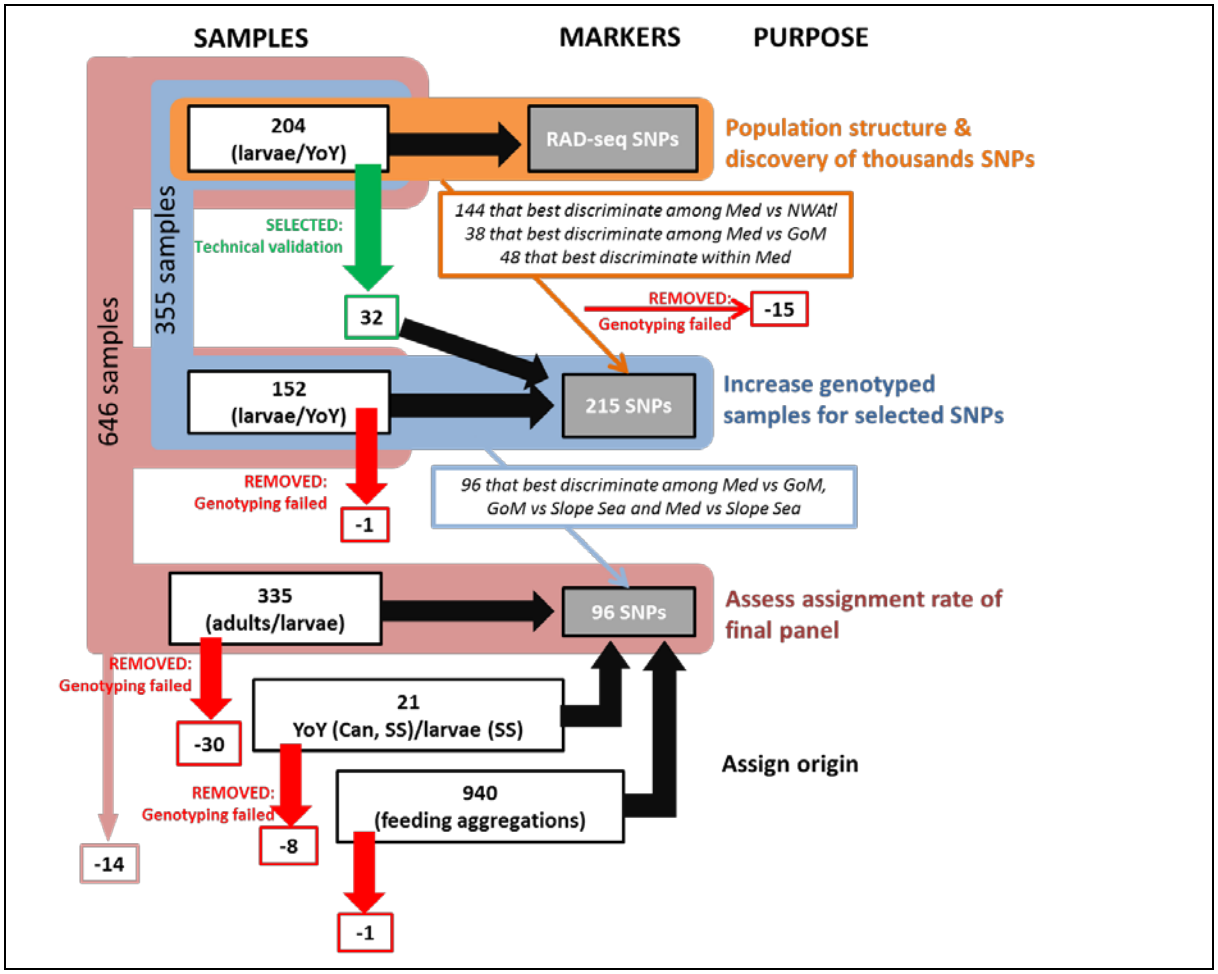
N Rodríguez-Ezpeleta *et al.* – Supporting Information

