Genomic consequences of a recent three-way admixture in supplemented wild brown trout populations revealed by local ancestry tracts

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Abstract
Understanding the evolutionary consequences of human-mediated introductions of domesticated strains into the wild and their subsequent admixture with natural populations is of major concern in conservation biology. However, the genomic impacts of stocking from distinct sources (locally derived vs. divergent) on the genetic integrity of wild populations remain poorly understood. We designed an approach based on estimating local ancestry along individual chromosomes to provide a detailed picture of genomic admixture in supplemented populations. We used this approach to document admixture consequences in the brown trout Salmo trutta, for which decades of stocking practices have profoundly impacted the genetic make-up of wild populations. In southern France, small local Mediterranean populations have been subject to successive introductions of domestic strains derived from the Atlantic and Mediterranean lineages. To address the impact of stocking, we evaluate the extent of admixture from both domestic strains within populations, using 75,684 mapped SNPs obtained from double-digested restriction site-associated DNA sequencing. Then, the chromosomal ancestry profiles of admixed individuals reveal a wider diversity of hybrid and introgressed genotypes than estimated using classical methods for inferring ancestry and hybrid pedigrees. In addition, the length distribution of introgressed tracts retained different timings of introgression between the two domestic strains. We finally reveal opposite consequences of admixture on the level of polymorphism of the recipient populations between domestic strains. Our study illustrates the potential of using the information contained in the genomic mosaic of ancestry tracts in combination with classical methods based on allele frequencies for analysing multiple-way admixture with population genomic data.

KEYWORDS
hybridization, introgression, local ancestry inference, Salmo trutta, stocking practices

1 | INTRODUCTION

From the early 1950s, hybridization between species, lineages or populations has been associated with intentional or unintentional disturbance of natural systems by human activities (Anderson, 1953; Anderson & Stebbins, 1954). Since then, human-mediated hybridization (HMH) resulting in gene flow between source and recipient populations has become an increasingly significant concern in conservation biology (Levin, Francisco-Ortega, & Jansen, 1996; Rhymer & Simberloff, 1996). Despite the continued technical improvements...
in the analysis of hybridization and admixture, the debate about the costs and benefits of HMH is still ongoing (Bohling, 2016; Grabenstein & Taylor, 2018; Wayne & Shaffer, 2016). On the one hand, intra- or interspecific hybridization can be sources of beneficial genes transfers and evolutionary novelties through adaptive introgression (Anderson & Stebbins, 1954; Heiser, 1951; Lamichhaney et al., 2017; Runemark et al., 2018; Schumer, Rosenthal, & Andolfatto, 2014; Stebbins, 1950; Whitney et al., 2015; Yakimowski & Rieseberg, 2014). On the other hand, they can disrupt local adaptations and genes coadaptation, leading to maladaptive introgression and a loss of adaptive diversity (Laikre, Schwartz, Waples, & Ryman, 2010; Randi, 2008; Rhymy & Simberloff, 1996; Todesco et al., 2016). The emerging view offered by genomewide surveys is that HMH between species, taxa or populations often results in a mix of positive and negative effects that are scattered across the genome (Allendorf, 2017; Edmands, 2007; Hedrick, 2013). This is consistent with the “haplotype block view” of hybridization introducing both adaptive and maladaptive “blocks of genic materials belonging to different adaptive systems” (Anderson & Stebbins, 1954).

The “genic block” metaphor refers to the mosaic of local ancestry tracts along individual genomes, which results from multigenerational gene flow and recombination events among hybridizing taxa (Buerkle & Lexer, 2008; Gompert & Buerkle, 2013; Gravel, 2012; Liang & Nielsen, 2014; Liu et al., 2013). The identification of ancestry tracts is based on the analysis of marker associations in admixed individuals with the goal of recovering continuous ancestry blocks inherited from ancestral taxa, sometimes including explicitly linkage information (Lawson, Hellenthal, Myers, & Falush, 2012; Loh et al., 2013; Price et al., 2009; Zhou, Qiu, & Xu, 2017). Compared to analyses based on allele frequencies, the study of ancestry tracts provides more detailed information for inferring population-level processes such as demography and admixture (Allendorf, 2017; Duranton et al., 2018). For instance, the number and the length distribution of ancestry tracts allow to estimate the admixture level and its variation at both a genomewide scale (i.e., the relative ancestry proportions of each admixed individual; Buerkle, 2005) and a local chromosomal scale (i.e., the ancestry at a particular locus; Falush, Stephens, & Pritchard, 2003). This is of prime importance for management decision such as putting under protection populations that have been described as “pure” (i.e., not introgressed by maladaptive alleles; e.g., Hansen & Mensberg, 2009). Moreover, because recombination progressively breaks down ancestry tracts across generations, the length of introgressed fragments allows to estimate the number of generations spent since initial admixture (Corbett-Detig & Nielsen, 2017; Gravel, 2012; Pool & Nielsen, 2009). Ultimately, the genomewide distribution of introgressed tracts could reveal genomic regions that might be important for providing selective advantages or disadvantages to introgressed individuals (Flori et al., 2014; Hedrick, 2013; Medugorac et al., 2017; Nelson, Wallberg, Simões, Lawson, & Webster, 2017; Racimo, Sankararaman, Nielsen, & Huerta-Sánchez, 2015; Wall et al., 2013; Wang, Vieira, Crawford, Chu, & Nielsen, 2017). The development of next-generation sequencing (NGS) technologies has offered an unprecedented way to study admixture and introgression in many hybridizing taxa including both model and non-model species (Allendorf, Hohenlohe, & Luikart, 2010; Angeloni, Wagemaker, Vergeer, & Ouborg, 2012; Hoffmann et al., 2015; Kohn, Murphy, Ostrander, & Wayne, 2006; Narum, Buerkle, Davey, Miller, & Hohenlohe, 2013; Ouborg, Pertoldi, Loeschke, Blijsema, & Hedrick, 2010; Primmer, 2009; Steiner, Putnam, Hoeck, & Ryder, 2013). However, only few studies have used local ancestry blocks to characterize the genomic landscape of admixture in conservation genomics (but see Duranton et al., 2018; Galavemi et al., 2017; VonHoldt, Kays, Pollinger, & Wayne, 2016).

