugge – BEL) and differed significantly between basins ($P = 0.028$). Variability at microsatellites seems higher in the Mediterranean Sea than in the Atlantic Ocean. Microsatellite loci DLA105 and DLA248 showed distinct gradients in allele frequency

between the Atlantic Ocean and Mediterranean Sea. Observed heterozygosity values ranged from 0.688 (Muravera – MUR) to 0.822 (Saint-Malo) among loci and did not differ between basins ($P = 0.14$). One sample (Aveiro – POR) appeared to deviate from Hardy–Weinberg equilibrium after Bonferroni correction (Supporting Information, Table S3). Both the Atlantic Ocean and the Western Mediterranean lineages showed a significant heterozygote deficiency over the 14 loci after Bonferroni correction. There was no linkage disequilibrium between all 14 microsatellite loci, which is congruent with their genomic position (Supporting Information, Table S1).

A total of 644 individuals from 22 sites were genotyped at 49 SNP loci. All but four LGs (LG 3, 10, 19 and 21) were represented by one or more SNP(s); LG 16 and LG 17 had four SNPs, LG 20 had five SNPs. The MAF ranged from 0.002 for locus *Dl_6k11* to 0.487 for locus *S159*. It was lower than 5% at six loci (*Dl_6k11*, *S9*, *NS35*, *S186*, *S106* and *S109*; Supporting Information, Table S2). Observed heterozygosity values ranged between 0.205 (Wexford – IRW) and 0.304 (Muravera – MUR), and differed significantly between basins ($P = 0.006$; Supporting Information, Table S4). Of 49 successfully genotyped SNPs, 15 were monomorphic in the mapping panel and four SNPs were homozygous in the mapping parents. Loci *S110*, *S170* and *S218* were discarded from further analyses because of non-Mendelian inheritance or failed genotyping, leaving the number of utilized SNPs to 46 loci. None of the samples, except the Eastern Mediterranean ones, appeared to deviate from Hardy–Weinberg equilibrium after Bonferroni correction. Linkage disequilibrium permutation tests revealed that the 46 SNPs were independent, which is congruent with the genomic position of the SNPs (Supporting Information, Table S2). At the lineage level, 24, seven and 19 cases of linkage disequilibrium involving 24, 11 and 22 loci were significant after Bonferroni correction in the Atlantic Ocean, Western Mediterranean and Eastern Mediterranean, respectively.

Genetic structure

Overall genetic differentiation (F_{ST}) between Mediterranean and Atlantic samples was five times lower based on microsatellites (0.057; $P < 0.001$) (Supporting Information, Table S5) than on SNP markers (0.295; $P < 0.001$) (Supporting Information, Table S7). Multi-locus pair-wise F_{ST} among Atlantic samples varied from 0.000 to 0.018 (microsatellites) and 0.000 to 0.027 (SNPs); the samples from Rabat (MRBT) and Ksar es-Seghir (MKS1) showed above average values. Differentiation between the Eastern and Western Mediterranean Sea was small (microsatellites: 0.005; $P < 0.001$; SNPs: 0.024; $P < 0.001$). Within the Mediterranean basin pair-wise F_{ST} values were more variable than in the Atlantic Ocean (microsatellites: $0.001 < F_{ST} < 0.030$; SNPs: $0.000 < F_{ST} < 0.094$); samples in the eastern basin, in particular, vary. The presence of population structure was confirmed with assignment analysis through both DAPC and Structure. DAPC identified membership of the microsatellite genotypes to three clusters, one involving Atlantic samples and two involving Mediterranean samples (Fig. 2A, B). Clustering of the SNP genotypes led to four clusters, with one cluster in the Atlantic Ocean and three weakly separated clusters in the Mediterranean Sea (Fig. 2C, D). Almost all Atlantic individuals were assigned to the Atlantic group while almost all Mediterranean individuals to the Mediterranean group. Individuals from the Strait of Gibraltar (Ksar es-Seghir – MKS1) clustered with the Atlantic samples. No substructure was found in the Atlantic Ocean except that three samples (Portugal – PFA, Rabat – MRBT, Ksar es-Seghir – MKS1) were influenced by Mediterranean genotypes. Mediterranean samples split into two groups: Eastern and Western Mediterranean basin (Fig. 2A, B) or an additional Adriatic group for the SNP genotypes (Fig. 2C, D). Results from the Structure analysis are comparable to the DAPC analysis with $k = 2$ providing the most probable groupings including one Atlantic and one Mediterranean group (Supporting Information, Figs S1, S2). Two groups were detected in the Atlantic basin based on the SNP markers with the samples of Faro (PFA) and Morocco (MRBT and MKS1) constituting a separate cluster (Fig. 1, Supporting Information, Fig. S3). Although the number of clusters of the SNP genotypes was evaluated to be two in the Mediterranean basin, a weak substructure of three groups was observed (Fig. 1, Supporting Information, Fig. S4).

