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The contribution of ancient admixture to reproductive isolation between European sea bass lineages

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Understanding how new species arise through the progressive establishment of reproductive isolation (RI) barriers between diverging populations is a major goal in Evolutionary Biology. An important result of speciation genomics studies is that genomic regions involved in RI frequently harbor anciently diverged haplotypes that predate the reconstructed history of species divergence. The possible origins of these old alleles remain much debated, as they relate to contrasting mechanisms of speciation that are not yet fully understood. In the European sea bass (*Dicentrarchus labrax*), the genomic regions involved in RI between Atlantic and Mediterranean lineages are enriched for anciently diverged alleles of unknown origin. Here, we used haplotype-resolved whole-genome sequences to test whether divergent haplotypes could have originated from a closely related species, the spotted sea bass (*Dicentrarchus punctatus*). We found that an ancient admixture event between *D. labrax* and *D. punctatus* is responsible for the presence of shared derived alleles that segregate at low frequencies in both lineages of *D. labrax*. An exception to this was found within regions involved in RI between the two *D. labrax* lineages. In those regions, archaic tracts originating from *D. punctatus* locally reached high frequencies or even fixation in Atlantic genomes but were almost absent in the Mediterranean. We showed that the ancient admixture event most likely occurred between *D. punctatus* and the *D. labrax* Atlantic lineage, while Atlantic and Mediterranean *D. labrax* lineages were experiencing allopatric isolation. Our results suggest that local adaptive introgression and/or the resolution of genomic conflicts provoked by ancient admixture have probably contributed to the establishment of RI between the two *D. labrax* lineages.

KEY WORDS: Ancient admixture, introgression, reproductive isolation, genomic conflicts, marine fish.

Impact Statement

Speciation is often viewed as a progressive accumulation of reproductive isolation (RI) barriers between two diverging lineages through time. However, because it is a progressive process, speciation can once initiated take different pathways, sometimes leading to the erosion of an established species barrier or the acquisition of new speciation genes transferred from another species boundary. Here, we describe such a case in the European sea bass. This marine fish species split 300,000 years ago into an Atlantic and a Mediterranean lineage, which remained partially reproductively isolated after experiencing postglacial secondary contact. For unknown reasons, genomic regions involved in RI between lineages started to diverge well before the split. We here show that diverged alleles were acquired by the Atlantic lineage from an ancient event of admixture with a parapatric sister species about 80,000 years ago. Introgressed foreign alleles that were locally driven to high frequencies in the Atlantic have subsequently experienced reduced gene flow between the Atlantic and Mediterranean populations during the post-glacial secondary contact, thus contributing to increased RI between two sea bass lineages. These results support the view

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that RI barriers can evolve via reticulate gene flow across multiple species boundaries.

Speciation is the evolutionary process that leads to the emergence of new species through the progressive establishment of reproductive isolation (RI) barriers among diverging populations (Coyne and Orr 2004). Identifying those barriers and understanding the eco-evolutionary context in which they evolved has been at the core of the speciation genetics research program (Presgraves 2010; Wolf et al. 2010). Over the last decade, progress in sequencing technologies has allowed important insights into the genetic basis of RI barriers through the study of genome-wide differentiation/divergence patterns among closely related species (Feder et al. 2012; Seehausen et al. 2014; Harrison and Larson 2016; Wolf and Ellegren 2016; Ravinet et al. 2017). An important result of speciation genomics studies is that the age of the alleles located within genomic regions involved in RI is often much older than the average coalescent time computed across the whole genome. This finding indicates that the regions involved in RI tend to be enriched for anciently diverged haplotypes. An example of this comes from the fixed chromosomal inversions involved in RI between *Drosophila pseudoobscura* and *Drosophila persimilis*, which show higher divergence than collinear regions of the genome (Fuller et al. 2018). Another case is provided by the large genomic regions of ancient ancestry that have been found across the threespine stickleback genome, which are involved in RI between marine and freshwater populations (Colosimo et al. 2005; Nelson and Cresko 2018). A third example, among others (see Marques et al. 2019 for a review), was described in Darwin's finches, whereby genomic regions showing increased divergence in several species pairs also display anciently diverged haplogroups that originated before the species splits (Han et al. 2017).

Different hypotheses can explain the origin and the maintenance of these highly divergent haplotypes. First, polymorphism has possibly been maintained over the long term in the ancestral population before being differentially sorted among the descendant lineages (Guerrero and Hahn 2017). This hypothesis has been proposed to explain the excess of haplotype divergence in the aforementioned examples (Colosimo et al. 2005; Han et al. 2017; Fuller et al. 2018). One mechanism that may explain the long-term maintenance of polymorphism is ancestral population structure, that is, subdivision owing to barriers to gene flow in the ancestral population (Slatkin and Pollack 2008). In addition to demography, balancing selection due to either frequency-dependent selection, heterozygote advantage (overdominance), or heterogeneous selection in space or time (Charlesworth 2006) can also promote the maintenance of ancient polymorphisms. For instance, in Darwin's finches, balancing selection has been proposed to explain the maintenance of divergent haplogroups associated with beak shape, due to the selective advantage of rare beak morphologies, or changing environmental conditions inducing heterogeneous selection (Han et al. 2017). An alternative explanation to the presence of anciently diverged alleles is admixture with a divergent lineage. Contemporary hybridization has long been recognized as a common phenomenon in plants and animals (Abbott et al. 2013; Payseur and Rieseberg 2016), and cases of ancient admixture are increasingly detected by genomic studies. One emblematic example is past admixture between modern humans and two extinct archaic hominin lineages, Neanderthal and Denisova (Green et al. 2010; Meyer et al. 2012; Pääbo 2015). More recently, ancient introgression from the extinct cave bear has also been detected in the genomes of living brown bears (Barlow et al. 2018). Therefore, past admixture is increasingly recognized as a source of anciently diverged alleles in contemporary genomes.

Understanding why and how divergent haplogroups tend to disproportionately contribute to the buildup of RI between nascent species remains, however, highly challenging. First, because retention of ancestral polymorphism and past admixture is notoriously difficult to distinguish and not mutually exclusive hypotheses to explain the presence of anciently diverged alleles (Durand et al. 2011; Eriksson and Manica 2012; Welch and Jiggins 2014; Racimo et al. 2015; Theunert and Slatkin 2017). Second, identifying the genomic regions that resist introgression is still a major obstacle to the detection of RI loci (Cruickshank and Hahn 2014). These tasks are now facilitated by the direct assessment of local ancestry along individual genome sequences (Schumer et al. 2018b; Duranton et al. 2018), thus paving the way for assessing the role of ancient admixture in speciation. Here, we use new haplotype-resolved whole-genome sequences to delineate the regions involved in RI between European sea bass lineages and understand the origin of the divergent haplogroups they contain.

The European sea bass (*Dicentrarchus labrax*) is a marine fish species subdivided into two glacial lineages, which correspond to current Atlantic and Mediterranean populations (Lemaire et al. 2005). The two lineages diverged in allopatry for about 300,000 years before experiencing a secondary contact since the last glacial retreat (Tine et al. 2014). Postglacial gene flow between the two lineages has been strongly asymmetrical, mostly occurring from the Atlantic to the Mediterranean genetic background (Tine et al. 2014). Using a detailed analysis of local ancestry tracts across Mediterranean and Atlantic sea bass genomes, we found direct evidence for highly heterogeneous rates of gene flow along most chromosomes (Duranton et al. 2018). This mosaic introgression pattern was attributed to the presence of multiple small effect RI loci mainly located in low-recombining regions. Importantly, genomic regions seemingly

involved in RI also present particularly high values of absolute nucleotide divergence (d_{XY}). It is generally assumed that increased d_{XY} indicates the presence of haplotypes that started to diverge earlier than the rest of the genome. However, regions of increased divergence may simply have resisted gene flow during secondary contact, whereas haplotypes in the remainder of the genome got rejuvenated due to recombination. This later hypothesis has however been rejected in the European sea bass based on simulations accounting for both background selection and selection against introgressed tracts (Duranton et al. 2018). Therefore, anciently diverged alleles are unlikely to have evolved within the 300,000-year divergence history inferred from genome-wide polymorphism data and are thus older. In the present study, we use new haplotype-resolved whole-genome sequences to accurately delineate regions involved in RI and investigate the mechanisms underlying their excess of divergence. We specifically test for past admixture with a closely related species using a new genome sequence from the parapatrically distributed spotted sea bass (*Dicentrarchus punctatus*). Our results show that gene flow occurred between *D. punctatus* and the Atlantic lineage of *D. labrax* about 80,000 years ago, resulting in a low background ancestry from *D. punctatus* in contemporary *D. labrax* genomes. By contrast, genomic regions involved in RI between the two *D. labrax* lineages generally display high frequencies of haplotypes derived from *D. punctatus* in the Atlantic, whereas these archaic tracts remain rare in the Mediterranean. This suggests that ancient admixture has played an important role in the evolution of RI between Atlantic and Mediterranean Sea bass lineages, consistently with predictions from models of local adaptive introgression and selection against genetic incompatibilities.

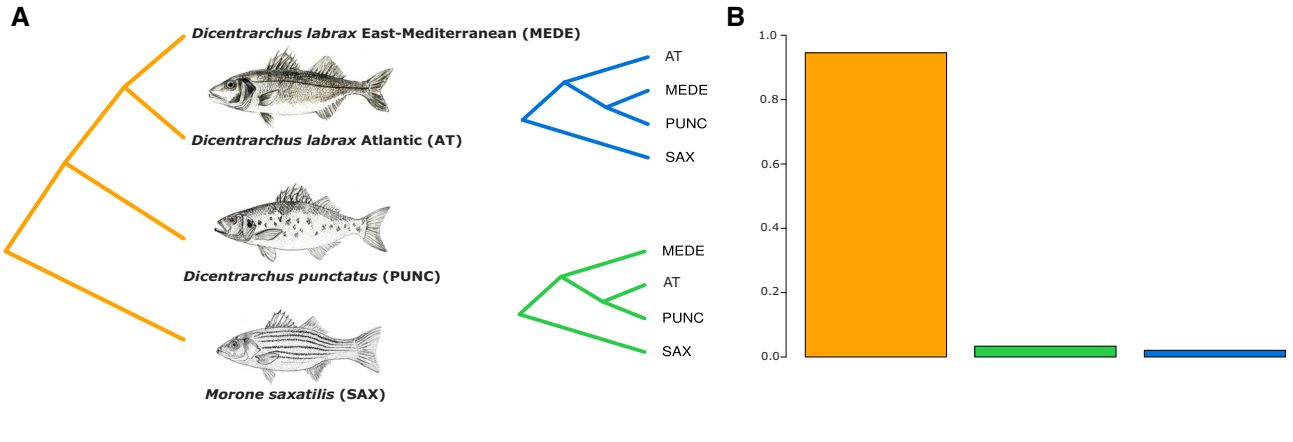
Material and Methods

WHOLE-GENOME RESEQUENCING AND HAPLOTYPING

We sequenced the whole genome of one *D. punctatus* individual from the Atlantic Ocean (Gulf of Cadiz, PUN) and 59 new *D. labrax* individual genomes. Fifty-two of them were wild individuals captured from the Atlantic Ocean (English Channel, 10 males $\male_{AT}$), the western Mediterranean Sea (Gulf of Lion, 14 females $\female_{WME}$ and nine males $\male_{WME}$), and the eastern Mediterranean Sea (Turkey, 10 males $\male_{NEM}$ and Egypt, nine males $\male_{SEM}$). Some of these specimens were experimentally crossed to generate first generation hybrids, which were used to phase the genome of their parents using a phasing-by-transmission approach. To this end, seven F1 hybrids obtained from seven different biparental families (pedigree $\male_{AT}$ x $\female_{WME}$) were also submitted to whole-genome sequencing. All captive breeding procedures were performed at Ifremer's experimental aquaculture facility (agreement for experiments with animals: C 34-192-6). Fish were reared in normal aquaculture conditions in agreement with the French decree no. 2013–118 (1 February 2013 NOR:AGRG1231951D).

