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Population-specific variations of the genetic architecture of sex determination in wild European sea bass *Dicentrarchus labrax* L.

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Abstract :

Polygenic sex determination (PSD) may show variations in terms of genetic and environmental components between populations of fish species exposed/adapted to different environments. The European sea bass (*Dicentrarchus labrax*) is an interesting model, combining both a PSD system and a genetic subdivision into an Atlantic and a Mediterranean lineage, with genetic substructures within the Mediterranean Sea. Here, we produced experimental progeny crosses (N = 927) from broodstock sampled in four wild populations (North Atlantic, NAT; Western Mediterranean, WEM; North-Eastern Mediterranean, NEM; South-Eastern Mediterranean, SEM). We found less females than males in the progeny, both in the global dataset (32.5%) and within each paternal group (from 25.1% for NEM to 39.0% for WEM), with significant variation among populations, dams, and sires. Sex, body weight (BW), and body length (BL) showed moderate heritability (0.52 ± 0.17, 0.46 ± 0.17, 0.34 ± 0.15, respectively) and sex was genetically correlated with BW and BL, with rAsex/BW = 0.69 ± 0.12 and rA sex/BL = 0.66 ± 0.13. A weighted GWAS performed both on the global dataset and within each paternal group revealed a different genetic architecture of sex determination between Atlantic and Mediterranean populations (9 QTLs found in NAT, 7 in WEM, 5 in NEM, and 4 in SEM, with a cumulated variance explained of 27.04%, 21.87%, 15.89%, and 12.10%, respectively) and a more similar genetic architecture among geographically close populations compared to geographically distant populations, consistent with the hypothesis of a population-specific evolution of polygenic sex determination systems in different environments.

Introduction

Sex determination is a mechanism of major evolutionary importance that exhibits a high variety of modalities, and this is especially true in fish (Mank et al. 2006; Heule et al. 2014). These modalities are generally classified as genotypic sex determination systems (or GSD, which includes male/female heterogamety and polygenic sex determination, or PSD) and environmental sex determination systems (ESD; Bull 1983).

PSD has been initially formalised by Bulmer and Bull (1982): they proposed an underlying "sex tendency" phenotype, with a polygenic determinism influencing the observed phenotype (male or female) depending on whether it lies below or beyond a fixed threshold. Under this model, any environmental or genotypic effect can equally affect the phenotype (here sex tendency) and bring its value below or beyond the threshold, therefore determining sex.

In the classical view, polygenic sex determination is thought to be unstable, and should evolve either towards GSD in a fluctuating environment generating biased sex-ratios, or towards ESD if some environments increase the fitness of a specific sex (Bulmer and Bull 1982). Modelling approaches have shown that the orientation of polygenic sex determination in one or the other direction depends on complex combinations of environmental variation between and within environmental patches, and on migration rates between patches (Van Dooren and Leimar 2003; Bateman and Anholt 2017). Therefore, starting with the same

ancestral polygenic sex determination system, we may hypothesize that there could be a population-specific evolution of the sex determination mechanism, in other words the components of the model may balance differently between sub-populations of the same species that are exposed to different environmental conditions (Vandeputte et al. 2012; Guinand et al. 2017).

In teleost fish, polygenic sex determination has been well-documented in the Atlantic silverside, *Menidia menidia* (Conover and Heins 1987), some populations of the zebrafish, *Danio rerio* (Liew et al. 2012; Wilson et al. 2014) and in the European sea bass, *Dicentrarchus labrax* (Vandeputte et al. 2007; Palaiokostas et al. 2015). However, some authors consider that it may be more frequent than classically thought (Moore and Roberts 2013) since it is difficult to characterize.

The European sea bass (*Dicentrarchus labrax* L.) offers an interesting model to investigate the evolution of polygenic sex determination. This species combines both a polygenic sex determination system (Vandeputte et al. 2007; Palaiokostas et al. 2015) and a clear genetic subdivision into an Atlantic and a Mediterranean lineage, in addition to population genetic structure within the Mediterranean Sea (Naciri et al. 1999; Bahri-Sfar et al. 2000; Lemaire et al. 2005; Quéré et al. 2012; Tine et al. 2014).

Experimental evidence show that early life rearing temperature has a strong influence on the sex-ratio of sea bass, with low rearing temperature

(between 13 and 17° C) during the first 60 days of life, favouring the production of female offspring (reviewed by Vandeputte and Piferrer 2018), which are preferred in aquaculture due to their higher growth rate (Saillant et al. 2001; Felip et al. 2006). In the natural environment, observed variation in yearly cohort sex-ratio may be indicative of natural variations in temperature influencing sex determination also in the wild populations of sea bass (Vandeputte et al. 2012).

Variation of sex-ratios between families in sea bass is consistent with the hypothesis that phenotypic sex is determined by an underlying continuous sex tendency combining the effects of polygenes and temperature (Vandeputte et al. 2007). However, at least three genome-wide significant sex determining quantitative trait loci (QTLs) were recently found by Palaiokostas et al. (2015), showing that some specific loci could have a stronger effect on sex tendency, at least in some populations (in this case the western Mediterranean).

Here, we hypothesize that the sex determination system of European sea bass could have a different genetic architecture in different populations exposed to different environmental conditions (especially temperature) in the wild. Experimental progeny crosses were produced from broodstock sampled in four wild populations (corresponding to the whole distribution of natural populations, from Northern Atlantic to Eastern Mediterranean Sea). We estimated the additive genetic variation for sex tendency and the

134 correlation between sex tendency and growth-related traits (weight and
135 length) and performed a weighted genome-wide association study
136 (wGWAS), both on the global dataset and within each group of paternal
137 origin, to assess possible population-specific variations in the architecture of
138 sex determination in sea bass.

139 ## Materials and methods

140 ### Broodstock origin, production and rearing of experimental fish

141 The male broodstock used in this study belonged to four different origins,
142 matching with most of the natural range of the species: North Atlantic
143 (NAT), Western Mediterranean (WEM), North-Eastern Mediterranean
144 (NEM) and South-Eastern Mediterranean (SEM). The female broodstock
145 belonged to the WEM population. The origin and collection of the
146 broodstock has been detailed by Vandeputte et al. (2014).

147 The NAT and WEM sires were reared at the IFREMER facilities of
148 Palavas-les-Flots (France) and the sperm stripped and cryopreserved
149 following Fauvel et al. (1998), while the sperm of SEM and NEM sires were
150 both cryopreserved in 2005 at IOLR, Eilat, Israel (SEM sperm) and at
151 Beymelek Lagoon, Turkey (NEM sperm) following the protocol of Sansone
152 et al. (2002). The dams were reared at the IFREMER station of Palavas-les-
153 Flots under natural photoperiod which was 11L:13D at the time of spawning
154 (3rd of March, 2014) and natural temperature, which decreased from 23.5°C
155 in August 2013 to 13.5°C at the time of spawning; dams with a suitable

stage of development of eggs (determined after ovarian biopsy) were hormonally injected and stripped 72 hours after the injection. Artificial fertilization was performed at the IFREMER station, using a full factorial mating scheme: 15 sires per origin (60 sires in total) were crossed with 9 WEM dams. The fertilization protocol and the rearing of experimental fish were described previously by Doan et al. (2017a). Briefly, after hatching, larvae were reared in a common garden at a temperature of 16.5 °C and 25‰ salinity until 58 dph (days post-hatching); the following seven days the temperature was gradually increased to 20 °C. Fish were then reared at a mean temperature of 21.5 °C (18.1-22.4 °C) and 30‰ salinity until 102 dph. Afterwards, fish were divided into five juvenile tanks (A, B, C, D, E), with a mean temperature of 22.1 °C (15.5-27.9 °C). Fish were fed using a classical hatchery feeding sequence (*Artemia* nauplii, Le Gouessant Marine Start and Neo Start pellets). At 180 dph, fish were individually tagged and measured for body weight (BW) and fork length (BL). At the same time, fin samples were collected for genomic DNA extraction. At 226 dph, 927 randomly chosen experimental fish were euthanized with an overdose of benzocaine, dissected, and the sex was recorded by visual observation of the gonads or using the squash technique (Menu et al. 2005) when macroscopic observation was ambiguous. The reliable identification of the phenotypic sex was possible for all 927 fish. Sex was coded as a binary trait, 1 for males and 2 for females.