We restricted the combined analysis of the microsatellite and SNP genotypes to DAPC, due to different assumptions underlying the analysis of the two marker types in Structure. Overall, population structure was confirmed. The scatter plot of the first two components of the DAPC fitted a hierarchical island model. The number of genetic clusters was parsimoniously estimated to be four, similar to the analysis based on SNP genotypes and one more than the microsatellite genotypes. One cluster grouped all Atlantic samples while the three others clustered all Mediterranean samples. Samples from each basin formed distinct populations, and the Mediterranean samples split into a western, Adriatic and eastern group (Fig. 2E, F). Samples from Morocco (MRBT and MKS1) clustered with the Atlantic lineage while the Murcia (EMUR) sample clustered with the Medi-

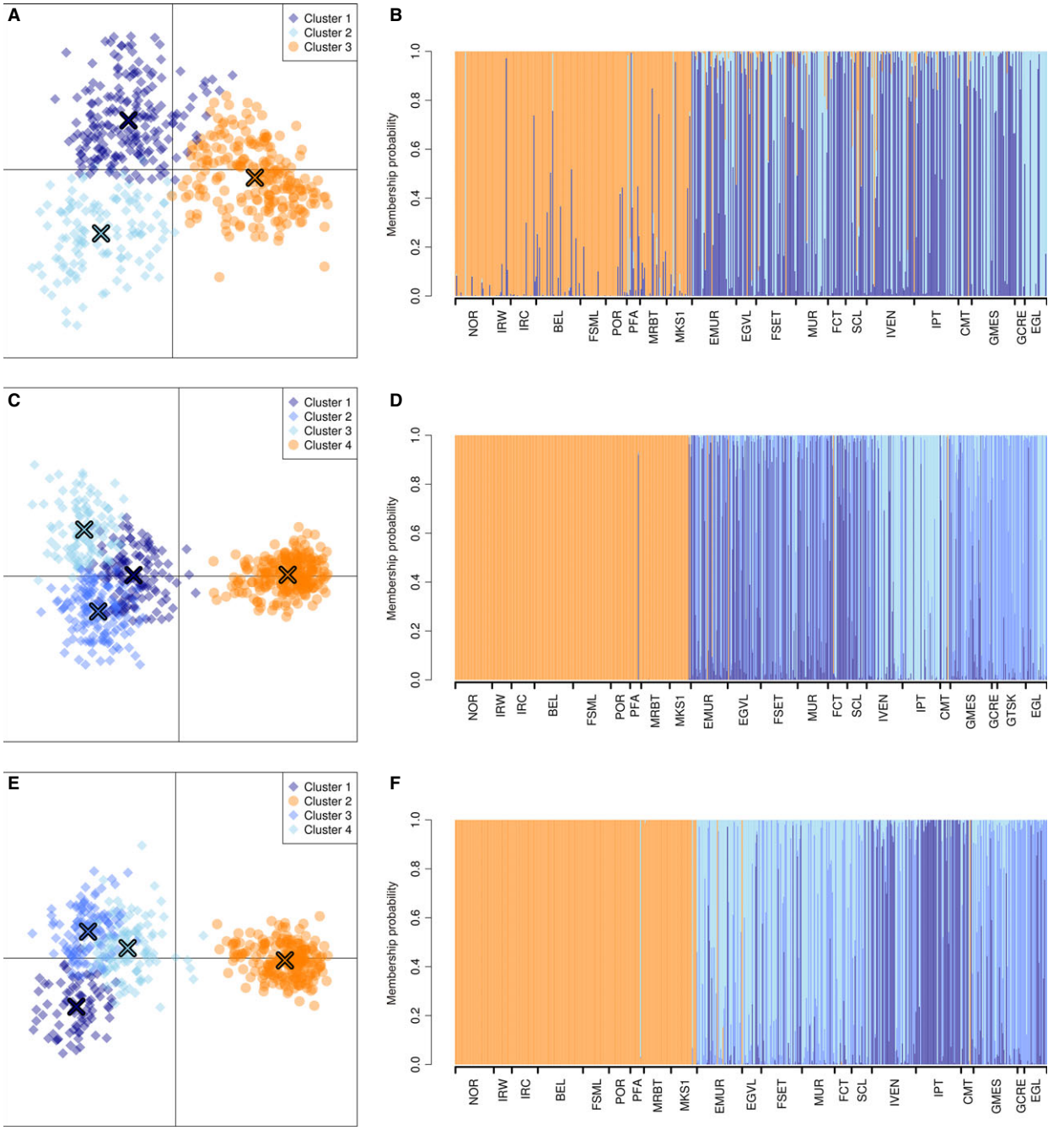

Figure 2. DAPC-based clustering of microsatellite genotypes (A and B), SNP genotypes (C and D) and the combined microsatellite and SNP genotypes (E and F) of European sea bass. A, C and E, the DAPC plot of the clusters; B, D and F, the results for membership probability per individual organized by sampling site (see Table 1 for codes). For full colour representation see the online version of the paper.

terranean lineage. Genotypes of a few individuals from Valencia (EGLV) and Sète (FSET) clustered with the Adriatic group.

Mantel tests indicated that Atlantic samples were isolated by distance (microsatellites: $Z = 196\ 260$, $r = 0.379$, $P = 0.04$; SNPs: $Z = 653\ 598$, $r = 0.683$, $P = 0.0001$). The correlation of genetic distances to geographical distances remained significant when controlling for latitude (microsatellites: $r = 0.371$, $P = 0.04$) and longitude (microsatellites: $r = 0.388$, $P = 0.03$; SNPs: $r = 0.799$, $P = 0.003$). There was a measurable effect of the south-east Atlantic samples

of Rabat (MRBT) and Ksar es-Seghir (MKS1) as none of the correlations remained significant after their removal (Supporting Information, Table S6).