Whole genome sequencing libraries were prepared separately for each individual using either the Illumina TruSeq DNA PCR-Free (40 individuals) or the TruSeq DNA Nano protocol (20 individuals), depending on DNA concentration (Table S1), and following Illumina standard recommendations. Pools of five individually barcoded libraries were then sequenced on 12 separate lanes of an Illumina HiSeq3000 using 2 × 150 bp PE reads at the GeT-PlaGe Genomics platform (Toulouse, France). Thirty-three individuals were sequenced twice due to insufficient amounts of sequence reads obtained in the first run (Table S1). For each individual, the alignment of PE reads to the sea bass reference genome (Tine et al. 2014) was performed using BWA-mem version 0.7.5a (Li 2013) with default parameters. Duplicate reads were marked using Picard version 1.112 before being removed, producing a mean coverage depth of 33.8× per individual (Fig. S1). We then followed GATK's (version 3.3-0-g37228af) best practice pipeline from individual variant calling (using HaplotypeCaller), to joint genotyping, genotype refinement, and variant filtering (using Filter Expression: QD < 10; MQ < 50; FS > 7; MQRankSum < -1.5; ReadPosRankSum < -1.5). We used the BQSR algorithm to recalibrate base quality scores using a set of high-quality variants identified in a previous study (Duranton et al. 2018), and to perform variant quality score recalibration using the VQSR algorithm. Hard filtering was then applied to exclude low-quality genotypes with a GQ score < 30. For the seven mother-father-offspring trios, we used family-based priors for genotype refinement. We obtained a total of 14,579,961 SNPs after filtering for indels, missing data (using –max-missing-count 8) and removing the mitochondrial and ungrouped scaffolds (chromosome UN) in VCFtools version 0.1.11 (Danecek et al. 2011).

We performed haplotype phasing in *D. labrax* after removing the *D. punctatus* individual and merging the 59 newly sequenced genomes with the 16 genomes already obtained in Duranton et al. (2018). Fifteen individuals that were involved in family crosses (i.e., newly sequenced or not already phased in the previous study) were submitted to phasing-by-transmission using the PhaseByTransmission algorithm in GATK with default parameters and a mutation rate prior of 10^{-8} for de novo mutations. For all individuals, variants located on a same read pair were directly phased using physical phasing information. Nonrelated *D. labrax* individuals were then statistically phased using the reference-based phasing algorithm implemented in Eagle2 (version 2.4) (Loh et al. 2016). The 22 parents phased with the phasing-by-transmission approach were used to build a European sea bass reference haplotype library (F1 genomes were



C

	East-Mediterranean *D. labrax*	West-Mediterranean *D. labrax*	Atlantic *D. labrax*	*D. punctatus*
East-Mediterranean *D. labrax*				
West-Mediterranean *D. labrax*	0.0007			
Atlantic *D. labrax*	0.0010	0.0009		
D. punctatus	0.0056	0.0055	0.0053	
M. saxatilis	0.0454	0.0453	0.0451	0.0461

Figure 1. **Phylogenetic relationships among *D. labrax* lineages, *D. punctatus*, and the outgroup species *M. saxatilis*. (A) Three different topologies rooted by *M. saxatilis*, representing the species tree (orange) and two discordant topologies grouping *D. punctatus* with the Atlantic (green) or the Mediterranean (blue) *D. labrax* lineage. (B) Proportion of each topology in nonoverlapping 50-Kb windows along the genome. (C) Genome-wide average pairwise sequence divergence between species/lineages measured by d_{xy} using one individual per lineage.**

excluded because their haplotype information was redundant with that of their parents), which was used in Eagle2 to improve statistical phasing. We finally filtered out SNPs that were not phased or not genotyped over all individuals (using –max-missing-count 0 and –phased in VCFtools). In this way, we generated a dataset of haplotype-resolved whole-genome sequences from 68 unrelated *D. labrax* individuals (14 AT, 31 WME, 11 SEM, and 12 NEM), containing 5,074,249 phased SNPs without missing data. The genetic relationships of the newly sequenced genomes with respect to the 16 already available were evaluated with a Principal Component Analysis (PCA; Fig. S2). Although we detected a slight genetic differentiation between North and South eastern Mediterranean samples on the PCA (Fig. 2), we later determined that they present similar genome-wide averages of Atlantic ancestry and introgressed tract length (18,148 bp for the South and 17,769 bp for the North; Fig. S5). Therefore, we regrouped these samples together within a single eastern Mediterranean population (MEDE), similarly to Duranton et al. (2018).

PHYLOGENOMIC ANALYSES

Phylogenetic relationships among Moronids genomes were studied using a method of topology weighting by iterative sampling of subtrees, as implemented in *Twisst* (Martin and Belleghem 2017) (github.com/simonhmartin/twisst). Neighbor-joining trees were generated for every nonoverlapping 50-kb window along the genome including *Morone saxatilis* (SAX), *D. punctatus* (PUNC), and the Atlantic (AT) and Mediterranean (MEDE) *D. labrax* lineages. We choose to use only the MEDE population because the western Mediterranean population (WME) is deeply introgressed by Atlantic alleles (31% on average; Duranton et al. 2018). We used 14 individuals for each of the two *D. labrax* lineages, corresponding to the total number of individuals available for the AT population. The resulting dataset contained a total of 9,606,462 SNPs. For each window, genealogies were computed using all possible combinations of haplotypes, generating three different types of topologies rooted by SAX (Fig. 1A). The proportion of each topology was estimated within each window and genome-wide. Due to its fragmented assembly, only 52% of the *M. saxatilis* genome could be aligned to the *D. labrax* genome (Duranton et al. 2018), and therefore, some windows could not be analyzed. Both Incomplete Lineage Sorting (ILS) and admixture between *D. punctatus* and either of the two *D. labrax* lineages are expected to generate discordant phylogenetic trees compared to the species tree. However, the proportions of

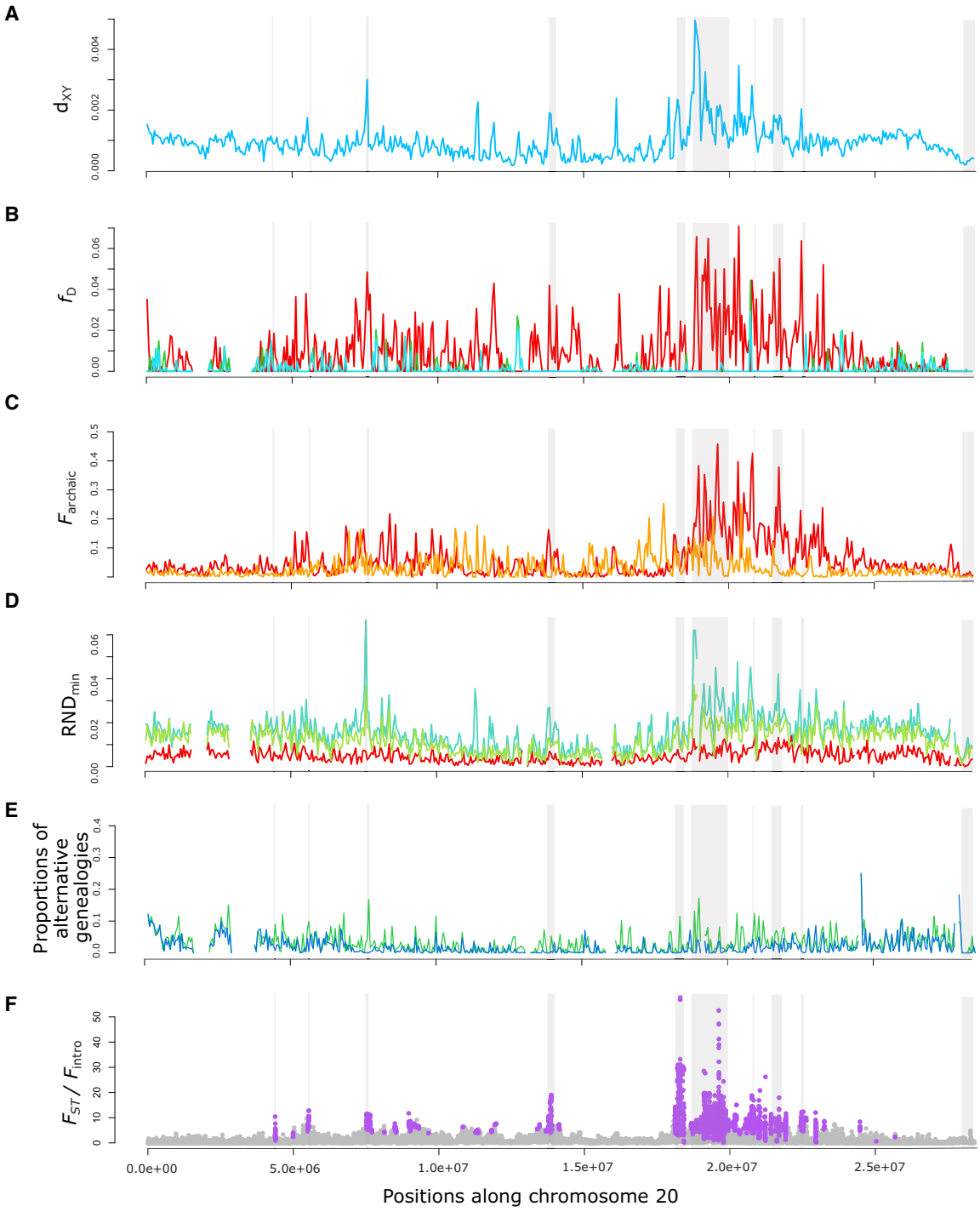


Figure 2. Divergence and introgression statistics measured in nonoverlapping 50-kb windows along chromosome 20. (A) d_{XY} measured between the Atlantic and Mediterranean (including eastern and western population) *D. labrax* lineages. (B) f_D statistics measured using (((MED, AT), PUN), SAX) in red, (((AT, WEM), PUN), SAX) in green, and (((ATL, EMED), PUN), SAX) in blue. (C) Fraction of archaic introgressed tracts ($F_{archaic}$) inferred in the eastern Mediterranean (orange) and Atlantic (red) populations of *D. labrax*. (D) RND_{min} measured between *D. punctatus* and *D. labrax* Atlantic (red), western (green), and eastern (blue) Mediterranean populations. (E) Proportions of genealogies grouping *D. punctatus* with the Atlantic (green) or the Mediterranean (blue) *D. labrax* lineage in the *Twisst* analysis. (F) F_{ST} between the Atlantic and western Mediterranean population of *D. labrax* divided by the fraction of Atlantic tracts introgressed into the western Mediterranean genomes for each SNP along the chromosome. Purple points show SNPs with significant associations to reproductive isolation after applying FDR correction to the probabilities determined with the HMM approach. Gray rectangles represent genomic regions identified as involved in reproductive isolation with our window-based HMM approach.

topologies grouping *D. punctatus* with the AT or the MEDE population are expected to be equal under ILS. Therefore, different proportions of these two discordant topologies across the genome can be the sign of uneven admixture between *D. punctatus* and the two *D. labrax* lineages. To provide indications for genome-wide average absolute sequence divergence among all pairs of species and lineages, we calculated d_{XY} using one haplome per species/lineages with MVFTools version 5.1.2 (Pease and Rosenzweig 2015) and averaged distance values calculated in nonoverlapping 50-kb windows. However, these values may be slightly underestimated because our initial filtering procedure only retained high-quality phased variants in *D. labrax*.