Genotyping, parentage assignment and descriptive statistics

Fin clips from the 927 experimental fish, from the 60 sires and from the 9 dams were sent to LABOGENA (Jouy-en-Josas, France) for genomic DNA extraction and genotyping. Genotyping was performed with an iSelect Custom Infinium Illumina® European sea bass 3K SNP array. The design of this SNP array was done by selecting 2 722 SNPs from an initial genome-wide variation map containing 2 628 725 SNPs phased into chromosome-wide haplotypes. These SNPs were discovered from 14 wild individuals from both the Atlantic and Mediterranean areas, using whole-genome sequencing as described by Duranton et al. (2018). A first filtering of the SNPs was made to remove variants closer than 80 bp from another known variant. Then, as recommended by Illumina®, we filtered the remaining SNPs to avoid A/T and C/G variants, that need a particular design (Infinium I Probe Design) involving setting up two probes instead of one. Among the remaining candidates, SNPs were chosen to cover all the chromosomes with a variable SNP density depending on the local nucleotide diversity (π), as reported by Tine et al. (2014). To do so, five π-classes were defined depending on the π estimated in non-overlapping 50kb windows: class 1 for $\pi < 10^{-3}$; class 2 for $10^{-3} < \pi < 2 \cdot 10^{-3}$; class 3 for $2 \cdot 10^{-3} < \pi < 3 \cdot 10^{-3}$; class 4 for $3 \cdot 10^{-3} < \pi < 4 \cdot 10^{-3}$; class 5 for $\pi > 4 \cdot 10^{-3}$. Based on "best quality criterion", one SNP was selected in class 1 windows (in interval 22.5-27.5 kb of the window), 2 SNPs in class 2 windows (in intervals 12.5-17.5 kb

and 35-40 kb), 3 SNPs in class 3 windows (in intervals 5.8-10.8 kb, 22.5-27.5 kb and 39-44 kb), 4 SNPs in class 4 windows (in intervals 3.7-8.7 kb, 16.2-21.2 kb, 28.7-33.7 kb and 41.2-46.2 kb) and 5 SNPs in class 5 windows (in intervals 2.5-7.5 kb, 12.5-17.5 kb, 22.5-27.5 kb, 32.5-37.5 kb and 42.5-47.5 kb). Since nucleotide diversity is negatively correlated to the local recombination rate in sea bass (which was estimated by Tine et al., 2014), this local adjustment in the density of SNPs aimed at homogenizing the density of markers along the recombination map instead of the physical map.

Parentage assignment was performed with an exclusion-based software, VITASSIGN (Vandeputte et al. 2006), using 2 722 markers and allowing 29 allelic mismatches to recover pedigree.

Proportion of individuals, males and females in the global dataset, per origin, per tank, per dam and per sire, pairwise comparisons and χ^2 tests were performed in R version 3.4.3 using the packages *stats* (R Core Team 2017) and *gmodels* (Warnes et al. 2015). All *P*-values were adjusted for multiple testing with the Bonferroni correction method.

Principal component analysis

To describe the overall genetic structure among the 927 individuals genotyped on the basis of genome-wide SNP data, we performed a principal component analysis (PCA) using *–pca* function in PLINK (Purcell et al. 2007). A two-dimension scatter plot of individuals coordinates on the two

first principal components was generated, indicating the percentages of variance explained.

Heritability, genetic and phenotypic correlations

Heritability was estimated on the entire dataset through a linear mixed sire model using the software VCE 6.0 (Groeneveld et al. 2010). The model was the following:

$$y_{ijkl} = o_i + t_j + s_{k(i)} + d_l + e_{ijkl}$$

where y_{ijkl} is the phenotype for the studied trait (coded as a binary trait, 1 for male and 2 for female in the case of sex); o_i is the fixed effect of the population of origin of the sires i; t_j is the fixed effect of the rearing tank j; $s_{k(i)}$ is the random additive genetic effect of sire k within origin i; d_l is the random effect of dam l; e_{ijkl} is the random residual.

As explained by Falconer and Mackay (1996), the sire component accounts for ¼ of the additive genetic variance; for this reason, the heritability was estimated as $h^2 = 4\sigma_s^2/\sigma_p^2$, with σ_s^2 being the sire component of variance and σ_p^2 the phenotypic variance. When the trait was sex, heritability on the observed (binary) scale was transformed to the value on the underlying liability scale (Dempster and Lerner 1950; Lynch and Walsh 1998) following the formula:

$$h_u^2 = h_o^2\, p\,(1\text{-}p) / z^2$$

where h_u^2 is the heritability on the liability scale, h_o^2 is the heritability on the observed scale, p is the incidence (proportion of females) in the population

and *z* is the value of the normal distribution density at the point where the cumulative distribution function of the normal distribution reaches incidence.

Genetic and phenotypic correlations between sex and growth-related traits (body weight and length at 180 dph) were assessed using VCE 6.0 software (Groeneveld et al. 2010) applying a three traits sire model with sex, weight and length as variables.

Genome-wide association study (GWAS)

GWAS was performed through the BLUPf90 family of programs for mixed-model computations (Misztal et al. 2015) in order to identify possible SNPs associated with phenotypic sex.

Owing to the genetic subdivision between Atlantic and Mediterranean sea bass lineages and the finer scale differentiation between Western and Eastern Mediterranean populations (Naciri et al. 1999; Bahri-Sfar et al. 2000; Lemaire et al. 2005; Quéré et al. 2012; Tine et al. 2014; Duranton et al. 2018), the dataset was split into four groups depending on paternal origin (NAT, WEM, NEM and SEM) and the analyses were performed separately within each group. The origin of the sire was taken into account as a fixed effect when we performed GWAS on the global dataset.

The raw SNP dataset was quality-filtered before running the GWAS. We firstly removed 8 individuals with a call rate lower than 0.9. We then applied variant filters to exclude SNPs showing either significant Mendelian

distortions, a minor allele frequency (MAF) lower than 0.05, or a proportion of missing genotypes greater than 0.1. This resulted in a final dataset containing 1 205 retained markers out of the total 2 722 SNPs which were genotyped in 919 offsprings and 69 parents (988 individuals in total). The high number of discarded SNPs is mainly due to technical design problems, which lead to target the wrong (i.e. non variable) base position for 50% of the markers. The mapping of the SNPs used for further analyses was thus reported by Doan (2017b), reconstructed from the genotypes of the same individuals.

For the weighted GWAS (wGWAS) the following model was applied:

$$y = \mathbf{X}b + \mathbf{W}u + e$$

where *y* is the vector of phenotypes, *b* vector of the fixed effects (intercept, tank and origin of the sires), ***X*** the incidence matrix relating phenotypes with the fixed effects, ***W*** the incidence matrix relating phenotypes with the random animal effects, u the vector of random animal effects ~ N(0, Gσg2) with ***G*** being the genomic relationship matrix (VanRaden 2008), σg2 the additive genetic variance, e the vector of residuals ~N(0, I) and the residual variance. The genomic relationship matrix ***G*** was established as follow:

$$\mathbf{G} = \mathbf{ZDZ'}/q$$

where ***D*** is a diagonal matrix with weights for SNP effects, ***Z*** is a matrix of gene content adjusted for allele frequencies and *q* is a weighting factor equal to $2\Sigma p_i (1 - p_i)$, where p_i is the MAF of SNP *i*.