Genetic and geographical distances of the Mediterranean samples (microsatellites: $Z = 1710\ 752$, $r = 0.509$, $P = 0.04$; SNP: $Z = 4309\ 638$, $r = 0.643$, $P = 0.0006$) and Eastern Mediterranean samples (microsatellites: $Z = 283\ 369$, $r = 0.899$, $P = 0.04$; SNP: $Z = 830\ 887$, $r = 0.815$, $P = 0.02$) were correlated but not the Western Mediterranean samples (microsatellites: $Z = 165\ 783$, $r = 0.167$, $P = 0.21$; SNP: $Z = 65\ 774$, $r = -0.221$, $P = 0.01$). None of the correlations was significant when controlling for either latitude or longitude in the case of the microsatellites (Supporting Information, Table S6).

Detection of outlier loci

The number of comparisons in which loci were detected under selection was lower than the number of false positives expected with a false positive rate of 0.05 in all LnRH tests. Global outlier tests by lineage showed weak signatures of directional selection at 2/0/9 microsatellite and 6/0 SNP loci with lnRH/Lositan/Bayescan and Lositan/Bayescan, respectively, in the Atlantic Ocean (Table 2). The signal was stronger in the Mediterranean lineage at 6/3/9 and 3/1 with lnRH/Lositan/Bayescan and Lositan/Bayescan, respectively. Because of the sharp distinction between the two Mediterranean basins, the Mediterranean samples were analysed separately in a Western and an Eastern Mediterranean group. Here the significant outlier tests were 2/0/4 and 2/0 for the Western Mediterranean and 3/1/1 and 1/1 for the Eastern Mediterranean, respectively. As the number of comparisons in which outlier loci were detected was smaller than the number of expected false positives, those loci are probably not under directional selection. Among the SNP loci, just one locus (*SL-UTR1*; somatolactin; LG 13) was identified as a strong candidate in the Mediterranean lineage and in the Eastern Mediterranean group. Unlike elsewhere, frequencies of allele *SL-UTR1-1* were higher than 0.3 in the Adriatic Sea and Northern Mediter-

Table 2. SNP and microsatellite loci detected under putative directional selection by the software packages Lositan and BayeScan in Atlantic, Mediterranean, Western Mediterranean and Eastern Mediterranean samples of European sea bass. Results from the LnRH tests are shown for microsatellites as the number of combinations in which loci were detected under selection (only numbers > 2 are reported). Loci indicated by asterisks were detected with a confidence interval of *99 and **99.5% for Lositan and *95 and **99% for BayeScan. Results are listed as LnRH/Lositan/BayeScan for microsatellites and Lositan/BayeScan for SNPs. Outlier loci detected by at least two approaches are indicated in bold.