TESTS FOR FOREIGN INTROGRESSION WITHIN *D. labrax*

We tested for admixture between *D. labrax* and another species using three different methods that capture complementary aspects of the data. Because *D. punctatus* is the only closely related species parapatrically distributed with *D. labrax*, we first tested for historical gene flow between these two species. To do so, we used the ABBA-BABA test (Green et al. 2010; Durand et al. 2011) with *M. saxatilis* as the outgroup (O), *D. punctatus* as the potential donor species (P3), and the two *D. labrax* lineages as potential recipient populations (P1 and P2). We used the dataset containing 14,579,961 SNPs from *D. punctatus* and unphased *D. labrax* samples, and only kept sites that were available for both *M. saxatilis* and *D. punctatus* in genome alignments, representing a total of 9,606,462 SNPs. This allowed testing for different amounts of gene flow between P3 and P2, and P3 and P1, by comparing the number of genealogies of type ((P1,(P2,P3),O) (i.e., ABBA genealogies) and ((P2,(P1,P3),O) (i.e., BABA genealogies). An excess of shared derived alleles between the donor and one of the two recipient populations (i.e., excess of ABBA over BABA genealogies, or vice versa) indicates gene flow from *D. punctatus* to *D. labrax* population P2 or P1, respectively. Although the ABBA-BABA test is adequate to detect introgression, the Patterson's *D* statistic that measures the imbalance between the two types of genealogies ($D = \frac{\text{sum(ABBA)}-\text{sum(BABA)}}{\text{sum(ABBA)}+\text{sum(BABA)}}$) is not appropriate to quantify introgression over small genomic windows (Martin et al. 2015). Therefore, we used the f_D statistics ($f_D = \frac{s(\text{P1, P2, P3, O})}{s(\text{P1, } PD\text{, } PD\text{, O})}$) to estimate admixture proportion between P2 and P3, where s = sum(ABBA-BABA) and *PD* corresponds to the most likely donor population (i.e., the population with the higher frequency of the derived allele). To test for admixture between *D. punctatus* and different populations of *D. labrax*, we made different tests using successively the Atlantic (AT), eastern (MEDE combining SEM and NEM individuals), western (WME), or the whole Mediterranean (MED) populations of *D. labrax* populations as P2 or P1. We used scripts from Martin et al. (2015) to estimate the number of ABBA and BABA genealogies and the f_D statistics in nonoverlapping 50-kb windows along the genome, keeping only windows containing at least 500 SNPs.

Second, we used a method that allows archaic introgressed tracts to be identified without using an archaic reference genome for the donor species (Skov et al. 2018). The main advantage of this method is that it makes no assumption on the identity of the donor species. Basically, it looks for local excesses of private variants in a candidate recipient population by comparison to another nonadmixed population (Skov et al. 2018). To test for archaic introgression within the Atlantic *D. labrax* lineage, we identified variants that were not shared with the eastern Mediterranean population, and conversely to test for introgression in the Mediterranean *D. labrax* lineage. We only analyzed the eastern Mediterranean population because the western Mediterranean is more strongly impacted by gene flow from the Atlantic (Tine et al. 2014; Duranton et al. 2018). We used the phased genomes dataset containing 5,074,249 SNPs, assuming a constant mutation and call rate to run the model in 1000-bp windows along each chromosome. For each window, the probability that an individual haplotype contains an archaic introgressed fragment was estimated to identify introgressed windows with a posterior probability superior to 0.8 (Skov et al. 2018). We then combined individual profiles of introgressed windows to estimate the fraction of introgressed archaic tracts in each population (F_{archaic}), as the fraction of haplotypes for which a window was identified as introgressed. The inferred fraction of introgressed archaic tracts was finally averaged in nonoverlapping 50-kb windows along the genome.

Finally, we used the RND_{min} statistics, which is sensitive to rare introgression while being robust to mutation rate variation across the genome (Rosenzweig et al. 2016). The main advantage of this statistic is that, unlike the two former methods, it does not rely on the comparison of two recipient populations that differ in their level of introgression. The RND_{min} corresponds to the ratio of the minimal pairwise distance between haplotypes from the potential donor and recipient populations (d_{min}) over the average divergence of those populations to an outgroup species (d_{out}). If gene flow has occurred genome-wide, then locally elevated RND_{min} values indicate regions where introgression has been limited or absent. We used MVFTools version 5.1.2 (Pease and Rosenzweig 2015) to measure d_{min} between *D. punctatus* and different population of *D. labrax* (AT, MEDE, WME, and MED). For this analysis, we used 4,943,488 polymorphic sites that were phased within *D. labrax* and nonmissing in *D. punctatus*. On one hand, this dataset excludes a large number of variants that are differentially fixed between *D. punctatus* and *D. labrax*, and therefore underestimates the real level of divergence between *D. punctatus* and *D. labrax*. On the other hand, excluding diagnostic SNPs rendered the test more sensitive to the detection of ancient

introgression, because the accumulation of divergence after introgression only adds noise to chromosomal variations in RND_{min}. We estimated d_{out} by averaging the divergence measures between *M. saxatilis* and the two *Dicentrarchus* species. All values were averaged in nonoverlapping 50-kb windows along the genome.

DETECTION OF INTROGRESSED TRACTS BETWEEN ATLANTIC AND MEDITERRANEAN *D. labrax* LINEAGES

To test whether ancient introgression has influenced genomic patterns of postglacial gene flow between Atlantic and Mediterranean *D. labrax* lineages, we mapped Atlantic tracts introgressed into Mediterranean genomes and vice versa. Local ancestry inference was performed with Chromopainter version 0.04 (Lawson et al. 2012), an HMM-based program that estimates the probability of Atlantic and Mediterranean ancestry for each variable position along each haplome. To do so, it compares a focal haplotype to reference populations composed of nonintrogressed Atlantic and Mediterranean haplotypes. Because Mediterranean individuals are introgressed to various extents by Atlantic alleles, we used a pure Mediterranean reference population reconstituted by Duranton et al. (2018) with the same model parameters. We then identified the starting and ending position of each introgressed tract within both Atlantic and Mediterranean genetic backgrounds by analyzing the ancestry probability profiles inferred by Chromopainter, following the same methodology as in Duranton et al. (2018). Identified tracts in each *D. labrax* population (Fig. S5) were then combined to estimate the fraction of introgressed tracts (F_{intro}) for each position along the genome, which was finally averaged in nonoverlapping 50-kb windows. For every variable position along the genome, F_{intro} is thus estimated as the fraction of individuals for which this position is located on an introgressed tract.

DELINEATION OF RI REGIONS BETWEEN ATLANTIC AND MEDITERRANEAN *D. labrax* LINEAGES

We adapted the HMM approach developed by Hofer et al. (2012) to precisely delineate genomic regions involved in RI between Atlantic and Mediterranean *D. labrax* lineages. Genomic regions involved in RI between European sea bass lineages are characterized by elevated genetic differentiation and increased resistance to gene flow (Tine et al. 2014; Duranton et al. 2018). Therefore, we combined both measures of F_{ST} (Hofer et al. 2012; Marques et al. 2016) and resistance to introgression measured as the inverse of F_{intro} (Cruickshank and Hahn 2014; Duranton et al. 2018). To identify true RI islands in our HMM strategy, we thus used the ratio of F_{ST} over F_{intro} (i.e., the frequency of Atlantic tracts within western Mediterranean *D. labrax* genomes). Our rationale was that these regions should be associated with both high F_{ST} (Figs. S6A and S6D) and low F_{intro} values (Figs. S6B and S6E) (Duranton et al. 2018), hence elevated F_{ST}/F_{intro} ratio values (Figs. S6C and S6F). We used the HMM approach to map RI at two different scales, a SNP-by-SNP (Fig. 6A-C) and a 50-kb window scale, which was more suitable to delineate regions (Fig. 6D-F). We used VCFtools version 0.1.15 (Danecek et al. 2011) to estimate F_{ST} between the Atlantic and the western Mediterranean *D. labrax* lineage for each SNP and every nonoverlapping 50-kb window along the genome. The HMM was designed with three different states corresponding to low (i.e., neutral genomic regions), intermediate (i.e., regions experiencing linked selection), and high F_{ST}/F_{intro} ratio values (i.e., regions involved in RI). The most likely state of each SNP/window was inferred by running the HMM algorithm chromosome-by-chromosome. Finally, we controlled for false discovery rate and retained only SNPs/windows with an FDR-corrected *P*-value inferior to 0.001 (Hofer et al. 2012).

ESTIMATION OF THE TIME SINCE FOREIGN INTROGRESSION WITHIN *D. labrax*

We used two different approaches to estimate the time since foreign introgression within *D. labrax*. First, we relied on the fact that the length of introgressed tracts is informative of the time elapsed since introgression. Recombination progressively shortens migrant tracts across generations following introgression into a new genetic background (Fisher 1954; Barton 1983; Baird 1995; Pool and Nielsen 2009; Liang and Nielsen 2014). Because we found a good correspondence between the inferred fraction of introgressed archaic tracts ($F_{intro\text{-}archaic}$) and f_D values (see results) using *D. punctatus* as a donor species, we used the length of archaic haplotypes that were identified with the method of Skov et al. (2018). Only archaic tracts found in Atlantic genomes within windows involved in RI between Atlantic and Mediterranean *D. labrax* lineages were considered, corresponding to 1310 windows of 50 kb. This choice was justified because archaic tract detection relies on a signal of differential introgression between two populations. Therefore, archaic tracts can be correctly identified and delimited only if they are present in one lineage (e.g., the Atlantic) but absent in the other (e.g., the Mediterranean), which was only the case in RI islands between Atlantic and Mediterranean *D. labrax* lineages (see Results).