The wGWAS was implemented through an iterative process (Zhang et al. 2010; Wang et al. 2012; Zhang et al. 2016) and using Gibbs sampling (THRGIBBS1F90) to estimate the *GEBV*s (genomic estimated breeding values) since this is specifically adapted to the analysis of binary traits. The following steps were performed:

1) in the first iteration, $\boldsymbol{D}$ was first set equal to $\boldsymbol{I}$, the identity matrix (VanRaden, 2008);

2) $\boldsymbol{G}$ was calculated as $\mathbf{G} = \mathbf{ZDZ'}/q$;

3) *DGV*s (direct genomic values) were obtained from *GEBV*s as $DGV_i=-(\Sigma_{j,\ j\neq i}\ g^{ij}GEBV^j/g^{ii})$ with g^{ij} elements of the $\mathbf{G}^{-1}$ matrix (Lourenco et al. 2015), and converted to SNP effects as $a_i = \mathbf{DZ'G^{-1}}DGV_i$;

4) new SNP weights over a 5 SNP window as $d_i=\Sigma_i\ a_i^2/5$ were calculated and normalized so that the total genetic variance remained constant;

5) the process was then iteratively repeated from step 2 with the new $\boldsymbol{D}$ matrix.

After 3 rounds there was no further modification of the variance explained by the SNPs (i.e. the correlation coefficient R^2 between round 1 and 2 was equal to 0.93, 0.97 between round 2 and 3 and 0.99 between round 3 and 4).

We calculated the regional variance explained by summing neighbouring SNP variance in overlapping windows of 5 adjacent SNPs, suggested by Habier et al. (2011) as the more appropriate method to infer the effect of

QTLs. We considered as QTLs the genomic segments that explained a proportion of genetic variance higher than 2%.

Results

Parentage assignment and descriptive statistics

Assignment to a unique parental pair was achieved for all 927 offspring. The number of fish per paternal origin varied from a minimum of 150 (NAT) to a maximum of 328 individuals (SEM). The number of offspring per sire varied from 1 to 36 and the number of offspring per dam varied from 2 to 250.

The proportion of females in the global dataset was 32.5% and was variable among groups of paternal origin (χ^2 = 12.27, df = 3, *P*-value = $7 \cdot 10^{-3}$), ranging from 25.1% for NEM to 39.0% for WEM (Table 1). Pairwise comparisons showed significant sex-ratio differences between NAT and NEM and between WEM and NEM (Table 1).

There were neither significant differences in the proportion of males and females between the five rearing tanks (χ^2 = 9.48, df = 4, *P*-value = $5.02 \cdot 10^{-2}$) nor in the proportion of animals belonging to the four paternal origins between tanks (χ^2 = 16.37, df = 12, *P*-value = $17.47 \cdot 10^{-2}$; Table S1). On the contrary, the proportion of females in the offspring strongly differed per sire and per dam (χ^2 = 124.56, df = 59, *P*-value = 10^{-6} for sires, χ^2 = 34.72 df = 8, *P*-value = $3 \cdot 10^{-6}$ for dams; Fig. 1). The proportion of females ranged from

330 0 to 100% in paternal half-sib families and from 0 to 55% in maternal half-
331 sib families.

332 Female offspring were, on average, heavier and longer than males at 180
333 dph; this was true both for the global dataset and within groups of paternal
334 origin (Table 1).

335 **Principal component analysis**

336 The principal component analysis performed on the global dataset revealed
337 that genotypic variance was mainly explained by paternal origin (first
338 principal component explaining 5.9% of the variance, Fig. 2a). Furthermore,
339 variations among dams were also detected (second axis explaining 4.1% of
340 the variance, Fig. 2b).

341 More precisely, the first PC axis distinguished three groups corresponding
342 to the population of origin of the sires; North Atlantic, Western and Eastern
343 Mediterranean groups were clearly separated, while the difference between
344 North and South Eastern Mediterranean group was more subtle. This
345 stratification explained by the population of origin of the sires was properly
346 taken into account as a fixed effect in the models used to estimate
347 heritability and to perform the wGWAS on the global dataset.

348 **Heritability, genetic and phenotypic correlations**

349 Heritability was moderately high for all variables (sex, body weight and
350 body length at 180 dph, Table 2). The genetic and phenotypic correlations

between sex and growth-related traits were moderately high and the genetic correlations were higher compared to the phenotypic correlations (> 0.65 *vs.* 0.42). The genetic and phenotypic correlations between body weight and length were close to unity (Table 2).

Genome-wide association study (GWAS)

Results from the wGWAS performed on the global dataset identified one major group of SNPs on LG6 explaining up to 3.41% of the variance for sex. Other important groups of SNPs were detected on LG7, LG12, LG15 and LG*x*, explaining up to 2.73% of variance, while a minor group explaining slightly more than 2% of variance was located on LG2 (Fig. 3a; Table 3).

The comparisons of the Manhattan plots of the wGWAS performed separately within each group of paternal origin (Fig. 3b, 3c, 3d, 3e; Table 3), revealed a clear pattern of similarity between samples belonging to adjacent paternal origins. Nevertheless, taking into account genomic regions explaining at least 2% of the variance showed a very variable architecture of sex determination, with some peaks being shared among populations, while others being clearly population-specific (Table 4 and Fig. 4).

A group of SNPs on LG5 explained 4.37% of variance for sex in ♀WEM×♂NAT, 3.12% in ♀WEM×♂WEM and 4.6% in ♀WEM×♂NEM, while in ♀WEM×♂SEM this peak was not observed. Peaks shared only between ♀WEM×♂NAT and ♀WEM×♂WEM were identified on LG8,

explaining 2.18% and 4.06% of the variance, respectively, and on LG19, explaining 4.25% and 2.80% of the variance, respectively. The crossings ♀WEM×♂NAT and ♀WEM×♂NEM shared two peaks on LG9 (2.17% and 2.38% of variance explained, respectively) and on LG10 (4.97% and 3.36% of variance explained, respectively).

One group of SNPs, which was shared between ♀WEM×♂NAT and ♀WEM×♂SEM was identified on LG1B, explaining 2.18% and 3.53%, respectively. One group of SNPs that was in common between ♀WEM×♂WEM and ♀WEM×♂SEM was identified on LG11 (3.48% and 2.54% of variance explained, respectively). Furthermore, ♀WEM×♂WEM cross share two groups of SNPs with ♀WEM×♂SEM cross, on LG7 (variance explained of 2.41% and 2.22%, respectively) and on LG20 (3.47% and 3.78% of the variance, respectively).

The ♀WEM×♂NAT cross showed specific peaks, that were not shared with any other paternal origin, on LG3 (2.41% of variance explained), LG12 (2.51%), LG16 (2.03%). One specific peak was found in the ♀WEM×♂WEM cross, as well (LG4, 2.55% of variance explained).

Two groups of SNPs were identified as specific to the Eastern Mediterranean populations, one in the ♀WEM×♂NEM cross (LG15, 3.04% of variance explained) and one in the ♀WEM×♂SEM cross (LG*x*, 2.59% of variance explained).

Interestingly, we did not identify any sex QTL explaining more than 2% of the variance that was common to all populations.