	Locus	Atlantic Ocean	Mediterranean Sea	Western Mediterranean Sea	Eastern Mediterranean Sea
Microsatellites	DLA0016	–/–/**	–/**/–		
	DLA0119	–/–/**	**2**/*/–		
	DLA0248		**7**/**/–		**2**/**/–
	DLA0110	4/–/–	–/–/**		
	DLA0008	**6**/–/**	**3**/–/**	–/–/**	**2**/–/**
	DLA0145	–/–/*	**3**/–/**	**3**/–/–	
	DLA0142	–/–/**	**5**/–/**	–/–/**	**2**/–/–
	DLA0146	–/–/**	**2**/–/**	**2**/–/**	
	DLA0140	–/–/*	–/–/**		
	DLA0020	–/–/**	–/–/*		
	DLA0105	–/–/**	–/–/**	–/–/**	
	DLA0228		–/–/*		
SNPs	Dl_21p3	*/–			
	Dl_26f8	**/–	**/–	**/–	
	Dl_32m8	**/–			
	Dl_36d23	**/–			
	Dl_38e22	**/–			
	ILB1-int2	**/–			
	SL-UTR1	**/**		**/*	
	SOX10		**/–	*/–	
	YY		*/–	**/–	

ranean Sea. As aquaculture escapees have been identified in the Bardawil (Bahri-Sfar *et al.*, 2005) and are suspected in the Messolonghi sample (Dimitriou *et al.*, 2007), the analysis was repeated without these samples. The LOSITAN analysis still recognized locus *SL-UTR1* as an outlier (data not shown). Its allele frequencies consistently grouped in an Eastern and Western Mediterranean area. An analysis of genetic structure without the *SL-UTR1* locus resulted in the same results as mentioned above.

DISCUSSION

In European sea bass, a set of 14 microsatellite and 46 SNP markers yielded well-known and new patterns of intrapopulation genetic differentiation. As expected, there is a clear difference between the Atlantic and Mediterranean lineage. Newly found is that the Atlantic lineage has a weak structure; it is introgressed in the south-eastern range of the Atlantic Ocean by the Mediterranean lineage. Sea bass inhabiting the Mediterranean basin is structured into three groups. The western Mediterranean population is homogenously structured, while the Eastern Mediterranean shows evidence of isolation by distance. Signatures of selection at two microsatellite loci and one SNP locus associated with the 3′ untranslated region of the somatolactin gene characterize the Eastern Mediterranean group.

THE POWER OF SNP AND MICROSATELLITE MARKERS TO DETECT HISTORICAL AND CONTEMPORARY SPATIAL PATTERNS

Our study combines a set of 46 mapped and annotated SNPs with an established resource of 14 mapped anonymous microsatellite markers (Chistiakov *et al.*, 2005, 2008). SNPs and microsatellite markers are firmly established in population genetics, each having distinct and complementary characteristics (Morin, Luikart & Wayne, 2004; Helyar *et al.*, 2011; Hagen *et al.*, 2013). With SNPs outperforming microsatellites at a finer scale in non-model organisms, greater power has been achieved to discriminate populations with SNP and microsatellite markers combined (Hess, Matala & Narum, 2011) or with genome scans based on thousands of SNP markers (Corander *et al.*, 2013; Roesti *et al.*, 2014). Overall, our 14 microsatellites and 46 SNPs harbour complementary information on genetic diversity and structure, with SNP observed heterozygosities being on average three times lower, estimates of F_{IS} on average comparable although not always congruent, and F_{ST} values on average several times higher between basins. Our values of genetic differentiation at the microsatellite loci (overall $F_{ST} = 0.041$) match published data of European sea bass (Lemaire *et al.*, 2005; Fritsch *et al.*, 2007; Coscia & Mariani, 2011; Quéré *et al.*, 2012). SNP loci show higher values (overall $F_{ST} = 0.194$) although three to four times lower than for mtDNA (Lemaire *et al.*, 2000; Coscia *et al.*, 2012). SNP genotypes separated the North African samples from the other Atlantic samples and distinguished better between Adriatic, and Western and Eastern Mediterranean samples. The combination of microsatellites and SNP genotypes provided a similar picture as the SNP genotypes, although the pattern changed somewhat in the Mediterranean Sea.

The power of markers required to discriminate among 'open' marine populations is linked to their characteristics and the degree of differentiation. Increasing the number of markers from tens of microsatellites to thousands of SNPs has enhanced the resolution and confidence in individual genotypes (Novembre *et al.*, 2008), although small numbers may suffice (Provan *et al.*, 2013), especially in the case of SNPs (Willing *et al.*, 2012). New genomic venues have been opened with a recent study of European sea bass based on 234 148 SNPs isolated through restriction enzyme associated DNA (RAD) genotyping (Tine *et al.*, 2014).