Under simple neutral assumptions, there is an analytical expectation for the average length of introgressed tracts ($\bar{L}$) as a function of the number of generations since introgression (t), the local recombination rate (r in Morgans per bp), and the proportion of admixture (f), which takes the form $\bar{L} = [(1-f)\, r\, (t-1)]^{-1}$ (Racimo et al. 2015). We used this equation to estimate the age of admixture between *D. punctatus* and the Atlantic lineage of *D. labrax* ($t_{labrax-punctatus}$), as well as between the two lineages of *D. labrax* ($t_{Atlantic-Mediterranean}$). For each estimation, we used the average value of the retained

windows. Hence, for $t_{\text{labrax – punctatus}}$: $f = 0.096$, $r = 3.693\times10^{-8}$ M/bp (Tine et al. 2014) and $\bar{L} = 5{,}513$ bp, and for $t_{\text{Atlantic – Mediterranean}}$: $f = 0.341$, $r = 3.23\times10^{-8}$ M/bp, and $\bar{L} = 52{,}026$ bp. Because we only considered a relatively small fraction of the genome to call archaic tracts, we could not obtain precise estimations of those parameters. Therefore, we estimated the age of contact between *D. punctatus* and *D. labrax* by reference to the age of the postglacial secondary contact between Atlantic and Mediterranean lineages of *D. labrax*, which has been more precisely estimated to 2300 generations using a larger fraction of the genome (Tine et al. 2014; Duranton et al. 2018).

Second, we transformed the estimated transition parameter values of the HMM model used to detect archaic introgressed tracts using $p \approx T_{admix} \cdot 2 \cdot r \cdot L \cdot a$ (Skov et al. 2018). In this equation, p is the probability of transition from the *D. labrax* to the archaic ancestry state, T_{admix} represents the admixture time in generations, r is the recombination rate in Morgan per bp, a is the admixture proportion, and L is the size of the window (here $L = 1000$ bp). Parameter p was estimated separately for each chromosome by averaging over the values estimated per individual haplome. We finally estimated T_{admix} chromosome-by-chromosome using the average recombination rate and the fraction of archaic introgressed tracts of each chromosome (Table S2). The time in generations was converted into years using a generation time of 5 years (Tine et al. 2014). We then obtained a distribution for T_{admix} across the 24 chromosomes, from which we identified the maximum and its 90% confidence interval by bootstrapping the distribution 10,000 times.

CHARACTERIZING FOREIGN ANCESTRY TRACTS WITHIN *D. labrax*

We used Spearman's correlation test to evaluate relationships among $F_{\text{intro-archaic}}$, f_{D}, d_{XY}, and RND_{min} statistics that relate to a series of predictive hypotheses. More specifically, if *D. punctatus* has anciently contributed to *D. labrax* in the Atlantic, the local abundance of archaic tracts inferred within Atlantic *D. labrax* genomes (F_{archaic}) should be positively correlated to f_{D}. Moreover, if the abundance of archaic tracts within Atlantic *D. labrax* explains the presence of anciently diverged alleles between Atlantic and Mediterranean Sea bass lineages, d_{XY} should increase with the amount of ancient admixture. Finally, if regions involved in RI between Atlantic and Mediterranean Sea bass lineages harbor reduced frequencies of *D. punctatus*-derived tracts in the Mediterranean, a positive correlation is expected between RND_{min} measured between *D. punctatus* and Mediterranean *D. labrax* and ancient admixture from *D. punctatus* within the Atlantic.

We then focused on SNP-level statistics to specifically address the frequency distributions of derived mutations from *D. punctatus* within *D. labrax* genomes, separately in the Atlantic and Mediterranean lineages. Because anciently introgressed alleles most likely originated from *D. punctatus* (see Results), we used *D. labrax* polymorphic sites for which *M. saxatilis* harbors the ancestral and *D. punctatus* the derived state (i.e., ABBA-BABA informative sites) to characterize ancient introgression. For each of these SNPs, we measured the frequency of the *D. punctatus*-derived allele separately in the Atlantic and Mediterranean *D. labrax* populations using VCFtools. We then separated SNPs associated to RI islands from those that were not associated to RI islands in the SNP-based HMM analysis to represent the site frequency spectrum of each *D. labrax* lineage, conditioned on *D. punctatus* being derived (*CSFS*). Finally, two conditioned joint site frequency spectra (*CJSFS*) were generated (i.e., for RI and non-RI SNPs) to represent the bi-dimensional SFS between Atlantic and Mediterranean *D. labrax* lineages, conditioning on sites that have the derived allele in *D. punctatus*. These analyses aimed at distinguishing two hypotheses with respect to the mechanisms underlying differential introgression of *D. punctatus*-derived mutations in RI islands between Atlantic and Mediterranean *D. labrax*. Our first hypothesis was that anciently introgressed alleles are not directly involved in RI but simply maintained at different frequencies because genetic barriers between *D. labrax* lineages (i.e., unrelated to the history of ancient admixture) have impeded their postglacial rehomogenization. In this case, we expected no excess of high-frequency *D. punctatus*-derived mutations in RI islands compared to non-RI regions. Alternatively, under the hypothesis that anciently introgressed alleles are associated with RI in sea bass, an excess of *D. punctatus*-derived mutations almost fixed within RI islands in the Atlantic but nearly absent in the Mediterranean was expected compared to the alternate configuration (i.e., almost fixed in the Mediterranean and nearly absent in the Atlantic).

Results

PHYLOGENOMIC ANALYSIS

We reconstructed the genetic relationships among the three Moronid species used in our study: the striped bass (*M. saxatilis*), the spotted sea bass (*D. punctatus*), and the European sea bass (*D. labrax*), which is further subdivided into two partially reproductively isolated populations: the Atlantic and Mediterranean Sea bass lineages. The most represented topology found genome-wide across most of the 50-kb windows corresponds to the expected species tree (Fig. 1B). However, we also found less than 10% discordant topologies, among which *D. punctatus* was grouped about twice as often with the Atlantic *D. labrax* lineage as with the Mediterranean lineage (Fig. 1B). We found 4.5% of absolute sequence divergence between the outgroup *M. saxatilis* and the two *Dicentrarchus* species. Absolute divergence between

D. labrax and *D. punctatus* (0.55%) was more than five times higher than divergence between Atlantic and Mediterranean *D. labrax* lineages (0.1%), consistent with previous estimates (Tine et al. 2014; Duranton et al. 2018). We found a slightly higher divergence between *D. punctatus* and the eastern Mediterranean (0.56%) compared to the Atlantic *D. labrax* lineage (0.53%). Within *D. labrax*, divergence to the Atlantic population was higher for the eastern (0.1%) compared to the western Mediterranean population (0.09%) (consistent with the PCA; Fig. S2), as expected due to gene flow between Atlantic and Mediterranean *D. labrax* lineages (Tine et al. 2014; Duranton et al. 2018).

TEST FOR FOREIGN INTROGRESSION WITHIN *D. labrax*

Chromosomal patterns of absolute sequence divergence (d_{XY}) between the Atlantic and Mediterranean lineages of *D. labrax* (Figs. 2A and S3A) showed highly heterogeneous divergence along the genome, as reported in previous studies (Tine et al. 2014; Duranton et al. 2018). To determine if local excesses of d_{XY} can be explained by past admixture with another lineage, we first looked for gene flow between *D. labrax* lineages and *D. punctatus* using the ABBA-BABA test. Some genomic regions showed particularly high values of the f_D statistics, thus reflecting locally elevated ancestry from *D. punctatus* within the *D. labrax* Atlantic lineage (Figs. 2B and S3B red curve). By contrast, when the f_D statistic was used to measure local *D. punctatus* ancestry within *D. labrax* Mediterranean populations, low and relatively homogeneous introgression was found across the entire genome (Figs. 2B and S3B blue and green curves). This finding thus indicates highly heterogeneous introgression of spotted sea bass alleles within the Atlantic *D. labrax* lineage, and comparatively lower introgression within the Mediterranean lineage.

We also searched for the presence of archaic introgressed tracts in *D. labrax* genomes. A relatively low fraction of archaic tracts ($F_{archaic}$) was found along the genome in both Atlantic (4.85% in non-RI islands) and Mediterranean (2.73% in non-RI islands) *D. labrax* individuals (Figs. 2C and S3C). In some regions, however, $F_{archaic}$ was particularly high in the Atlantic (i.e., >30%) compared to the Mediterranean lineage. Interestingly, those regions also presented the highest f_D values (Fig. 2B red curve), and there was a highly significant positive correlation between f_D and $F_{archaic}$ in Atlantic *D. labrax* genomes (Fig. S4E Spearman's rho = 0.281***). These results thus support the hypothesis that the detected archaic segments that locally reach high frequencies in some regions of Atlantic *D. labrax* genomes have been inherited from *D. punctatus* at some time in the past. Furthermore, regions of particularly increased *D. punctatus* ancestry also showed the highest absolute divergence values between Atlantic and Mediterranean *D. labrax* lineages, with positive genome-wide correlations being found with d_{XY} for both f_D (Fig. S4A, Spearman's rho = 0.281***) and $F_{archaic}$ (Fig. S4C, Spearman's rho = 0.531***).

Lastly, we used the RND_{min} statistic to detect chromosomal variations in ancient introgression. Values of RND_{min} measured between *D. punctatus* and the Atlantic *D. labrax* lineage were low and relatively constant along chromosomes (Figs. 2D and 4D red curves), indicating widespread (although locally rare) introgression across the genome. By contrast, RND_{min} was higher and highly variable when measured with the Mediterranean *D. labrax* populations (Figs. 2D and 4D blue and green curves), indicating that introgression from *D. punctatus* is absent or nearly absent in some genomic regions of the Mediterranean lineage. These regions, which seem resistant to *D. punctatus* introgression in Mediterranean *D. labrax* genomes, also showed elevated values of $F_{archaic}$ (Fig. S4F genome-wide Spearman's rho = 0.472***) and f_D (Fig. S4D genome wide spearman's rho = 0.223***) in Atlantic genomes, along with increased d_{XY} between Atlantic and Mediterranean *D. labrax* lineages (Fig. S4B genome-wide Spearman's rho = 0.717***). These results thus indicate the existence of outlying patterns of *D. punctatus* ancestry in the most divergent genomic regions between *D. labrax* lineages, due to increased and decreased frequencies of anciently introgressed tracts in the Atlantic and Mediterranean lineages, respectively, compared to the background level. Interestingly, the relative proportions of the two discordant topologies were similar along most of the genome, except for regions with locally increased values of d_{XY}, f_D (for the AT population), $F_{archaic}$ (for the AT population), and RND_{min} (for the Mediterranean populations). In these regions, we found an excess of discordant genealogies grouping *D. punctatus* with the Atlantic *D. labrax* lineage (Fig. 2E). This corroborates our other results indicating local excesses of *D. punctatus* ancestry in some regions of Atlantic *D. labrax* genome.

Finally, our HMM approach allowed 70,738 SNPs to be categorized as being likely associated with RI islands between the two *D. labrax* lineages (Figs. 2F and S3G). We found a good concordance between the positions of RI islands identified with the SNP and window-based methods, although the former allowed us to detect narrower RI-associated regions with a higher resolution (Figs. S6C and S6F). As expected, all these regions displayed increased levels of ancient *D. punctatus* introgression in the Atlantic but decreased *D. punctatus* ancestry in the Mediterranean (Fig. 2). They also tended to be at the extreme part of all measured genome-wide correlations between introgression and divergence statistics (Fig. S4). Furthermore, $F_{archaic}$ values were significantly more elevated within RI regions in Atlantic compared to Mediterranean genomes (Fig. S8), thus strengthening the association of RI-islands to differential rates of archaic ancestry.