Discussion

In this study we explored the genetic basis of the sex determination system in the European sea bass by implementing a genome-wide association study approach in a factorial crossing experiment. For the first time, sea bass belonging to different origins across the whole distribution range of natural populations were compared to assess variation in the genetic architecture of sex, including a comparison between the Atlantic and Mediterranean sea bass lineages. We found different QTLs underlying sex determination between Atlantic and Mediterranean populations, with a gradient of similarities from Western to Eastern Mediterranean populations, reflecting the previously documented introgression of Atlantic genes within the Mediterranean genetic background (Guinand et al. 2017, Duranton et al. 2018). This finding is consistent with the hypothesis of a population-specific evolution of polygenic sex determination systems in different evolutionary lineages occupying different environments.

An important result was the increased sharing of QTLs for sex determination in adjacent populations, which could result from an ongoing admixture between two evolutionary lineages (i.e. Atlantic and Mediterranean) characterized by different genetic architectures of sex determination systems. The detected geographical gradient in the

416 architecture, from NAT to SEM, would then reflect the level of
417 introgression and indeed corresponds to the admixture gradient recently
418 found in sea bass population genomic studies (Duranton et al. 2018).
419 The ancestral architecture of the sex determination in sea bass might have
420 evolved differently during the 300 000 years of divergence between Atlantic
421 and Mediterranean lineages, explaining the origin of the variation that now
422 has population-specific influences on sexual determination. Indeed, we did
423 not find any linkage group common to all populations with groups of SNPs
424 explaining more than 2% of the variance, which support the hypothesis put
425 forward by Guinand et al. (2017) that the most important genes affecting sex
426 may differ between sea bass populations.
427 ♀WEM×♂NAT cross showed some similarities compared to
428 ♀WEM×♂WEM and ♀WEM×♂NEM, that have gradually reduced in
429 ♀WEM×♂SEM. This finding can be more likely explained by the recent
430 history of inter-basins connectivity, since Atlantic alleles have been
431 progressively diffused from the Western to the Eastern Mediterranean since
432 the end of the last glacial maximum (Tine et al. 2014; Duranton et al. 2018).
433 The resulting longitudinal gradient of admixture across the Mediterranean
434 populations makes the WEM population (31% of Atlantic ancestry) more
435 similar to the Atlantic than the NEM and SEM population (13% of Atlantic
436 ancestry) in most of the genome (Duranton et al. 2018).

437 Therefore, a gradient in similarity of genomic architecture is expected if
438 sex determination QTLs introgress similarly to neutral genes. We do not
439 reject, however, the possibility that differential adaptations between Atlantic
440 and Mediterranean environments have also contributed to the patterns we
441 observed, although this hypothesis is difficult to distinguish from historical
442 admixture. Finally, the presence of a biogeographical barrier to gene flow
443 located in the Siculo-Tunisian Strait (Quignard 1978; Bahri-Sfar et al.
444 2000), which limits the connections between Western and Eastern
445 Mediterranean, may explain the further reductions of similarities between
446 Western and Eastern Mediterranean populations.

447 In our case, the four paternal groups are all related by the WEM dams (i.e.
448 all the individuals have 50% of the genome coming from WEM), with the
449 result that even the Eastern Mediterranean group contains a higher level of
450 Atlantic ancestry than what is expected in "pure" wild ♀NEM×♂NEM or
451 ♀SEM×♂SEM crosses. This leads to the conclusion that the real differences
452 existing in nature could be even stronger than what we observed here, due to
453 our experimental design.

454 We interpreted our results as differences between male origins with the
455 implicit assumption that they mostly reflect additive QTLs effects from the
456 sire population of origin. Still, some QTLs could be due to the dam
457 population (WEM). This is not the preferred hypothesis as no QTLs are
458 shared between all paternal group, although they all share the same dams.

459 Another possibility is that some of the QTLs observed are not linked to
460 additive genetic variation but to dominance. The higher number of QTLs in
461 the ♀WEM×♂NAT cross could be indicative of dominant alleles involved
462 in sex determination (especially since some heterosis in sex-ratio has been
463 shown by Guinand et al. 2017 when mating Atlantic and Mediterranean
464 individuals).

465 Within each paternal origin, we performed a cross validation analysis of
466 QTLs, by removing four times 25% of the offspring along the second axis
467 of the PCA (representative of variation between dams, see Fig. 2b). Most of
468 the QTLs were identified in all subgroups, suggesting they were linked to
469 the sire origin studied, but several of them were absent in some of the
470 subgroups, which may be indicative of sire origin by dam interaction, i.e.
471 dominance variation (see Supplementary material S2).

472 Parental effects on phenotypic sex were clearly significant: the dams (χ^2 =
473 34.72, df = 8, *P*-value = $3 \cdot 10^{-6}$) and sires (χ^2 = 124.56, df = 59, *P*-value =
474 10^{-6}) variation for the proportion of females in the offspring was strongly
475 different, similar to Vandeputte et al. (2007), where both sires and dams had
476 a similar-size effect on the sex-ratio of the progeny.

477 The heritability of sex tendency we estimated in the present study through
478 a linear mixed sire model was relatively high (h^2 = 0.52 ± 0.17), similar to
479 the estimate obtained for sire heritability by Vandeputte et al. (2007) on a
480 larger dataset consisting of individuals of Northern Atlantic origin (0.52 ±

481 0.13), suggesting that the influence of the genetic and the environmental
482 components on sex-ratio variance should be roughly equivalent.
483 The genetic correlation between sex and growth-related traits was
484 significant (h^2 = 0.69 ± 0.12 between sex and weight, h^2 = 0.66 ± 0.13
485 between sex and length) and higher compared to previous studies (r_A
486 between sex and size in the range of 0.23 and 0.59; Vandeputte et al. 2007;
487 Palaiokostas et al. 2015). Overall, these results confirm the hypothesis of a
488 strong link between genes affecting sex and growth (reviewed by
489 Vandeputte and Piferrer, 2018), with a clear sexual growth dimorphism (at
490 the age of 180 dph, females were 34.4% heavier and 9.63% longer than
491 males).
492 The sex-ratio in the global dataset was strongly skewed towards males,
493 with a percentage of females less than half the percentage of males (32.5%
494 versus 67.5%, respectively). This is consistent with the general observation
495 that cultured sea bass, because of the hatchery environment (especially
496 temperature), show an unbalanced sex-ratio in favour of males (Saillant et
497 al. 2003; Piferrer et al. 2005), different if compared to wild-born sea bass,
498 where in younger fish the sex-ratio seems to be balanced (Vandeputte et al.
499 2012).
500 The percentage of females was slightly higher in Atlantic/West-Med
501 populations compared to Eastern Mediterranean populations, suggesting a
502 possible different tendency in sex-ratio related to the origin of the

503 individuals under aquaculture conditions. This is consistent with the study
504 by Guinand et al. (2017), where the ♀WEM×♂Atlantic and
505 ♀WEM×♂WEM crosses showed a higher mean proportion of females
506 compared to the ♀WEM×♂NEM and ♀WEM×♂SEM crosses.

507 A limitation in our study is represented by the fact that the between-
508 populations variation of sex-ratio could be confounded by non-additive
509 genetic effects, as NAT, NEM and SEM broodstock were used only as sires.
510 As previously reported by Guinand et al. (2017), sex-ratio can show non-
511 additive components of genetic variance, and we have no possibility to
512 disentangle additive and non-additive genetic effects in our case.

513 Finally, a better understanding of the genetic architecture of sex tendency
514 in sea bass could have applications in aquaculture production. European sea
515 bass is one of the most important marine species widely cultured in the
516 Mediterranean areas and represents 49% of the marine Mediterranean
517 aquaculture production (FEAP Annual report 2016). The strong bias
518 towards males under aquaculture condition has been recognised by farmers
519 as a problem for different reasons (lower growth rates of males compared to
520 females, reduced flesh quality and general decrease of the commercial
521 values of the product; Felip et al. 2006). Uncovering the population-specific
522 sex determination system may help to produce stocks with higher
523 proportions of females, through selective breeding and genomic selection.
524 Moreover, the choice of broodstock coming from a specific origin could be

interesting to start new breeding programs, due to the between-population differences in sex-ratio we found.