WebPanel 1. Methodological procedures

A detailed schematic view of the methods followed is provided below. First, thousands of single nucleotide polymorphism (SNP) markers were discovered using RAD-seq on 204 larvae and young of the year (YoY) samples (orange). From those, the 230 most discriminant SNP markers (144 + 38 + 48) were selected and genotyped in 32 previously used and 152 new samples (blue). From those, the 96 most discriminant SNP markers were selected and genotyped for panel validation in 335 spawning adults and larvae (pink). The panel was used to assign origin of Canary Islands (Can) YoY, Slope Sea (SS) larvae and YoY, and of feeding aggregations. The baseline for assignment was composed of 646 individuals resulting from summing up all reference samples used for panel selection and validation, excluding the Slope Sea YoY.



Tissue sampling and DNA extraction

Larvae, YoY, juveniles, and medium-to-large adult bluefin tuna (ABFT, *Thunnus thynnus*) samples were obtained from scientific surveys and commercial fisheries operating throughout the species distribution range, including spawning grounds (WebTables 1–5). From each fish, a ~1-cm^3 piece of muscle or fin tissue sample was excised and immediately stored in RNA-later or 96% molecular grade ethanol at –20ºC until DNA extraction. Larvae were collected using bongo net tows in the known ABFT spawning areas, and immediately preserved in ethanol. The larvae were identified morphologically (Richards 2005) and/or genetically (Puncher *et al*. 2015). Genomic DNA was extracted from about 20 mg of tissue or from whole or partial larvae (eyeballs or tails) using the Wizard Genomic DNA Purification kit (Promega, WI) following manufacturer's instructions for "Isolating Genomic DNA from Tissue Culture Cells and Animal Tissue". Extracted DNA was suspended in Milli-Q water and concentration was determined with the Quant-iT dsDNA HS assay kit using a Qubit 2.0 Fluorometer (Life Technologies; Carlsbad, CA). DNA integrity was assessed by electrophoresis, migrating about 100 ng of GelRed-stained DNA on an agarose 1.0% gel.

RAD-seq library preparation and data analysis

Restriction-site-associated DNA libraries of 204 larvae and YoY samples (WebTable 1) were prepared following the methods of Etter *et al*. (2011). Starting DNA (ranging from 50–250 ng) was digested with the *SbfI* restriction enzyme and ligated to modified Illumina P1 adapters containing 5 base pair (bp) unique barcodes. Pools of 33 individuals were sheared using the Covaris M220 Focused-ultrasonicator Instrument (Life Technologies) and size selected to 300–500 bp by cutting agarose migrated DNA. After Illumina P2 adaptor ligation, each library was amplified using 14 PCR cycles. Each pool was paired-end sequenced (100 bp) on an Illumina HiSeq2000. Raw sequences are available at NCBI SRA Bioproject # SUB4348994. Generated RAD-tags were analyzed using Stacks version 1.32 (Catchen *et al*. 2013). Quality filtering and demultiplexing was performed with process_radtags truncating all reads to 90 nucleotides to avoid the lower quality bases at the end of the read. PCR duplicates were removed applying clone_filter to reads whose forward and reverse pairs passed quality filtering. The following steps were applied to non-clone filtered data (ie all forward reads passing quality filtering, even if their reverse pair failed) and to clone-filtered data (ie single representatives of each PCR clone).