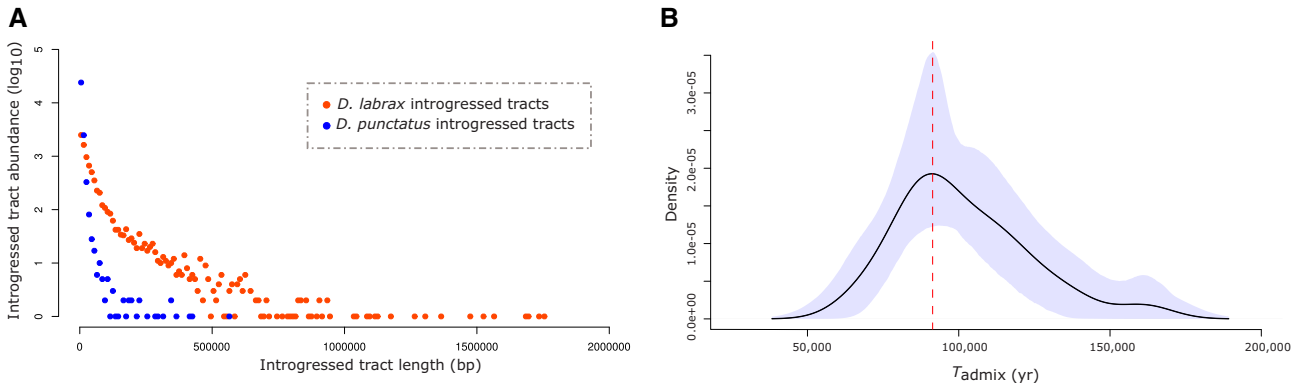


Figure 3. Estimation of the time since admixture between *D. punctatus* and Atlantic *D. labrax*. (A) Length distributions of *D. punctatus* tracts introgressed into Atlantic *D. labrax* genomes (blue) and Atlantic *D. labrax* tracts introgressed into western Mediterranean *D. labrax* genomes (orange). Both distributions were generated using similar sequence lengths (totalizing 65.6 Mb) along the genomes of 14 Atlantic and 14 Mediterranean individuals, so that tract abundances can be compared. (B). Distribution of estimated time since admixture between *D. punctatus* and *D. labrax* (T_{admix}) obtained from estimated transition parameter values of the HMM model over the 24 chromosomes. The maximum of the distribution is represented by the vertical red dashed line and the blue shape represents the 95% credibility envelope of the distribution obtained using 10,000 bootstrap resampling.

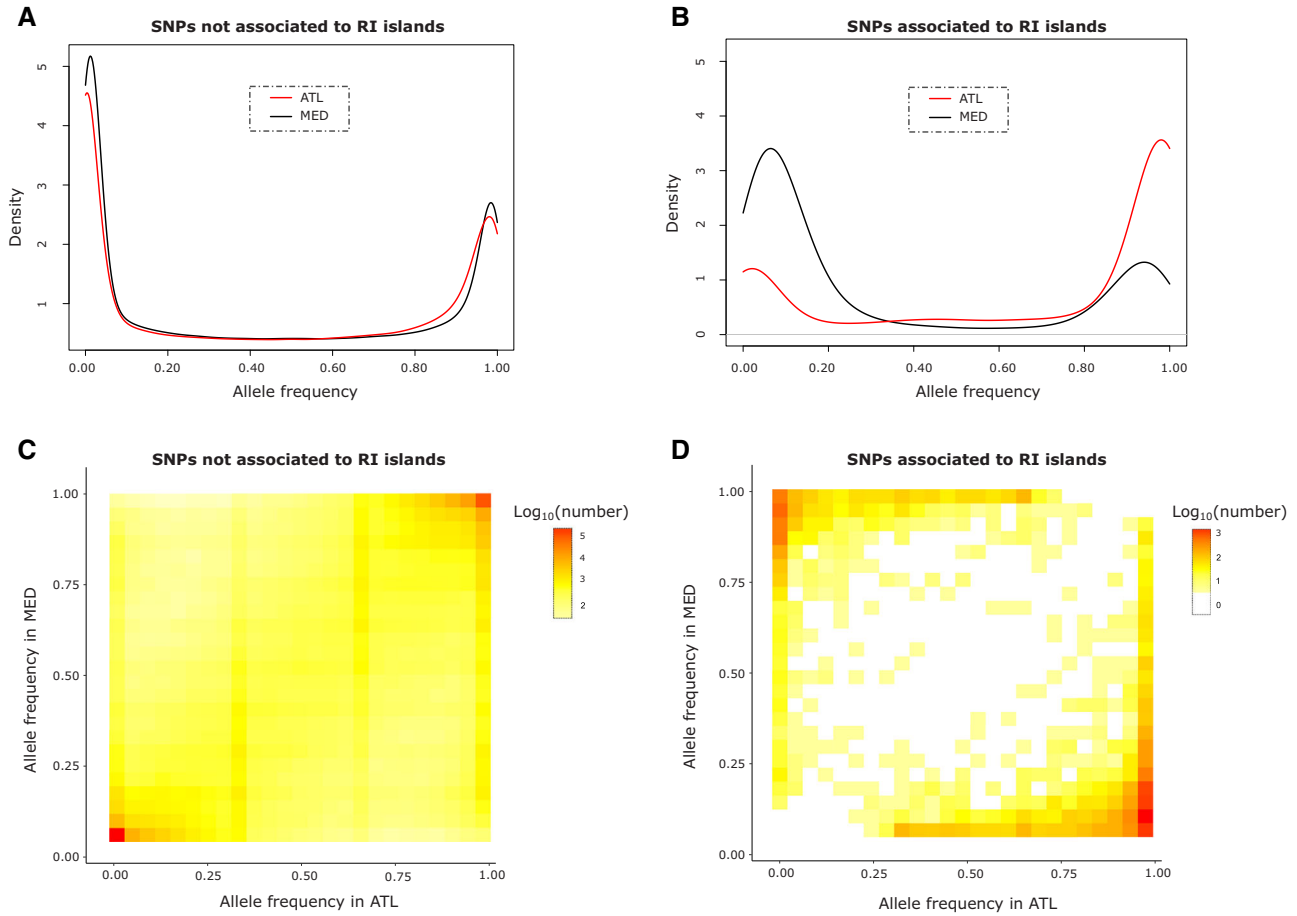


Figure 4. One- and two-dimensional Site Frequency Spectra of *D. punctatus*-derived alleles segregating in *D. labrax*. (A) Conditional Site Frequency Spectra (*CSFS*) of *D. punctatus*-derived alleles in AT (red) and MED (black) *D. labrax* lineages for categories of SNPs that are either not associated or (B) associated to RI islands identified between the two *D. labrax* lineages. (C) Conditional Joint Site Frequency Spectra (*CJSFS*) of derived *D. punctatus* alleles between MED (54 individuals) and AT (14 individuals) lineages based on 618,842 SNPs not involved in RI, and (D) 7372 SNPs involved in RI.

ESTIMATION OF THE TIME SINCE INTROGRESSION BETWEEN *D. punctatus* AND *D. labrax*

We estimated the timing of past gene flow between *D. punctatus* and *D. labrax* by first comparing the length distribution of *D. punctatus* tracts introgressed into Atlantic *D. labrax* genomes to that of Atlantic *D. labrax* tracts introgressed into western Mediterranean *D. labrax* genomes (Fig. 3A). The two distributions showed similar shapes, although *D. punctatus* tracts were on average almost 10 times shorter ($\bar{L}_{punctatus} = 5513$ kb) than Atlantic *D. labrax* tracts ($\text{CI}_{90} = (\bar{L}_{labrax} = 52,026$ kb)). *Dicentrarchus punctatus* tracts were also less abundant in almost all length classes except for the shortest tracts (Fig. 3A). We estimated the average time since introgression for both distributions as $t_{\text{labrax – punctatus}} = \frac{1}{((1-0.096)\cdot 3.693e^{-8}\cdot 5513)} + 1$ and $t_{Atlantic-Mediterranean} = \frac{1}{((1-0.341)\cdot 3.23e^{-8}\cdot 52026)} + 1$, which placed the timing of admixture between *D. punctatus* and *D. labrax* approximately six times earlier than the secondary contact between the two *D. labrax* lineages. Using the age of secondary contact previously estimated between Atlantic and Mediterranean Sea bass lineages (i.e., 11,500 years; Tine et al. 2014; Duranton et al. 2018) as a calibration time point, ancient gene flow between the two species was dated to about 70,000 years ago. Second, we converted the estimated values of the transition parameter (p) of the HMM model used to detect archaic introgressed tracts to estimate one value of T_{admix} for each chromosome (Table S2). From the obtained time distribution (Fig. 3B), we estimated the most probable time of ancient admixture to $T_{\text{admix}} = 91{,}149$ ($\text{CI}_{90\%} = [85,831,\ 110,645]$) years.

THE FREQUENCY OF *D. punctatus*-DERIVED MUTATIONS IN *D. labrax*

We used the conditioned site frequency spectrum between Atlantic and Mediterranean lineages as a way to represent how derived *D. punctatus* alleles segregate in *D. labrax*. For SNPs that were not associated to RI-islands by the HMM approach, the one-dimensional distribution of allele frequencies (*CSFS*) was highly similar between Atlantic and Mediterranean *D. labrax* lineages, showing a bimodal shape with few intermediate frequency variants (Fig. 4A). Most *D. punctatus*-derived alleles were present at either low or high frequencies, with ancestral mutations almost fixed in both *D. labrax* lineages being about 100 times more abundant than *D. punctatus*-derived mutations almost fixed in both *D. labrax* lineages in the *CJSFS* (Fig. 4C). This result showed that the combined effects of ILS and introgression during species divergence have resulted in very similar amounts of *D. punctatus*-derived mutations between Atlantic and Mediterranean *D. labrax* lineages. By contrast, SNPs found to be associated with RI islands showed a large excess of *D. punctatus*-derived alleles that were fixed or almost fixed in the Atlantic population, while segregating at low frequencies in the Mediterranean populations (Figs. 4B and 4D). This remained true whatever the Mediterranean population (east, west, or both) considered in the analysis (Fig. S7). The excess of high-frequency *D. punctatus*-derived mutations in the Atlantic sea bass lineage was also clearly visible in the reversal of the *CSFS* in RI islands compared to non-RI regions (Figs. 4A and 4B). Therefore, differential introgression of *D. punctatus*-derived mutations in RI islands is most likely due to their direct role in RI, rather than a delayed postglacial rehomogenization due to already-existing genetic barriers between *D. labrax* lineages in these regions.

Discussion

Recent speciation genomics studies have revealed that genomic regions involved in RI often contain anciently diverged alleles (e.g., Meier et al. 2017; Han et al. 2017; Nelson and Cresko 2018). One of the competing hypotheses to explain their origin is ancient admixture with an already diverged lineage. Our main objective here was to determine if such a scenario could explain the excess of divergence observed in RI regions between Atlantic and Mediterranean *D. labrax* lineages (Duranton et al. 2018). To achieve this goal, we used different complementary approaches that collectively provided strong support for ancient introgression from the sister species *D. punctatus*. Despite low divergence ($d_{XY} = 0.55\%$), partially overlapping range distributions, and interfertility in artificial crosses (Ky et al. 2012), contemporary hybridization has not been observed in the wild between *D. labrax* and *D. punctatus* (Tine et al. 2014). We here show that interspecies admixture has likely happened earlier in the past, bringing new key elements to understand the complex evolutionary history of incomplete speciation between Atlantic and Mediterranean Sea bass lineages.