GENETIC PATTERNS IN THE ATLANTIC OCEAN

Detecting subtle genetic structure in outbred marine species with large effective population sizes requires careful analysis of neutral and non-neutral genetic variation to understand the balance between geographical fragmentation, connectivity and adaptation (André *et al.*, 2011; Hemmer-Hansen *et al.*, 2013; Pujolar *et al.*, 2014). In agreement with the literature on European sea bass (see Introduction) we found evidence for a sharp separation between the Mediterranean Sea and Atlantic Ocean with microsatellite and SNP markers alike. Separation has been attributed to vicariance during the Pleistocene due to changing sea levels and hydrodynamic patterns, and shifting climate zones. Genomic analysis has revealed secondary nuclear introgression from the Atlantic to the Mediterranean lineage (Tine *et al.*, 2014) and cytoplasmic secondary introgression from the Mediterranean to the Atlantic lineage (Lemaire *et al.*, 2005) following re-established contact in the Holocene. The AOF functions as a barrier, separating cold and less saline Atlantic water from denser Mediterranean water masses. Many, although not all, marine taxa are affected by this strong environmental barrier (see review of Patarnello *et al.*, 2007).

Thus far, no distinct genetic spatial pattern of the Atlantic lineage has been detected, although a large

latitudinal range from 33°N (southern Morocco) to 60°N (Norway) was sampled. Southern populations did not seem to have retained an ancestral identity under the influence of latitudinal range shifts during the Pleistocene. Kettle *et al.* (2011) identified in the south-eastern Atlantic Ocean two refuges: the Azores, Canaries and north-west Africa, and the Atlantic Iberian peninsula (Roman & Palumbi, 2004; Chevolot *et al.*, 2006; Borrero-Perez *et al.*, 2011; Xavier *et al.*, 2011). The weak pattern judged from F_{ST} values, tests for isolation by distance and assignment analysis do not suggest vicariance but gene flow of nuclear genetic material from the Mediterranean Sea into the Atlantic Ocean (as observed at Rabat and Ksar es-Seghir). While Tine *et al.* (2014) document the introgression of the Atlantic nuclear genome into the Mediterranean, our evidence points to introgression in the opposite direction. This is in agreement with previous studies (Lemaire *et al.*, 2005; Coscia & Mariani, 2011; Coscia *et al.*, 2012), which identify introgression of mitochondrial genomes from the Mediterranean Sea in the Atlantic Ocean. This raises an interesting question on the permeability of the AOF system, the stability of which is influenced by seasonal variation in coastal currents. There is no further evidence for sea bass in the literature as unfortunately the coast of North Africa remains vastly undersampled (but see Bonhomme *et al.*, 2002; Lemaire *et al.*, 2005; Naciri *et al.*, 1999).

European sea bass has the capacity to migrate up to several hundred kilometres to the spawning grounds along the North-eastern Atlantic coasts (Pickett & Pawson, 1994; Fritsch *et al.*, 2007; Pawson *et al.*, 2008). The last-named authors attribute the lack of genetic differentiation to low-level exchanges between populations. Also, Coscia & Mariani (2011), covering sea bass populations from the Bay of Biscay up to Norway, revealed a homogeneous genetic structure. However, the microsatellite-based genotypes integrate historical (Holocene) gene flow and contemporaneous exchange, and are limited in their power to separate populations. With non-neutral markers differentiation at a finer resolution can be detected. For example, Quéré *et al.* (2010) found the somatolactin (SL) gene to differentiate the Bay of Biscay from the southern North Sea while other loci associated with candidate genes did not. Although the SL locus was included in our study, albeit genotyped with a different marker, we could not confirm the latter pattern.

The conservation implications of the weakly structured and heavily exploited sea bass populations are two-fold. First, although we find no genetic differentiation, we favour the delineation of spawning stocks (Pawson, Kupschus & Pickett, 2007a; Reiss *et al.*, 2009). Hence, we concur with Pawson *et al.* (2007b) to assign stock units in the North Sea, the Eastern English Channel, the Western English Channel, and the combined Irish and Celtic Sea. We propose three additional stocks: the Bay of Biscay following Quéré *et al.* (2010), the coasts off Portugal to Morocco and the Alboran Sea (this study). We speculate that the so far unexplored transition zone between north-west Iberia (Neiva *et al.*, 2012) and Cape Sagres (Portugal) (Martinez *et al.*, 1991; Castilho & McAndrew, 1998) might also play a role in stock delineation. As stock assessments under the guidance of the International Council for the Exploration of the Sea (ICES) have only recently been introduced for the data-limited stocks of sea bass, management measures have been implemented under the precautionary rule (ICES, 2014). It is expected that with access to numerous high-resolution markers (Tine *et al.*, 2014; project AQUATRACE – https://aquatrace.eu) the subtle genetic pattern of European sea bass will become better understood.