Respectively, only samples with at least 500,000 quality-filter passing forward reads or 100,000 PCR clone representatives were kept. Putative orthologous tags (stacks) per individual were assembled using "ustacks" with a minimum depth of coverage required to create a stack (*m*) of five or three and a maximum nucleotide mismatches (*M*) allowed between stacks of two or four. Catalogs of RAD loci were assembled based on two subsets of individuals (all samples or only Mediterranean Sea samples) using "cstacks" with a number of mismatches allowed between sample tags when generating the catalog (*n*) of three or six. Matches of individual RAD loci to the catalog were searched using "sstacks". From each generated catalog, SNPs present in RAD loci found in at least 75% of the

individuals under study were selected and exported into PLINK format using "populations". Using PLINK version 1.07 (Purcell *et al*. 2007), SNPs with a minimum allele frequency (MAF) smaller than 0.05, a genotyping rate smaller than 0.95, and those that failed the Hardy-Weinberg equilibrium test at $P < 0.05$ in at least two areas of study were excluded. Only samples with genotyping rate above 0.8 were retained per dataset. Each genotype dataset was exported to Structure and Genepop formats using PGDSpider version 2.0.8.3 (Lischer and Excoffier 2012). Genotype tables are available at https://github.com/rodriguez-ezpeleta/ABFT_popgentrace

Genetic diversity and population structure

F_{ST} values per population pair were calculated on each genotype dataset following the Weir and Cockerham (1984) formulation as implemented in Genepop 4.3 (Rousset 2008). Principal component analyses (PCAs) were performed with the R package "adegenet" (Jombart and Ahmed 2011) with no a priori population assignment of samples. For each genotype dataset, 10 subsets of 5000 randomly chosen SNPs were created and analyzed with the Bayesian clustering approach implemented in STRUCTURE (Pritchard *et al*. 2000). For each value of *K* (number of potential ancestral populations, which ranged from 1 to the number of presumed populations + 1), the genetic ancestry of each individual was evaluated without any prior population assignment, based on the admixture model and a burn-in period of 100,000 iterations followed by 300,000 iterations from which estimates were obtained. The results obtained from the 10 subsets considered for each value of *K* were analyzed with CLUMPP (Jakobsson and Rosenberg 2007) to identify common modes, and results were plotted using DISTRUCT (Rosenberg 2004). Best *K* was identified according to the Evanno method (Evanno *et al*. 2005) as implemented in StructureHarvester (Earl and vonHoldt 2012). Statistical significance among the distributions of belonging to one of two hypothetical ancestral populations was assessed with the Wilcoxon rank-sum test.

SNP selection and genotyping

For each catalog based on all samples, the 200 SNPs with the highest F_{ST} values among Northwest Atlantic (Gulf of Mexico larvae and Slope Sea YoY) and Mediterranean Sea (larvae and YoY) samples and the 100 SNPs with the highest F_{ST} values among Gulf of Mexico larvae and Mediterranean Sea larvae and YoY were selected. For each catalog based on only the Mediterranean Sea samples, the 30 SNPs with the highest F_{ST} values among each pair of intra Mediterranean Sea areas (West, Central, and East) were retrieved, and from those, the 50 SNPs that provided the highest sum of pairwise F_{ST} values for each catalog were selected. The flanking regions of the 1400 selected SNPs were obtained by matching their corresponding tags against the ABFT reference genome (Puncher *et al*. 2018) using an in-house script (see https://github.com/rodriguez-ezpeleta/ABFT_popgentrace). Obtained sequences were submitted to the Assay Design Group at Fluidigm Corporation (San Francisco, CA), and from the ones fulfilling the Fluidigm design criteria, the 144 that most discriminated between Northwest Atlantic