EXTENT OF ANCIENT ADMIXTURE

Overall, the average fraction of contemporary genomes derived from ancient admixture was lower than 6% (i.e., 5.39% in the Atlantic and 2.82% in the Mediterranean lineage), which is only slightly higher than the estimated persistence of archaic ancestry in humans and brown bears (Sankararaman et al. 2016; Barlow et al. 2018). Whether these low background levels reflect a relatively limited contribution of genetic material from *D. punctatus* during admixture, or the impact of long-term selection against admixed foreign ancestry (Harris and Nielsen 2016; Juric et al. 2016; Schumer et al. 2018b) was outside the scope of this study. Instead, we focused on understanding the marked excess of shared derived mutations found between *D. punctatus* and the Atlantic compared to the Mediterranean *D. labrax* lineage in RI-associated regions. This finding was strengthened by the locally increased frequency of archaic introgressed tracts found in Atlantic genomes within regions associated to RI with the

Mediterranean lineage. Such locally elevated differences in the frequency of *D. punctatus* derived alleles explain the increased sequence divergence previously observed in RI islands between Atlantic and Mediterranean lineages (Duranton et al. 2018). Below, we consider potential limitations to the detection of archaic introgression using contemporary genomes, and the related challenge of dating ancient admixture. We then discuss how the genomic mosaicism of species ancestry may relate to different mechanisms potentially involved in European sea bass speciation.

SEPARATING ANCIENT INTROGRESSION FROM SHARED ANCESTRAL VARIATION

Distinguishing past introgression and shared ancestral variation with ABBA-BABA and f_D statistics can be difficult, especially in regions of reduced divergence (Martin et al. 2015). Therefore, the positive correlations observed among f_D, the inferred frequency of archaic segments, and d_{XY} provided good support that regions of high *D. punctatus* ancestry in the Atlantic are responsible for increased divergence between *D. labrax* lineages. Admittedly, past gene flow may also have occurred with another now extinct species rather than with *D. punctatus*, as previously shown for other species (Meyer et al. 2012; Barlow et al. 2018; Gopalakrishnan et al. 2018; Foote et al. 2019; Kuhlwilm et al. 2019). However, because *D. labrax* harbors shared derived alleles with *D. punctatus*, any alternative ghost donor lineage must have shared a long common history with the spotted sea bass.

Another potential issue with the tests performed to detect ancient admixture is that they often rely on differential introgression patterns between two candidate recipient populations (Martin et al. 2015; Skov et al. 2018). Therefore, these tests only enabled us to detect regions where the level of archaic introgression differs between Atlantic and Mediterranean *D. labrax* lineages. This problem could be particularly acute outside RI regions, where postglacial gene flow between *D. labrax* lineages has almost completely rehomogenized allele frequencies (Tine et al. 2014; Duranton et al. 2018). To determine whether *D. punctatus* ancestry was simply absent or present but at similar levels in both lineages, we used the RND_{min} statistic that does not rely on the comparison of two populations (Rosenzweig et al. 2016). Low and nearly constant RND_{min} values indicated a widespread presence (although most of the time at low frequencies) of anciently introgressed tracts along Atlantic *D. labrax* genomes. By contrast, regions of elevated RND_{min} that coincided with the location of RI-islands revealed local resistance to introgression in Mediterranean *D. labrax* genomes. These results tend to be confirmed by the excess of genealogies grouping *D. punctatus* with the Atlantic compared to those grouping it with the Mediterranean lineage of *D. labrax* within RI-islands. Therefore, both lineages contain *D. punctatus* introgressed tracts at relatively similar levels outside RI islands, which contrasts with strong archaic ancestry differences found within RI-islands.

TIMING OF ANCIENT INTROGRESSION

To understand why *D. punctatus* alleles were rare within RI genomic regions in the Mediterranean, we reconstructed the history of ancient admixture by estimating the time of contact between *D. punctatus* and *D. labrax*. The two different methods respectively inferred a contact taking place approximately 70,000 and 90,000 years ago. This difference in estimated admixture times might be due to selective effects. Indeed, the formula used to estimate admixture time from the distribution of introgressed tracts length assumes that introgressed tracts are neutral. If positive selection has driven some *D. punctatus* tracts to high frequencies within Atlantic *D. labrax* genomes, these tracts would be longer than under neutrality, biasing the time estimate toward more recent admixture. In contrast, the HMM approach might be more robust to selective effects by attributing them to variations in *N*e along the genome. Although the two estimates slightly differ, they both place ancient admixture during the last glacial period (Snyder 2016), when Atlantic and Mediterranean *D. labrax* lineages were inferred to be geographically isolated (Tine et al. 2014; Duranton et al. 2018). The current distribution range of *D. punctatus* partially overlaps with the southern part of the *D. labrax* distributional area in both the Atlantic (i.e., from southern Biscay to Morocco) and southern Mediterranean Sea (i.e., North African shores). It is thus likely that the latitudinal range shifts that occurred during quaternary ice ages (Hewitt 2000) have favored hybridization by further increasing the range overlap between the two species, as they were coexisting in the Iberian or the northwestern African Atlantic refugium (Maggs et al. 2008). Once the two *D. labrax* lineages came into secondary contact after the last glacial maximum, the *D. punctatus* alleles already introgressed within Atlantic genomes could have readily introgressed into Mediterranean genomes. This hypothesis was supported by the observed gradient of decreasing *D. punctatus* ancestry from the Atlantic to the eastern Mediterranean lineage, which mirrored the gradient in Atlantic ancestry generated by the postglacial secondary contact (Duranton et al. 2018). The fact that *D. punctatus* tracts have most probably introgressed into the Mediterranean lineage secondarily indicates that ancient hybridization has only occurred in the Atlantic during the last glacial period. A possible explanation is the absence of sympatry between *D. punctatus* and *D. labrax* within the Mediterranean during the last glacial period. However, a missing piece of the reconstructed historical scenario remains with respect to the role of *D. punctatus* alleles in RI.

CAUSATIVE ROLE OF HIGH-FREQUENCY *D. punctatus* ALLELES IN RI-ISLANDS

If most of the currently observed RI-islands between *D. labrax* lineages already existed before ancient admixture with *D. punctatus*, such genetic barriers would have impeded the introgression of *D. punctatus* alleles into the Mediterranean lineage (Duranton et al. 2018). However, they would not account for increased frequencies of *D. punctatus*-derived alleles within RI-islands in the Atlantic lineage. The fact that, in Atlantic *D. labrax*, regions associated with RI exhibited closely fixed *D. punctatus*-derived alleles that comparatively occurred at low frequencies elsewhere in the genome strongly supports their direct role in the establishment of RI. This finding thus indicates that *D. punctatus* alleles have been first locally driven to high frequencies in the Atlantic *D. labrax* lineage, while being secondarily prevented from introgression within the Mediterranean lineage.

WHY DO ANCIENTLY INTROGRESSED ALLELES CONTRIBUTE TO RI?

Locally adaptive introgression

Understanding the underlying evolutionary mechanisms through which admixture has contributed to the buildup of RI remains highly challenging (Schumer et al. 2014, 2018a). One evolutionary force that can drive an allele to fixation is local positive selection. *Dicentrarchus punctatus* alleles may have fixed in the Atlantic *D. labrax* lineage following admixture because they provided a selective advantage in the Atlantic environment compared to ancestral *D. labrax* alleles, a process called adaptive introgression (Fisher 1937; Racimo et al. 2017). Several studies have revealed that the acquisition of adaptive phenotypes can be done through hybridization, such as altitude adaptation in humans (Huerta-Sánchez et al. 2014), mimicry in *Heliconius* butterflies (Dasmahapatra et al. 2012) or among others, seasonal camouflage in the snowshoe hares (Jones et al. 2018). Indeed, adaptive introgression allows the rapid transfer of linked variants that have already been tested by natural selection in their original environment, thus facilitating local adaptation (Martin and Jiggins 2017). Therefore, it is theoretically possible that the Atlantic *D. labrax* lineage has received from *D. punctatus* advantageous alleles in the Atlantic environment that were revealed to be deleterious in the Mediterranean Sea. Nevertheless, adaptive introgression is usually difficult to prove because it can be confounded with other processes such as uncoupling of an incompatibility from a multilocus genetic barrier (Fraïsse et al. 2014). Furthermore, it has been argued that adaptive introgression cannot play an important role in RI, because unconditionally favorable alleles spread easily among diverging lineages until RI is nearly complete (Barton 2013).

Fixation-compensation of deleterious mutations

Another evolutionary force that may have driven *D. punctatus*-derived alleles to fixation is genetic drift, which can induce the fixation of deleterious mutations and thus increase mutation load (Haldane 1937; Kimura et al. 1963). When gene flow occurred between *D. punctatus* and *D. labrax* during the last glacial period, populations of each species were probably experiencing bottlenecks (Hewitt 2000), which decreased the efficiency of selection and enhanced the probability of fixation of deleterious mutations by drift. Weakly deleterious *D. punctatus* alleles may therefore have introgressed and fixed within the *D. labrax* Atlantic population. Another related mechanism that may have influenced the outcome of hybridization is associative overdominance, due to the masking of recessive deleterious mutations in admixed genotypes (Ohta 1971; Whitlock et al. 2000; Bierne et al. 2002). Heterosis can locally increase the introgression rate of foreign alleles, even if interbreeding populations have similar amounts of deleterious variation (Kim et al. 2018). Therefore, heterosis may have favored the introgression of weakly deleterious *D. punctatus* variants in a bottlenecked Atlantic *D. labrax* lineage. Subsequently, when Atlantic and Mediterranean *D. labrax* lineages reconnected following postglacial recolonizations, expanding populations would have been sufficiently large to reveal the deleterious effects of the introgressed alleles, generating hybrid depression and hybridization load (Shpak 2005; Schumer et al. 2018b). Furthermore, the Atlantic population may have had enough time to evolve compensatory mutations (Kimura 1985), which could have become substrate for increased RI. The fact that most genomic regions involved in RI between *D. labrax* lineages exhibit low recombination rates (Tine et al. 2014; Duranton et al. 2018) could indicate a role of slightly deleterious alleles in RI, because selection is less efficient when linkage is strong.