Data archiving

The dataset underlying our findings is available in the institutional public data repository (SEANOE: http://www.seanoe.org/), http://doi.org/10.17882/55576

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Compliance with ethical standard

Conflict of interest The authors declare that they have no competing interests.

Supplementary information is available at *Heredity*'s website.

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679 **Table 1** Number of individuals (N), sex-ratio, mean body weight (g) and length
680 (mm) at 180 dph (total and separately for males and females) with the coefficient
681 of variation expressed in percentage (CV%); the results are showed for the global
682 dataset and separately per group of paternal origin; different letters indicate a
683 significant difference at $P < 0.05$.

		NAT N = 150	WEM N = 182	NEM N = 267	SEM N = 328	Global N = 927
Sex-ratio	proportion of males	0.620[a]	0.610[a]	0.749[b]	0.677[ab]	0.675
	proportion of females	0.380[a]	0.390[a]	0.251[b]	0.323[ab]	0.325
Mean $BW_{180\ dph}$ (CV%)		17.57 (36.4%)	14.70 (30.3%)	16.22 (34.5%)	16.09 (34.7%)	16.09 (34.7%)
	Males	16.00 (38.4%)	13.02 (28.1%)	14.90 (31.5%)	14.19 (30.5%)	14.48 (32.7%)
	Females	20.11 (29.9%)	17.32 (25.0%)	20.17 (30.8%)	20.08 (28.9%)	19.46 (29.4%)
Mean $BL_{180\ dph}$ (CV%)		113.01 (11.4%)	108.89 (9.4%)	111.48 (10.5%)	110.46 (10.7%)	110.86 (10.6%)
	Males	109.46 (11.4%)	105.23 (8.9%)	108.90 (10.0%)	106.54 (9.4%)	107.50 (9.9%)
	Females	118.79 (9.6%)	114.62 (7.8%)	119.18 (9.0%)	118.68 (9.4%)	117.85 (9.1%)

684

685 **Table 2** Heritability (± *s.e*, in bold on the diagonal) for sex (on the liability scale)
686 and growth-related traits (body weight, BW, and body length, BL, at 180 dph),
687 genetic (± *s.e.*; below the diagonal) and phenotypic correlations (above the
688 diagonal) among traits, estimated with VCE 6.0 (Groeneveld et al. 2010).

Trait	Sex	$BW_{180\ dph}$	$BL_{180\ dph}$
Sex	**0.52 ± 0.17**	0.42	0.42
$BW_{180\ dph}$	0.69 ± 0.12	**0.46 ± 0.17**	0.97
$BL_{180\ dph}$	0.66 ± 0.13	0.99 ± 0.005	**0.34 ± 0.15**

689

690 **Table 3** Identification of European sea bass chromosomes with a QTL explaining
691 more than 2% of the variance in the global dataset and in each of the four offspring
692 groups with the same paternal origin.

	1A	1B	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18-21	19	20	22-25	24	*x*
Global			●				●	●					●			●								●
NAT		●		●		●			●	●	●		●				●			●				
WEM					●	●		●	●			●								●	●			
NEM						●				●	●	●				●								
SEM		●						●													●			●

693

694 **Table 4** Total number of QTL for each paternal origin (in bold on the diagonal),
695 total number of shared QTL between origins (below the diagonal) and total number
696 of QTL that differ between origins (above the diagonal).

	NAT	WEM	NEM	SEM
NAT	**9**	10	8	11
WEM	3	**7**	8	7
NEM	3	2	**5**	9
SEM	1	2	0	**4**

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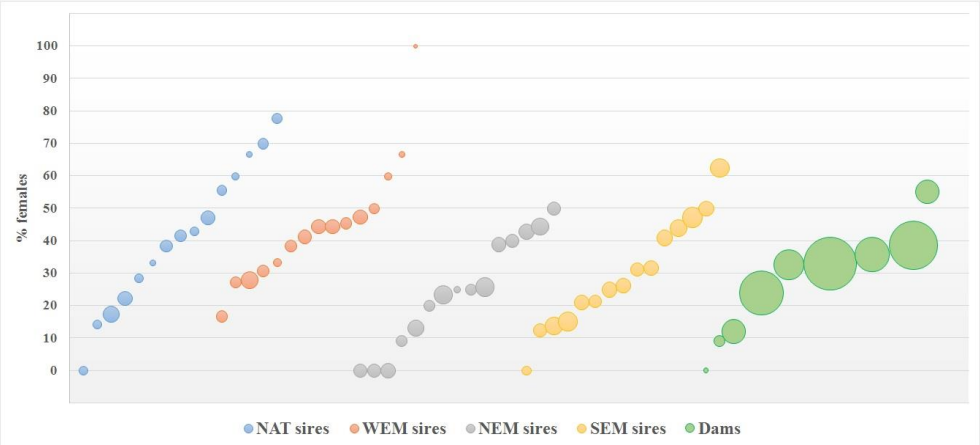

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700 **Fig. 1** Percentage of females per sire and per dam; blue, red, grey and yellow
701 bubbles identify the different origins of the sires, the green ones identify the dams;
702 the size of the bubble represents the total number of offspring per sire/dam.

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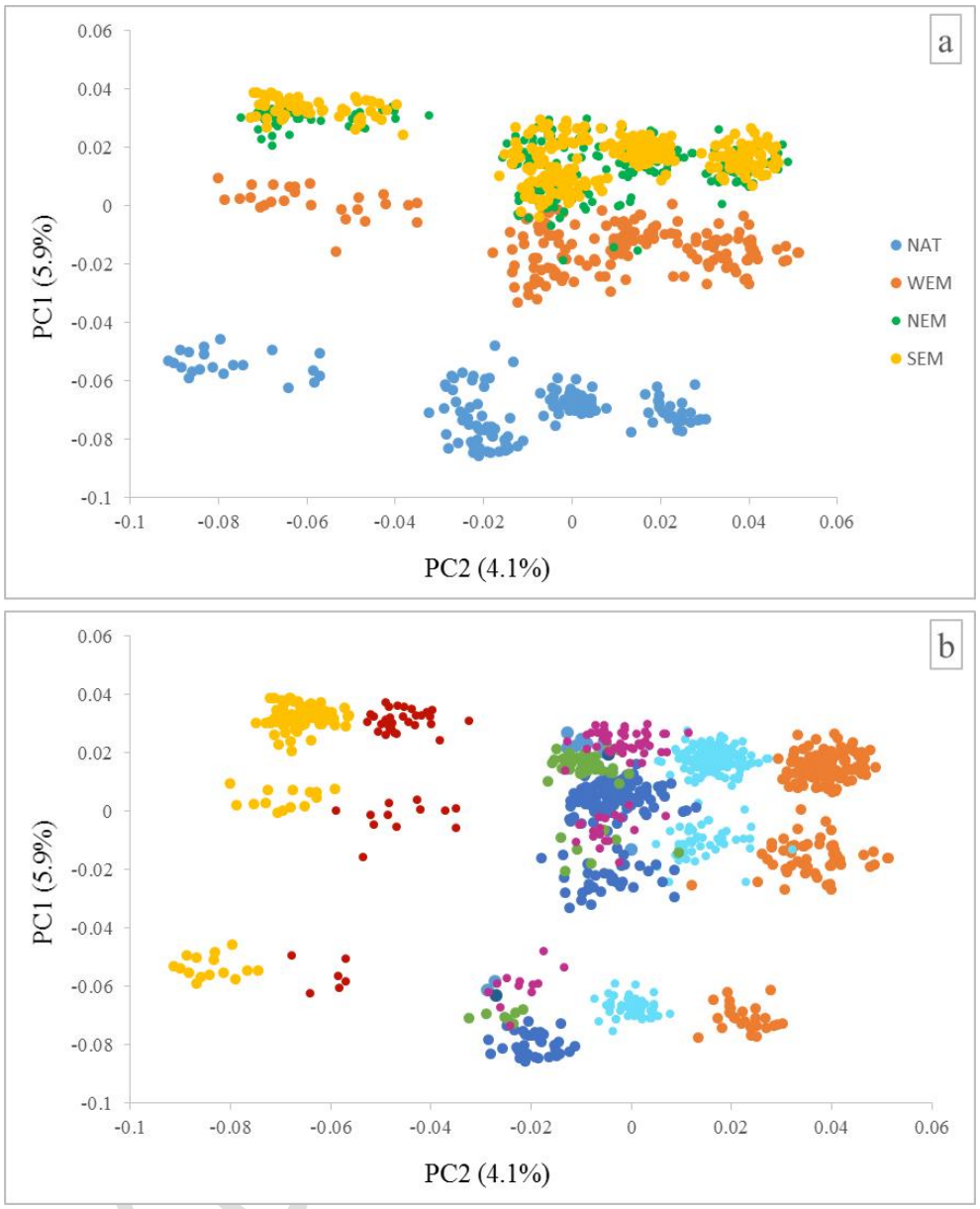