The second aspect of conservation relates to climate change and its effects on the population dynamics at the border of the distribution range. European sea bass has steadily expanded its range into northern Atlantic waters (Pawson *et al.*, 2007a) in response to changing local conditions (Davis & Shaw, 2001; Beaugrand *et al.*, 2013; Cheung, Watson & Pauly, 2013). There is also the putative disappearance of populations in the southern range, although without any firm evidence due to limited research in North African waters. Here the unique south-eastern population merits close attention because global change impacts dramatically populations at the southern border of their distribution range (Xavier *et al.*, 2011; Provan & Maggs, 2012).

GENETIC PATTERNS IN THE MEDITERRANEAN SEA

Two main physical features have shaped the biogeography of the oligotrophic Mediterranean Sea. First, the Western and Eastern Mediterranean Sea have been influenced by different oceanographic conditions throughout the Pleistocene and Holocene (Patarnello *et al.*, 2007). Geographically separate units (refuges) have led to incipient allopatric speciation as observed in the phylogeographical and phylogenetic patterns of taxa such as the fan mussel *Pinna nobilis* (Sanna *et al.*, 2013), the seagrass *Posidonia oceanica* (Arnaud-Haond *et al.*, 2007) and *Pomatoschistus* gobies (Larmuseau *et al.*, 2010; Mejri *et al.*, 2011). Second, water masses vary in salinity and temperature along a west to east gradient and have been influencing spatial divergence, for example in European hake *Merluccius merluccius* (Milano *et al.*, 2014) and Atlantic bluefin tuna *Thunnus thynnus* (Riccioni

et al., 2013). In the case of sea bass, Pleistocene cycling does not seem to have had that much influence while contemporary environmental features have impacted Mediterranean populations to some degree. They are fragmented into three slightly differentiated groups, each of them associated with a subbasin. As expected, European sea bass samples split into a eastern and western group, each linked to the Eastern and Western Mediterranean basin, respectively. The Siculo-Tunisian transition represents the boundary between both, similar to several other taxa such as seagrass *Posidionia* (Serra *et al.*, 2010) and the goby *Pomatoschistus tortonesei* (Mejri *et al.*, 2009). The separation of sea bass fits with previous evidence from allozyme markers (Allegrucci *et al.*, 1997) and microsatellite markers (Bahri-Sfar *et al.*, 2000; Quéré *et al.*, 2012). Interestingly, mtDNA polymorphism and differentiation do not differ between basins (Rondon, 2011).

European sea bass caught in the Western Mediterranean Sea show genetic homogeneity (García de León *et al.*, 1997; Naciri *et al.*, 1999; Lemaire *et al.*, 2005), a feature that has been attributed to hybrid swarming (Quéré *et al.*, 2012). Allopatric populations of the Atlantic Ocean and Eastern Mediterranean Sea seem to have come into secondary contact in the Western Mediterranean Sea and introgressed asymmetrically (Bierne *et al.*, 2011; Tine *et al.*, 2014). This is clear in the allelic profile and the genomic architecture.

The second group, inhabiting the Adriatic basin, has been overlooked previously, largely because no sample from that region had been incorporated in the analyses (but see Bahri-Sfar *et al.*, 2000). The subtle differentiation shows a pattern of isolation by distance probably in response to the local physical oceanography. The Pelagosa Sill and Strait of Otranto have been determining factors in isolating the biota of the Adriatic Sea by forming a northern and central cyclonic gyre. For example, the distribution of the Mediterranean shore crab *Carcinus aestuarii* fits the current pattern and splits into three populations, each matching with a gyre (Schiavina *et al.*, 2014). However, European sea bass inhabiting the Adriatic Sea did not show evidence of additional subdivision.

European sea bass shows more genetic structure in the Eastern Mediterranean Sea than in any other basin, although some small sample sizes could have influenced the outcome. The Messolonghi fish has two genetic backgrounds, which might be attributed to escapees from local aquaculture (Dimitriou *et al.*, 2007). The above average genetic distances observed by (Bahri-Sfar *et al.*, 2000; Castilho & Ciftci, 2005; Quéré *et al.*, 2012) fit the isolation by distance pattern we observed. An alternative interpretation that local populations bear the impact of a fragmented geography at low sea-level stands during the Pleistocene and hence changing currents and water masses (Rohling *et al.*, 2014) seems less likely. Unlike the mitochondrial haplotypes, only the microsatellite and SNP genotypes show some structure. Also, the low number of microsatellite markers used in earlier studies might have artificially inflated differentiation.