Reciprocal sorting of Bateson-Dobzhansky-Muller incompatibilities

Reproductive isolation may also have evolved through the resolution of genetic conflicts resulting from the contact between two diverged populations (Schumer et al. 2015; Blanckaert and Bank 2018). Because each population has almost inevitably fixed new adaptive or nearly neutral variants that form incompatibilities when combined in hybrid genomes (Dobzhansky 1937), Bateson-Dobzhansky-Muller incompatibilities (BDMIs) are recognized as a common substrate for speciation (Presgraves 2010). A genomic conflict induced by a two-locus BDMI can be resolved by fixing one of either parental alleles. In a hybrid population generated by an equal mixture of individuals from both parental populations, there is a 50% chance of fixing either parental combination (Schumer et al. 2015). Therefore, the resolution of multiple

BDMIs in an admixed population offers ample opportunity to reciprocally resolve independent BDMIs with respect to the origin of the parental allelic combination, which results in RI from both parental populations. Even in the presence of skewed initial admixture proportions, fixation of the minor parent combination can still happen with a sufficient number of BDMIs (Schumer et al. 2015). Therefore, the resolution of genetic conflicts between *D. punctatus* and *D. labrax* alleles in the Atlantic lineage may have induced the fixation of *D. punctatus* alleles at some incompatibility loci. Upon contact between Atlantic and Mediterranean *D. labrax* lineages, fixed *D. punctatus* alleles may have recreated the BDMIs, thus contributing to RI. This nonadaptive speciation model due to selection against genetic incompatibilities has the advantage of explaining both the fixation of *D. punctatus* alleles within the *D. labrax* Atlantic population, and their incompatibility with the Mediterranean lineage. Verbally, it can be seen as a case whereby speciation reversal between lineages A and B contributes to strengthen RI between lineages B and C through the transfer of incompatibilities between two porous species boundaries. At first sight, one might find surprising that BDMIs that were resolved following ancient admixture between *D. punctatus* and *D. labrax* Atlantic lineage could still act as barriers to gene flow between the two *D. labrax* lineages. However, this might be due to contrasting spatiotemporal modes of admixture. Indeed, the resolution of genomic conflicts possibly differs between a pulse of admixture (which could be the case for ancient admixture with *D. punctatus*) and a hybrid zone, as it exists between Atlantic and Mediterranean Sea bass lineages.

Conclusion

To conclude, our results show that divergent haplotypes that were introgressed from *D. punctatus* about 80,000 year ago have contributed to the strengthening of nascent RI between Atlantic and Mediterranean *D. labrax* lineages. The resulting genomic architecture of RI between contemporary *D. labrax* lineages is thus constituted by a mosaic of fixed blocks of different ancestries, that is, a mixture of genetic barriers inherited from the divergence history particular to *D. labrax* and the contribution of ancient admixture. Although additional analyses will be needed to fully understand which process has driven the fixation of *D. punctatus* alleles within Atlantic genomes, the resolution of genetic conflicts between *D. punctatus* and *D. labrax* seems the most parsimonious hypothesis (Schumer et al. 2015; Blanckaert and Bank 2018). This speciation mechanism can be thought of as a transfer of incompatibilities between two species boundaries, from the strongest to the weakest barrier, which is eventually strengthened by the displacement of genetic conflicts inherited from an ancient episode of admixture. Our findings add to previous reports showing that postglacial and recent hybridization events have played a role in the buildup of RI between admixed and parental lineages by generating similar genomic mosaics of ancestries (Runemark et al. 2018; Schumer et al. 2018b; Eberlein et al. 2019). The contribution of ancient admixture in European sea bass speciation suggests that significantly older admixture events, which may have left cryptic signatures in contemporary genomes, can be involved in seemingly recent speciation histories.

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AUTHOR CONTRIBUTIONS

MD and P-AG wrote the manuscript with inputs from FA and FB. Experimental crosses were managed by FA, genome sequencing was managed by SV and OB, genome alignment and SNP calling were performed by MD and P-AG, and MD performed all population genomic analyses. P-AG conceived the project and P-AG and FA managed financial support.

DATA ARCHIVING

Individual whole-genome resequencing data are available from the NCBI SRA under the accession code BioProject PRNJXXXXXX. VCF files and genome-wide statistics are available via Dryad at https://doi.org/10.5061/dryad.6t1g1jww2.

LITERATURE CITED

Abbott R., D. Albach, S. Ansell, J. W. Arntzen, S. J. E. Baird, N. Bierne, et al. 2013. Hybridization and speciation. J. Evol. Biol. 26:229–246. https://doi.org/10.1111/j.1420-9101.2012.02599.x

Baird S. J. E. 1995. A simulation study of multilocus clines. Evolution 49:1038–1045. https://doi.org/10.1111/j.1558-5646.1995.tb04431.x

Barlow A., J. A. Cahill, S. Hartmann, C. Theunert, G. Xenikoudakis, G. G. Fortes, et al. 2018. Partial genomic survival of cave bears in living brown bears. Nat. Ecol. Evol. 2:1563–1570. https://doi.org/10.1038/s41559-018-0654-8

Barton N. H. 1983. Multilocus clines. Evolution 37:454–471. https://doi.org/10.1111/j.1558-5646.1983.tb05563.x

———. 2013. Does hybridization influence speciation? J. Evol. Biol. 26:267–269. https://doi.org/10.1111/jeb.12015

Bierne N., T. Lenormand, F. Bonhomme, and P. David. 2002. Deleterious mutations in a hybrid zone: can mutational load decrease the barrier to gene flow? Genet. Res. 80:197–204. https://doi.org/10.1017/S001667230200592X

Blanckaert A., and C. Bank. 2018. In search of the Goldilocks zone for hybrid speciation. PLOS Genet. 14:e1007613. https://doi.org/10.1371/journal.pgen.1007613

Charlesworth D. 2006. Balancing selection and its effects on sequences in nearby genome regions. PLOS Genet. 2:e64. https://doi.org/10.1371/journal.pgen.0020064

Colosimo P. F., K. E. Hosemann, S. Balabhadra, G. Villarreal, M. Dickson, J. Grimwood, et al. 2005. Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. Science 307:1928–1933. https://doi.org/10.1126/science.1107239

Coyne J. A., and A. H. Orr. 2004. Speciation. Oxford Univ. Press, Sunderland, MA.

Cruickshank T. E., and M. W. Hahn. 2014. Reanalysis suggests that genomic islands of speciation are due to reduced diversity, not reduced gene flow. Mol. Ecol. 23:3133–3157. https://doi.org/10.1111/mec.12796

Danecek P., A. Auton, G. Abecasis, C. A. Albers, E. Banks, M. A. DePristo, et al. 2011. The variant call format and VCFtools. Bioinformatics 27:2156–2158. https://doi.org/10.1093/bioinformatics/btr330

Dasmahapatra K. K., J. R. Walters, A. D. Briscoe, J. W. Davey, A. Whibley, N. J. Nadeau, et al. 2012. Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. Nature 487:94–98. https://doi.org/10.1038/nature11041

Dobzhansky T. 1937. Genetics and the origin of species. Columbia University Press, New York.

Durand E. Y., N. Patterson, D. Reich, and M. Slatkin. 2011. Testing for ancient admixture between closely related populations. Mol. Biol. Evol. 28:2239–2252. https://doi.org/10.1093/molbev/msr048

Duranton M., F. Allal, C. Fraïsse, N. Bierne, F. Bonhomme, and P. A. Gagnaire. 2018. The origin and remolding of genomic islands of differentiation in the European sea bass. Nat. Commun. 9:2518. https://doi.org/10.1038/s41467-018-04963-6

Eberlein C., M. Hénault, A. Fijarczyk, G. Charron, M. Bouvier, L. M. Kohn, et al. 2019. Hybridization is a recurrent evolutionary stimulus in wild yeast speciation. Nat. Commun. 10:923. https://doi.org/10.1038/s41467-019-08809-7

Eriksson A., and A. Manica. 2012. Effect of ancient population structure on the degree of polymorphism shared between modern human populations and ancient hominins. Proc. Natl. Acad. Sci. USA 109:13956–13960. https://doi.org/10.1073/pnas.1200567109

Feder J. L., S. P. Egan, and P. Nosil, 2012. The genomics of speciation-with-gene-flow. Trends Genet. 28:342–350. https://doi.org/10.1016/j.tig.2012.03.009

Fisher, R. A. 1937. The wave of advance of advantageous genes. Ann. Eugen. 7:355–369.

———. 1954. A fuller theory of "Junctions" in inbreeding. Heredity 8:187–197. https://doi.org/10.1038/hdy.1954.17

Foote, A. D., Martin, M. D., Louis, M., Pacheco, G., Robertson, K. M., Sinding, M. H. S., et al. 2019. Killer whale genomes reveal a complex history of recurrent admixture and vicariance. Mol. Ecol. 28:3427–3444.

Fraïsse C., C. Roux, J. J. Welch, and N. Bierne. 2014. Gene-flow in a mosaic hybrid zone: is local introgression adaptive? Genetics 197:939–951. https://doi.org/10.1534/genetics.114.161380

Fuller Z. L., C. J. Leonard, R. E. Young, S. W. Schaeffer, and N. Phadnis. 2018. Ancestral polymorphisms explain the role of chromosomal inversions in speciation. PLOS Genet. 14:e1007526. https://doi.org/10.1371/journal.pgen.1007526

Gopalakrishnan S., M.-H. S. Sinding, J. Ramos-Madrigal, J. Niemann, J. A. S. Castruita, F. G. Vieira, et al. 2018. Interspecific gene flow shaped the evolution of the genus *Canis*. Curr. Biol. 28:P3441–3449.E5. https://doi.org/10.1016/j.cub.2018.08.041

Green R. E., J. Krause, A. W. Briggs, T. Maricic, U. Stenzel, M. Kircher, et al. 2010. A draft sequence of the Neandertal genome. Science 328:710–722. https://doi.org/10.1126/science.1188021

Guerrero R. F., and M. W. Hahn. 2017. Speciation as a sieve for ancestral polymorphism. Mol. Ecol. 26:5362–5368. https://doi.org/10.1111/mec.14290

Haldane, J. B. 1937. The effect of variation of fitness. Am. Nat. 71:337–349.

Han F., S. Lamichhaney, B. R. Grant, P. R. Grant, L. Andersson, and M. T. Webster. 2017. Gene flow, ancient polymorphism, and ecological adaptation shape the genomic landscape of divergence among Darwin's finches. Genome Res. 27:1004–1015. https://doi.org/10.1101/gr.212522.116

Harris K., and R. Nielsen. 2016. The genetic cost of Neanderthal introgression. Genetics 203:881–891. https://doi.org/10.1534/genetics.116.186890

Harrison R. G., and E. L. Larson. 2016. Heterogeneous genome divergence, differential introgression, and the origin and structure of hybrid zones. Mol. Ecol. 25:2454–2466. https://doi.org/10.1111/mec.13582

Hewitt G. 2000. The genetic legacy of the Quaternary ice ages. Nature 405:907–913. https://doi.org/10.1038/35016000

Hofer T., M. Foll, and L. Excoffier. 2012. Evolutionary forces shaping genomic islands of population differentiation in humans. BMC Genomics 13:107. https://doi.org/10.1186/1471-2164-13-107

Huerta-Sánchez E., X. Jin, Asan, Z. Bianba, B. M. Peter, N. Vinckenbosch, et al. 2014. Altitude adaptation in Tibetans caused by introgression of Denisovan-like DNA. Nature 512:194–197. https://doi.org/10.1038/nature13408

Jones M. R., L. S. Mills, P. C. Alves, C. M. Callahan, J. M. Alves, D. J. R. Lafferty, et al. 2018. Adaptive introgression underlies polymorphic seasonal camouflage in snowshoe hares. Science 360:1355–1358. https://doi.org/10.1126/science.aar5273

Juric I., S. Aeschbacher, and G. Coop. 2016. The strength of selection against Neanderthal introgression. PLOS Genet. 12:e1006340. https://doi.org/10.1371/journal.pgen.1006340

Kim B. Y., C. D. Huber, and K. E. Lohmueller. 2018. Deleterious variation shapes the genomic landscape of introgression. PLOS Genet. 14:e1007741. https://doi.org/10.1371/journal.pgen.1007741

Kimura M. 1985. The role of compensatory neutral mutations in molecular evolution. J. Genet. 64:7. https://doi.org/10.1007/BF02923549

Kimura, M., T. Maruyama, amd J. F. Crow. 1963. The mutation load in small populations. Genetics 48:1303–1312.