704

705 **Fig. 2** Two-dimension scatterplots showing the population stratification in the
706 global dataset (N = 927) by paternal origin (a) and by dams (b). The first principal
707 component was plotted against the second; the percentages of variance explained
708 by each axis is indicated.

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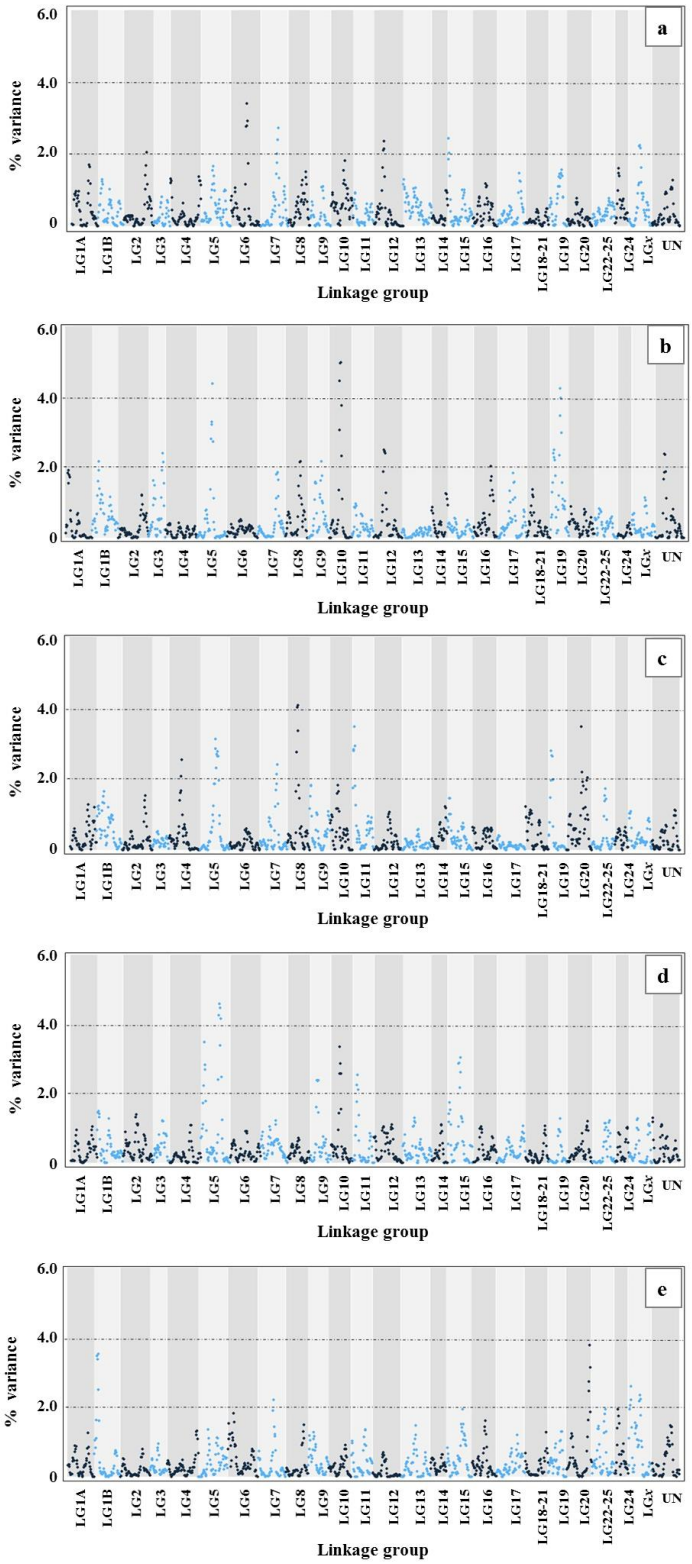


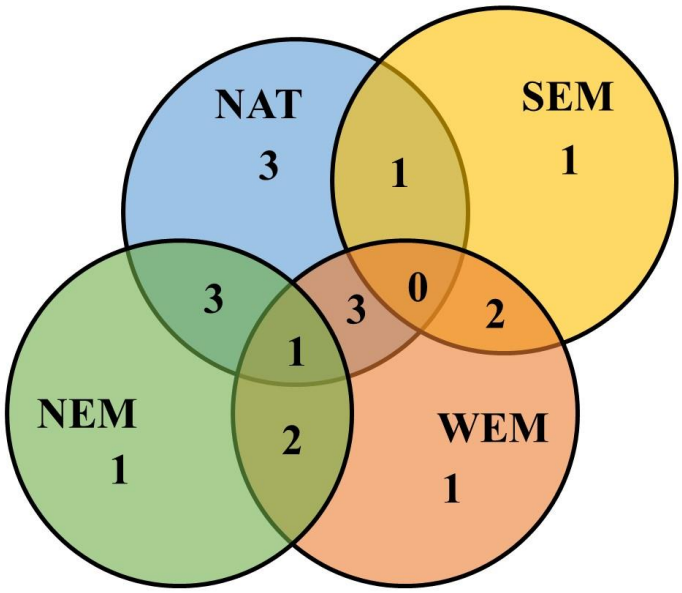
710

711 **Fig. 3** Manhattan plots showing the percentage of variance explained for sex. The

712 results were obtained with a weighted GWAS in a sliding window of 5 adjacent

713 SNPs in BLUPf90 (Misztal et al. 2015). a. global dataset; b. WEM×NAT; c.

714 WEM×WEM; d. WEM×NEM; e. WEM×SEM.



715

716 **Fig. 4** Venn diagram showing the number of QTLs for sex explaining more than

717 2% of the variance that were specific for each paternal group or that were shared

718 between groups of paternal origin.

Supplementary information for "Population-specific variations of the genetic architecture of sex determination in wild European sea bass *Dicentrarchus labrax* L."

Sara Faggion, Marc Vandeputte, Pierre-Alexandre Gagnaire, François Allal

Supplementary Table S1 Proportion of individuals, sex-ratio and proportion of individuals belonging to the different groups of paternal origin (NAT, WEM, NEM and SEM) per each rearing tank (A, B, C, D, E); different letters indicate a significant difference at $P \leq 0.05$.