A possible role for a candidate gene: somatolactin

An interesting outcome is the geographical distribution of the outlier locus SL. It is increasingly appreciated in natural populations that specific genomic regions underlie variation in adaptive traits and hence may be used to delineate population units (Funk *et al.*, 2012). In particular non-neutral markers, often linked to and possibly affected by local environmental conditions, have been effective in revealing subtle patterns in the ocean with its high potential for advection and animal dispersal (Cimmaruta, Bondanelli & Nascetti, 2005; Nielsen *et al.*, 2012; Hemmer-Hansen *et al.*, 2014). The challenge has been to link allelic variance and phenotypic change functionally, either through linkage analysis (quantitative trait loci), ecological genetic approaches or combined field- and lab-based studies. Increasingly cases with good evidence of genes implicated have been documented (e.g. Larmuseau *et al.*, 2009; Williams & Oleksiak, 2011; Eizaguirre *et al.*, 2012; Jones *et al.*, 2012). While so far only the *EIF3E* marker gene has been identified to play a role in local adaptation of sea bass to salinity (Guinand *et al.*, 2015), evidence of the growth hormone, prolactin and SL genes on a regional scale (Quéré *et al.*, 2010) has been criticized on the grounds of experimental bias (Guinand *et al.*, 2015). However, the SL hormone appears also in our genome scan.

The hormone SL is expressed in the pituitary and involved in a broad spectrum of functions including maturation, calcium regulation, body-colour regulation, lipid metabolism, cortisol secretion *in vivo*, acid–base regulation, fat metabolism and background adaptation (e.g. Kaneko & Hirano, 1993; Vargas-Chacoff *et al.*, 2009). It plays a significant role in the osmoregulation of sea bass (Varsamos *et al.*, 2006). The architecture of the sea bass somatolactin gene (*dlSL*) includes five exons and a promoter region with a polymorphic simple sequence repeat (SSR) and several transcription factor binding sites (including *cis*-regulatory elements such as Pit-1a) (Quéré *et al.*, 2010). SNP *SL-UTR1* genotyped in this study is located in the 3′ untranslated region, 220 bp from the end of the coding sequence. A second SNP was found 82 bp from the first SNP.

While SSR polymorphism might modulate SL expression, other elements located either upstream of the promoter or elsewhere in (non-)coding regions might also play a role. In this study, the significant differences between the Eastern and Western Mediterranean populations at the SL locus originate from higher values of the A allele in the Adriatic, Ionian and Aegean Sea, which are regions of more variable salinities along the longitudinal salinity gradient. Quéré *et al.* (2012) reported a clinal pattern of genetic differentiation possibly supporting adaptive variation at one single anonymous microsatellite locus (*DLA0068*) among the 21 loci of sea bass studied (but see Guinand *et al.*, 2015). The only other documented case in the Mediterranean Sea suggests that European hake carrying the $Gapdh^{120}$ and $Gpi2^{96}$ alleles might be better adapted to salinity (Cimmaruta *et al.*, 2005). However, given the long separate histories in the Mediterranean and Atlantic basins under diverse environmental conditions, sea bass have evolved two phenotypes (Gorshkov *et al.*, 2004; Mylonas *et al.*, 2005; Vandeputte *et al.*, 2014) with a distinct genomic architecture (Tine *et al.*, 2014).

Two points should be mentioned with regards to the management of the sea bass stocks. First, management of the commercial and recreational fishery should take into account the (genetic) stock structure revealed here. While this study identified three genetically distinct stocks in the Mediterranean Sea, the eastern Mediterranean basin and the Black Sea might harbour additional diversity. Accordingly, species-specific quota and ecosystem-based management should account for this by implementing at least three management units: Western Mediterranean Sea, Eastern Mediterranean Sea and Adriatic Sea.

Second, massive anthropogenic impacts on the natural populations of European sea bass raise major concerns. Expanding aquaculture in sea pens is associated with massive escapes (Arechavala-Lopez *et al.*, 2011), a phenomenon also observed in other marine fish (Jørstad *et al.*, 2008). Overall documentation is poor, even though there are testimonies and occasional records based on ecological (Toledo-Guedes, Sanchez-Jerez & Brito, 2014) and genetic evidence (Bahri-Sfar *et al.*, 2005; Katsares *et al.*, 2005; Triantafyllidis, 2007). Concerns for genetic introgression are high because domesticated sea bass have a different phenotypic and genetic profile from their natural conspecifics. This is either because of the source of the stock or because of intensive selection. As a consequence, reared fish may be sources of local infections (Arechavala-Lopez *et al.*, 2013) and interbreeding with wild conspecifics, especially in the vicinity of the spawning grounds. We could not test for escapees because our analysis did not include reference samples from aquaculture and our genome sampling is too sparse to detect hybrids.