Kuhlwilm, M., S. Han, V. C. Sousa, L. Excoffier, and T. Marques-Bonet. 2019. Ancient admixture from an extinct ape lineage into bonobos. Nat. Ecol. Evol. 3:957–965.

Ky C.-L., A. Vergnet, N. Molinari, C. Fauvel, and F. Bonhomme. 2012. Fitness of early life stages in F1 interspecific hybrids between *Dicentrarchus labrax* and *D. punctatus*. Aquat. Living Resour. 25:67–75. https://doi.org/10.1051/alr/2012006

Lawson D. J., G. Hellenthal, S. Myers, and D. Falush. 2012. Inference of population structure using dense haplotype data. PLOS Genet. 8:e1002453. https://doi.org/10.1371/journal.pgen.1002453

Lemaire C., J.-J. Versini, and F. Bonhomme. 2005. Maintenance of genetic differentiation across a transition zone in the sea: discordance between nuclear and cytoplasmic markers. J. Evol. Biol. 18:70–80. https://doi.org/10.1111/j.1420-9101.2004.00828.x

Li H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv:1303.3997v2.

Liang M., and R. Nielsen. 2014. The lengths of admixture tracts. Genetics 197:953–967. https://doi.org/10.1534/genetics.114.162362

Loh P.-R., P. Danecek, P. F. Palamara, C. Fuchsberger, Y. A. Reshef, H. K Finucane, et al. 2016. Reference-based phasing using the Haplotype Reference Consortium panel. Nat. Genet. 48:1443–1448. https://doi.org/10.1038/ng.3679

Maggs C. A., R. Castilho, D. Foltz, C. Henzler, M. T. Jolly, J. Kelly, et al. 2008. Evaluating signatures of glacial refugia for North Atlantic benthic marine taxa. Ecology 89:S108–S122. https://doi.org/10.1890/08-0257.1

Marques D. A., K. Lucek, J. I. Meier, S. Mwaiko, C. E. Wagner, L. Excoffier, et al. 2016. Genomics of rapid incipient speciation in sympatric threespine stickleback. PLoS Genet. 12:710–722. https://doi.org/10.1371/journal.pgen.1005887

Marques D. A., J. I. Meier, and O. Seehausen. 2019. A combinatorial view on speciation and adaptive radiation. Trends Ecol. Evol. 34:P531–P544. https://doi.org/10.1016/j.tree.2019.02.008

Martin S. H., and S. M. V. Belleghem. 2017. Exploring evolutionary relationships across the genome using topology weighting. Genetics 206:429–438. https://doi.org/10.1534/genetics.116.194720

Martin S. H., and C. D. Jiggins. 2017. Interpreting the genomic landscape of introgression. Curr. Opin. Genet. Dev. 47:69–74. https://doi.org/10.1016/j.gde.2017.08.007

Martin S. H., J. W. Davey, and C. D. Jiggins, 2015. Evaluating the use of ABBA–BABA statistics to locate introgressed loci. Mol. Biol. Evol. 32:244–257. https://doi.org/10.1093/molbev/msu269

Meier J. I., D. A. Marques, S. Mwaiko, C. E. Wagner, L. Excoffier, and O. Seehausen. 2017. Ancient hybridization fuels rapid cichlid fish adaptive radiations. Nat. Commun. 8:14363. https://doi.org/10.1038/ncomms14363

Meyer M., M. Kircher, M.-T. Gansauge, H. Li, F. Racimo, S. Mallick, et al. 2012. A high-coverage genome sequence from an archaic Denisovan individual. Science 338:222–226. https://doi.org/10.1126/science.1224344

Nelson T. C., and W. A. Cresko. 2018. Ancient genomic variation underlies repeated ecological adaptation in young stickleback populations. Evol. Lett. 2:9–21. https://doi.org/10.1002/evl3.37

Ohta, T. 1971. Associative overdominance caused by linked detrimental mutations. Genet. Res. 18:277–286.

Pääbo S. 2015. The diverse origins of the human gene pool. Nat. Rev. Genet. 16:313–314. https://doi.org/10.1038/nrg3954

Payseur B. A., and L. H. Rieseberg. 2016. A genomic perspective on hybridization and speciation. Mol. Ecol. 25:2337–2360. https://doi.org/10.1111/mec.13557

Pease J., and B. Rosenzweig. 2015. Encoding data using biological principles: the multisample variant format for phylogenomics and population genomics. IEEE/ACM Trans. Comput. Biol. Bioinform. 15:1231–1238. https://doi.org/10.1109/TCBB.2015.2509997

Pool J. E., and R. Nielsen. 2009. Inference of historical changes in migration rate from the lengths of migrant tracts. Genetics 181:711–719. https://doi.org/10.1534/genetics.108.098095

Presgraves D. C. 2010. The molecular evolutionary basis of species formation. Nat. Rev. Genet. 11:175–180. https://doi.org/10.1038/nrg2718

Racimo F., S. Sankararaman, R. Nielsen, and E. Huerta-Sánchez. 2015. Evidence for archaic adaptive introgression in humans. Nat. Rev. Genet. 16:359–371. https://doi.org/10.1038/nrg3936

Racimo F., D. Marnetto, and E. Huerta-Sánchez. 2017. Signatures of archaic adaptive introgression in present-day human populations. Mol. Biol. Evol. 34:296–317. https://doi.org/10.1093/molbev/msw216

Ravinet M., R. Faria, R. K. Butlin, J. Galindo, N. Bierne, M. Rafajlović, et al. 2017. Interpreting the genomic landscape of speciation: a road map for finding barriers to gene flow. J. Evol. Biol. 30:1450–1477. https://doi.org/10.1111/jeb.13047

Rosenzweig B. K., J. B. Pease, N. J. Besansky, and M. W. Hahn. 2016. Powerful methods for detecting introgressed regions from population genomic data. Mol. Ecol. 25:2387–2397. https://doi.org/10.1111/mec.13610

Runemark A., C. N. Trier, F. Eroukhmanoff, J. S. Hermansen, M. Matschiner, M. Ravinet, et al. 2018. Variation and constraints in hybrid genome formation. Nat. Ecol. Evol. 2:549–556. https://doi.org/10.1038/s41559-017-0437-7

Sankararaman S., S. Mallick, N. Patterson, and D. Reich. 2016. The combined landscape of Denisovan and Neanderthal ancestry in present-day humans. Curr. Biol. 26:1241–1247. https://doi.org/10.1016/j.cub.2016.03.037

Schumer M., G. G. Rosenthal, and P. Andolfatto. 2014. How common is homoploid hybrid speciation? Evolution 68:1553–1560. https://doi.org/10.1111/evo.12399

Schumer M., R. Cui, G. G. Rosenthal, and P. Andolfatto. 2015. Reproductive isolation of hybrid populations driven by genetic incompatibilities. PLOS Genet. 11:e1005041. https://doi.org/10.1371/journal.pgen.1005041

Schumer M., G. G. Rosenthal, and P. Andolfatto. 2018a. What do we mean when we talk about hybrid speciation? Heredity 120:379–382. https://doi.org/10.1038/s41437-017-0036-z

Schumer M., C. Xu, D. L. Powell, A. Durvasula, L. Skov, C. Holland, et al. 2018b. Natural selection interacts with recombination to shape the evolution of hybrid genomes. Science 360:656–660. https://doi.org/10.1126/science.aar3684

Seehausen O., R. K. Butlin, I. Keller, C. E. Wagner, J. W. Boughman, et al. 2014. Genomics and the origin of species. Nat. Rev. Genet. 15:176–192. https://doi.org/10.1038/nrg3644

Shpak M. 2005. The role of deleterious mutations in allopatric speciation. Evolution 59:1389–1399. https://doi.org/10.1111/j.0014-3820.2005.tb01789.x

Skov L., R. Hui, V. Shchur, A. Hobolth, A. Scally, M. H. Schierup, et al. 2018. Detecting archaic introgression using an unadmixed outgroup. PLOS Genet. 14:e1007641. https://doi.org/10.1371/journal.pgen.1007641

Slatkin M., and J. L. Pollack. 2008. Subdivision in an ancestral species creates asymmetry in gene trees. Mol. Biol. Evol. 25:2241–2246. https://doi.org/10.1093/molbev/msn172

Snyder C. W. 2016. Evolution of global temperature over the past two million years. Nature 538:226–228. https://doi.org/10.1038/nature19798

Theunert C., and M. Slatkin. 2017. Distinguishing recent admixture from ancestral population structure. Genome Biol. Evol. 9:427–437. https://doi.org/10.1093/gbe/evx018

Tine M., H. Kuhl, P.-A. Gagnaire, B. Louro, E. Desmarais, R. S. T. Martins, et al. 2014. European sea bass genome and its variation provide insights into adaptation to euryhalinity and speciation. Nat. Commun. 5:5770.

Welch J. J., and C. D. Jiggins. 2014. Standing and flowing: the complex origins of adaptive variation. Mol. Ecol. 23:3935–3937. https://doi.org/10.1111/mec.12859

Whitlock M. C., Pä. K. Ingvarsson, and T. Hatfield. 2000. Local drift load and the heterosis of interconnected populations. Heredity 84:452–457. https://doi.org/10.1046/j.1365-2540.2000.00693.x

Wolf J. B. W., and H. Ellegren. 2016. Making sense of genomic islands of differentiation in light of speciation. Nat. Rev. Genet. 18:87–100. https://doi.org/10.1038/nrg.2016.133

Wolf Jochen B. W., L. Johan, and B. Niclas. 2010. Speciation genetics: current status and evolving approaches. Philos. Trans. R. Soc. B Biol. Sci. 365:1717–1733. https://doi.org/10.1098/rstb.2010.0023

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Supplementary Table 1. Summary statistics of sequencing and mapping data for each individual.
Supplementary Figure 1. Depth of coverage per individual.
Supplementary Figure 2. Principal Component Analysis of the European sea bass population genetic structure.
Supplementary Figure 3. Statistics measured in non-overlapping 50 kb windows along the genome.
Supplementary Figure 4. Genome-wide correlations between pairs of statistics measured in non-overlapping 50 kb windows.
Supplementary Figure 5. Introgressed tract length distributions.
Supplementary Figure 6. Data and results for the SNPs and 50 kb window based HMM approach to identify regions involved in reproductive isolation between the two lineages of *D. labrax* along chromosome 7.
Supplementary Figure 7. Distributions and joint allele-frequency spectrums of derived *D. punctatus* alleles present in *D. labrax*.
Supplementary Figure 8. Distributions of the fraction of archaic tracts for Atlantic and Mediterranean genomes inside and outside regions involved in RI.
Supplementary Table 2. Values used to estimate T_{admix} for each chromosome.