		Tank				
		A	**B**	**C**	**D**	**E**
Proportion of individuals		0.223[a]	0.180[a]	0.219[a]	0.187[a]	0.191[a]
Sex-ratio	proportion of males	0.667[ab]	0.587[a]	0.729[b]	0.676[ab]	0.706[ab]
	proportion of females	0.333[ab]	0.413[a]	0.271[b]	0.324[ab]	0.294[ab]
Proportion of individuals per origin	NAT	0.097[a]	0.186[a]	0.172[a]	0.168[a]	0.198[a]
	WEM	0.256[a]	0.210[a]	0.172[a]	0.168[a]	0.169[a]
	NEM	0.266[a]	0.251[a]	0.315[a]	0.301[a]	0.305[a]
	SEM	0.382[a]	0.353[a]	0.340[a]	0.364[a]	0.328[a]

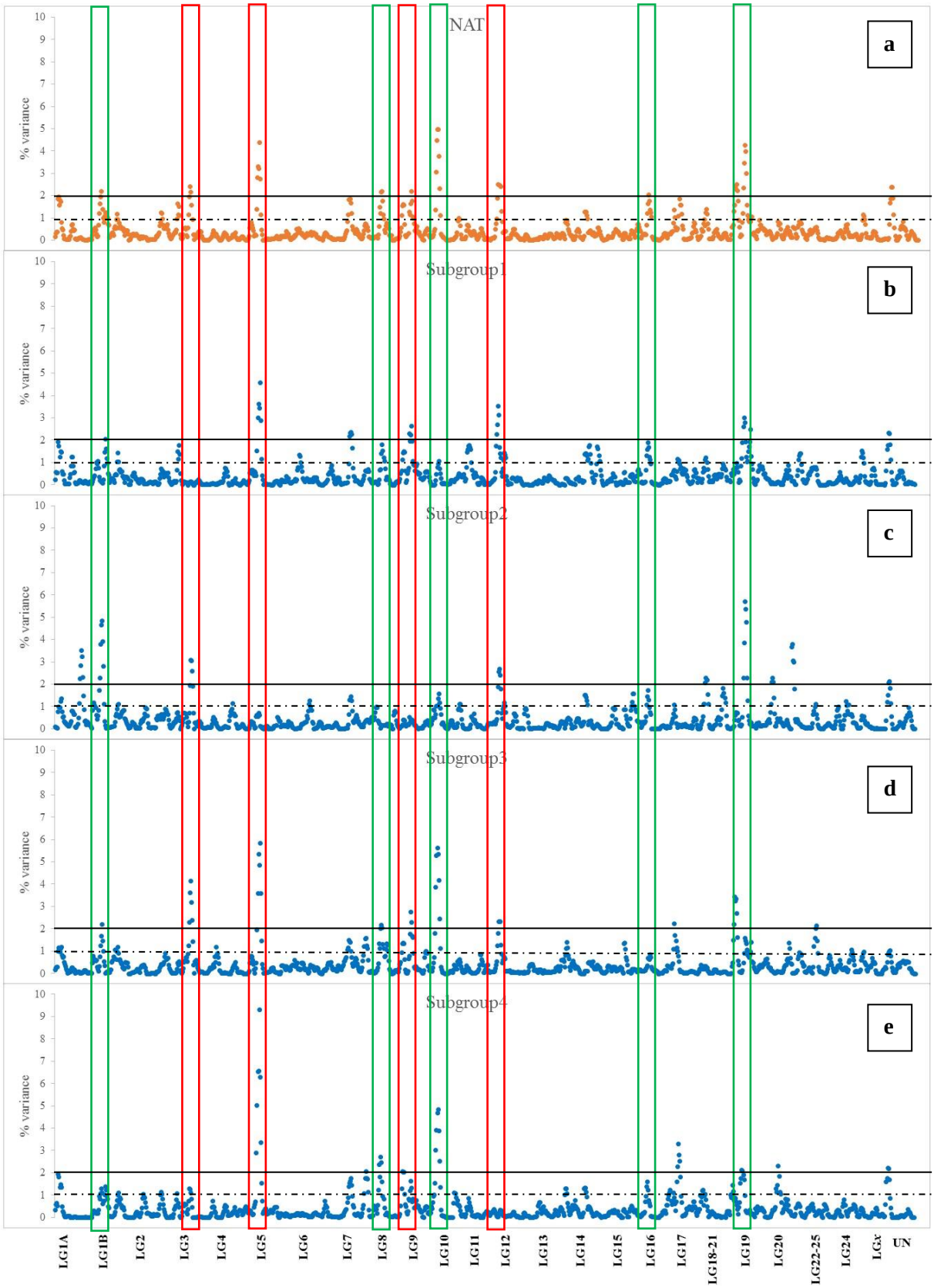
Supplementary material S2 Cross validation of QTLs

Method: in order to assess the stability of the QTLs examined, for each paternal origin, we generated four subgroups of offspring by removing for each 25% of the fish along the second axis of the PCA presented in fig 2b. Fish were then removed along an axis representing variation between dams, but on average all shared the same proportion of the different sires. For each of the subsets, which represented 75% of the offspring of a given paternal origin, we performed the wGWAS through the same iterative process and the same model described in the material and methods.

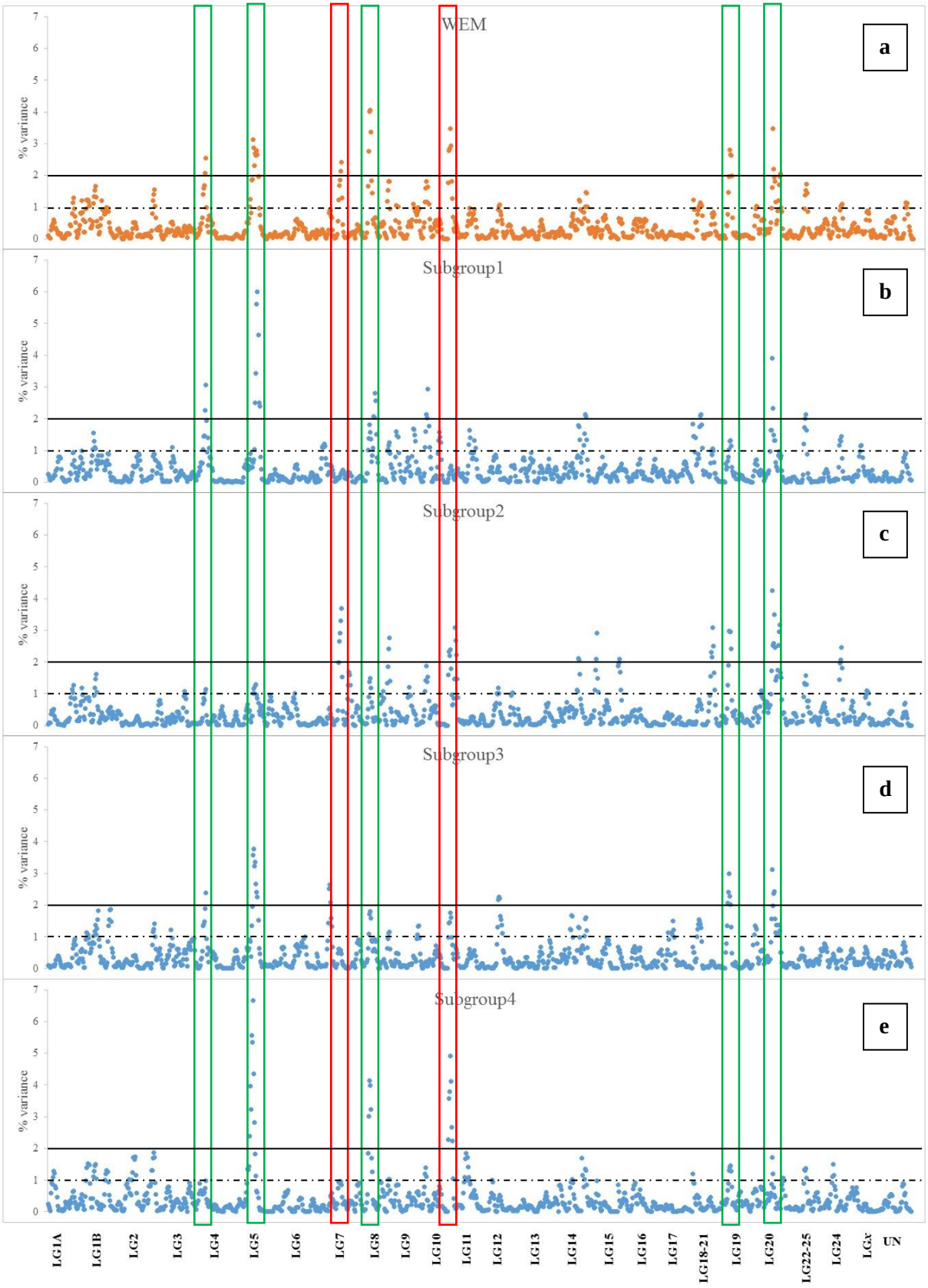
Results: In the following figures (supplementary figures 1 to 4), we compare the QTLs identified on the whole sample of the given sire origin (>2% variance explained) to the peaks observed in the subgroups. QTLs present in the whole sample of the given sire origin (>2% variance explained) and present in all subgroups tested within this population (>1% variance explained) are highlighted in green. In red QTLs absent in at least one of the subgroups.