versus Mediterranean samples, the 38 that most discriminated between Gulf of Mexico versus Mediterranean samples, and the 48 that most discriminated among Mediterranean Sea locations were selected (a total of 230 originating from the eight catalogs; WebTable 6) and sent for design and manufacture of primers for a SNPtype genotyping panel. Genotyping of 184 larvae and YoY samples (WebTable 2), including 32 already genotyped with RAD-seq, was performed on the Biomark HD platform using Flex Six, 48.48 and 96.96 Dynamic Array IFCs, and the resulting data were analyzed with the Fluidigm Genotyping Analysis Software (one sample was removed for failing for more than 20% of the SNPs). Genotypes derived from RAD and Fluidigm were combined for the 230 SNPs to make a dataset of 355 individuals (204 + 151 [184–32 repeated – 1 failed]). Successfully genotyped SNPs were ranked according to the average F_{ST} values for each of the following pairs: Gulf of Mexico versus Mediterranean Sea, Gulf of Mexico versus Slope Sea, and Slope Sea versus Mediterranean Sea. SNPs were checked for linkage disequilibrium using Genepop 4.3 (Rousset 2008), and only one per linked group ($P < 0.001$) and/or per genome contig was selected. Ninety-six SNPs with the lowest averaged F_{ST} value-based rank across the three pairs were selected (WebTable 7). 328 adults captured in the Gulf of Mexico and the Mediterranean Sea during the spawning season, along with seven larvae from the Gulf of Mexico (WebTable 3), six Canary Islands YoY, one Slope Sea YoY, 14 Slope Sea larvae (WebTable 4), and 940 feeding aggregations (WebTable 5), were genotyped for the 96 selected SNPs using 96.96 Dynamic Array IFCs. The resulting data were analyzed using Fluidigm Genotyping Analysis Software. Of these, 39 failed genotyping and were excluded from further analyses.

Origin assignment of samples of known and unknown origin

Self-assignments were performed by calculating assignment scores (that is, the probability of belonging to each of the baseline populations) with GeneClass2 (Piry *et al*. 2004) using the Rannala and Mountain (1997) criterion (0.05 threshold) of (1) 204 individuals (WebTable 1) using the RAD-seq derived genotypes for 169 SNPs (excluding, from the 230 selected, the 15 that failed, and the ones selected for discrimination among Mediterranean areas); (2) 355 individuals genotyped for 230 SNPs (WebTables 1 and 2, minus the 32 already genotyped with RAD-seq and the one that failed) using the RAD-seq and Fluidigm derived genotypes for the 96 final SNP set considering two (Gulf of Mexico and Mediterranean Sea) or three (Gulf of Mexico, Slope Sea, and Mediterranean Sea) populations as baselines; and (3) 165 Mediterranean Sea individuals (WebTable 1) using the RAD-seq derived genotypes for 46 SNPs (48 selected minus two that failed) considering three Mediterranean Sea populations as baselines. Samples with assignment scores below 90% were considered “unassigned”.

Using 341 reference individuals (WebTables 1 and 2, minus the 32 already genotyped with RAD-seq, the one that failed, and 14 Slope Sea YoY), loci were ranked according to their discriminative power to assign samples to their known origin following the allele frequency differential method implemented in WHICHLOCI (Banks *et al*. 2003). Assignment power of the developed 96 SNP panel and derived subsets was assessed by

calculating percentages of correctly and incorrectly assigned of samples of known origin not used for SNP selection. For that aim, assignment scores of each sample were calculated with GeneClass2 (Piry *et al*. 2004) using the Rannala and Mountain (1997) criterion (0.05 threshold). Assignments of natal origin (Gulf of Mexico and Mediterranean Sea) were calculated for 305 adults and larvae captured within one of the two spawning areas during the spawning season (WebTable 3) using a leave-one-out approach with a baseline of 646 individuals: that is, the 341 previously genotyped samples (355 minus 14 Slope Sea YOY) and the 305 spawning adults. True positive stock of origin assignment rate, true negative assignment rate, positive predictive value, negative predictive value, and accuracy were calculated for 70%, 80%, and 90% probability of assignment thresholds; the 80% assignment score threshold was selected as being the one providing the largest number of assigned individuals without compromising accuracy. Origins of Canary Islands YoY, Slope Sea larvae and YoY (WebTable 4), and of 940 feeding aggregation samples (WebTable 5) were calculated using the combined set of 646 reference samples as baseline. Samples with assignment scores below 80% were considered unassigned.

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