In conclusion, two distinct lineages of European sea bass inhabit the Atlantic Ocean and Mediterranean Sea. Within each basin isolation by distance has further shaped the spatial structure; the pattern is weak in the Atlantic Ocean and shows evidence for three divisions in the Mediterranean Sea, probably the result of geographical isolation. Further genomic studies might resolve this question, together with other open questions related to the nature of introgression in the Atlantic–Mediterranean contact zone, adaptation to longitudinal and latitudinal gradients, the relationship between lagoon and offshore populations, and the impact of fishing pressure.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. STRUCTURE bar graphs for 536 individuals of European sea bass showing membership to k = 2, 3 and 4 clusters. Each vertical bar represents an individual genotyped at 14 microsatellite loci, and each colour a cluster. For full colour representation consult the e-paper.

Figure S2. STRUCTURE bar graphs for 644 individuals of European sea bass showing membership to k = 2, 3 and 4 clusters. Each vertical bar represents an individual genotyped at 46 SNP loci, and each colour a cluster. For full colour representation consult the e-paper.

Figure S3. STRUCTURE bar graphs for all individuals of European sea bass from the Atlantic Ocean showing membership to k = 2 and 3 clusters genotyped at (A) 14 microsatellite (N =214) and (B) 46 SNP loci (N = 256).

Each vertical bar represents an individual genotyped at 14 microsatellite loci, and each colour a cluster. For full colour representation consult the e-paper.

Figure S4. STRUCTURE bar graphs for all individuals of European sea bass from the Mediterranean Sea showing membership to k = 2, 3 and 4 clusters genotyped at (A) 14 microsatellite (N = 322) and (B) 46 SNP loci (N = 392). Each vertical bar represents an individual genotyped at 14 microsatellite loci, and each colour a cluster. For full colour representation consult the e-paper.

Table S1. Characteristics of the sequences containing microsatellites, including the name of the microsatellite, the functional annotation of the sequence, the linkage group of the sequence in the sea bass genome, the repeat motif, the number of alleles and the GenBank accession number.

Table S2. Characterisation of the sequences containing SNPs by the procedure of development, including the name of the SNP, the functional annotation of the sequence, the SNP annotation (S, synonymous; NS, non-synonymous), the location of the sequence in the sea bass genome (linkage group number), the frequency of the minor allele (MAF) and the GenBank accession number. Loci that were discarded from the analysis because of non-Mendelian inheritance or putative presence of null alleles are indicated by *.

Table S3. Genetic variability and multi-locus F_{IS} estimates at 14 microsatellites of *Dicentrarchus labrax*. A, average number of alleles per locus; H_{exp}, unbiased expected heterozygosity; H_{obs}, observed heterozygosity; F_{IS}, multi-locus F_{IS} estimate (significant values are listed before and after Bonferroni correction underlined and in bold respectively).

Table S4. Genetic variability and multi-locus F_{IS} estimates at 46 SNPs of *Dicentrarchus labrax*. A, average number of alleles per locus; H_{exp}, unbiased expected heterozygosity; H_{obs}, observed heterozygosity; F_{IS}, multi-locus F_{IS} estimate. Significant values before and after Bonferroni correction are listed underlined and in bold respectively.

Table S5. Matrix of pair-wise estimates of F_{ST} (Θ) using all 14 microsatellites of *Dicentrarchus labrax* and sample sites with at least 15 individuals. Significant values before and after sequential Bonferroni correction are indicated underlined and bold respectively. Atlantic and Alboran samples are indicated in italics. For sample codes see Table 1.

Table S6. Results of the Mantel tests for evidence of isolation by distance for microsatellite and SNP loci of European sea bass, including partial Mantel tests when controlling for latitude, longitude, geographic and genetic distances. Z, *m*antel coefficient; r, correlation index; P, significance level (significant values are listed in bold).

Table S7. Matrix of pair-wise estimates of F_{ST} (Θ) using all the 46 SNPs of *Dicentrarchus labrax* and sample sites with at least 15 individuals. Significant values before and after sequential Bonferroni correction are indicated underlined and bold respectively. Atlantic and Alboran samples are indicated in italics. For sample codes see Table 1.

SHARED DATA

Data deposited in the Dryad digital repository (Souche *et al.*, 2015).