Discussion: In this cross validation, the subgroups were generated to maximize the genetic divergence (mainly due to the variation between dams) between them. Most of the QTLs found from the offspring of a given sire origin were also observed in all subgroups, suggesting they were linked to the sire origin studied. However, several of them were absent in some of the subgroups and some additional QTLs (>2% variance explained) were detected. This may indicate sire origin by dam interaction, i.e. dominance variation.

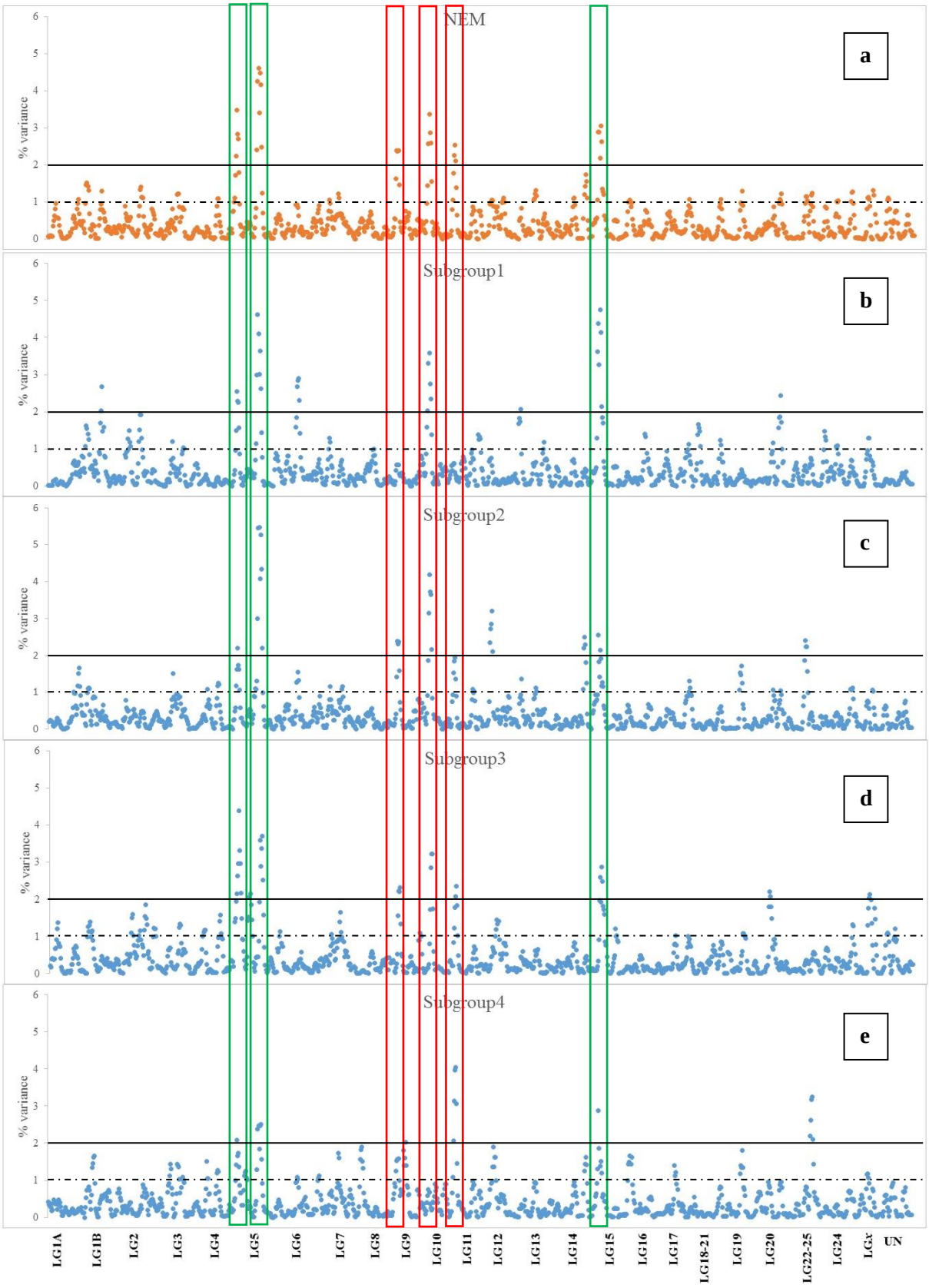
Supplementary Figure 1 Manhattan plots showing the percentage of variance explained for sex in North Atlantic (NAT) progenies. The results were obtained with a weighted GWAS in a sliding window of 5 adjacent SNPs in BLUPf90 (Misztal et al. 2015). a. all NAT sires offspring; b. subgroup 1; c. subgroup 2; d. subgroup 3; e. subgroup 4.



Supplementary Figure 2 Manhattan plots showing the percentage of variance explained for sex in Western Mediterranean (WEM) progenies. The results were obtained with a weighted GWAS in a sliding window of 5 adjacent SNPs in BLUPf90 (Misztal et al. 2015). a. all WEM sires offspring; b. subgroup 1; c. subgroup 2; d. subgroup 3; e. subgroup 4.



Supplementary Figure 3 Manhattan plots showing the percentage of variance explained for sex in Northern-Eastern Mediterranean (NEM) progenies. The results were obtained with a weighted GWAS in a sliding window of 5 adjacent SNPs in BLUPf90 (Misztal et al. 2015). a. all NEM sires offspring; b. subgroup 1; c. subgroup 2; d. subgroup 3; e. subgroup 4.



Supplementary Figure 4 Manhattan plots showing the percentage of variance explained for sex in Southern-Eastern Mediterranean (SEM) progenies. The results were obtained with a weighted GWAS in a sliding window of 5 adjacent SNPs in BLUPf90 (Misztal et al. 2015). a. all SEM sires offspring; b. subgroup 1; c. subgroup 2; d. subgroup 3; e. subgroup 4.