Because of their socio-economic importance, salmonids are certainly among fish, the most extensively managed worldwide (Antunes et al., 1999; Davidson et al., 2010; Fraser, 2008). Stocking practices and enhancement using hatchery fishes from distinct lineage as well as from nearby population with a closely related genetic background have been common in Salmonids (Angeloni et al., 2012; Antunes et al., 1999; Aprahamian, Martin Smith, McGinnity, McKelvey, & Taylor, 2003; Sundt-Hansen, Huisman, Skoglund, & Hindar, 2015) and have often resulted in gene flow between domestic and wild populations. Understanding the fitness consequences of such gene flow has become a central issue to many conservation programmes (Fraser, 2008; Glover et al., 2017; Harbicht, Wilson, & Fraser, 2014; Naish et al., 2007; Skaala et al., 2014; Waples, 1991). The impact of HMH has been classically studied using methods based on allele frequencies, highlighting neutral genetic diversity losses (Fernández-Cebrián Araguas, Sanz, García-Marín, & Fraser 2014; Hansen & Mensberg, 2009; Laikre et al., 2010), and altered local adaptation, fitness or reproductive success (Le Cam, Perrier, Besnard, Bernatchez, & Evanno, 2015; McGinnity et al., 2003; Mulhfeld et al., 2009). Genetic homogenization (i.e., loss of fine-scale population structure) among recipient populations has also been repeatedly pointed out (Eldridge, Myers, & Naish, 2009; Ozerov et al., 2016; Valiquette, Perrier, Thibault, & Bernatchez, 2014). However, most of these studies did not take into account the linkage information contained in ancestry blocks, and therefore could not provide estimates of local ancestry and time since introgression (but see Ozerov et al., 2016). Now that extensive genomic resources are available in salmonids, including dense linkage maps (Gagnaire, Pavéy, Normandeau, & Bernatchez, 2013; Gonen et al., 2014; Leitwein et al., 2017; Lien et al., 2011; McKinney et al., 2015; Sutherland et al., 2016; Tsai et al., 2016; Brieuc, Waters, Seeb, & Naish, 2014) and reference genomes (Berthelot et al., 2014; Lien et al., 2016), it becomes possible to explore the potential of local ancestry inference for studying the genomic consequences of HMH.

The brown trout (Salmo trutta L.) is a widely distributed Eurasian species (Sanz, 2017) which is heavily impacted by human activities and subject to intense stocking practices. The resulting introgression of foreign domestic alleles is suspected to be maladaptive to wild fish, even though large variation in levels of introgression has been reported (Almódovar, Suárez, Nicola, & Nuevo, 2001; Berrebi, Poteaux, Fissier, & Cattaneo-Berrebi, 2000; Hansen, 2002; Hansen, Fraser, Meier, & Mensberg, 2009; Poteaux, Bonhomme, & Berrebi, 1999). In France, a domestic Atlantic hatchery lineage which has

![Image](366x747 to 460x752)
been distributed worldwide has been used for decades for restocking and enhancement (Bohling, Haffray, & Berrebi, 2016), including in Mediterranean rivers where a distinct evolutionary lineage naturally occurs (Bernatchez, 2001). To avoid genetic admixture among lineages and the potentially negative consequences of HMH, several recent attempts have been made to develop local domestic Mediterranean strains, with the aim to substitute the release of foreign Atlantic genotypes by local genotypes (Bohling et al., 2016). However, the evolutionary consequences and the potential fitness outcomes of these different stocking practices on the genetic make-up of wild populations have not been assessed yet at the genomic level.

The aim of this study was to describe the mosaic of ancestry tracts resulting from the introduction of two different source strains, in order to investigate the evolutionary consequences of admixture and their implications for conservation genomics in a context of intraspecific HMH. We introduce an individual-level ancestry metrics inspired from the hybrid index and the interspecific heterozygosity (Anderson, 1953; Buerkle, 2005; Gompert & Buerkle, 2016), which quantifies the “chromosomal ancestry imbalance” (CAI) between homologues. We show that local ancestry inference is an efficient approach to distinguish early- and late-generation hybrids, without relying on the use of highly differentiated loci. Finally, we estimate the relative timing of hybridization with the two introduced strains using the length distribution of introgressed tracts and reveal opposite consequences of introgression on the level of polymorphism of the local populations.

2 | MATERIALS AND METHODS

2.1 | Sampling

Wild Mediterranean brown trout were sampled in southern France in April–May 2015 from three tributaries of the Orb River watershed, which belong to a long-term monitoring programme of the Hérault French Fishing Federation. Eighty-two individuals, with individual size ranging from 101 to 337 mm, were caught by electrofishing, fin-clipped and then locally released under the supervision of the Hérault French Fishing Federation. The study includes a total of 45 individuals from the Gravezon River, 14 from the Mare River and 23 from the upper Orb River (Table 1). Farmed fish (N = 102) were sampled in 2014 at the Babeau hatchery to genetically characterize each of the two domestic strains that were used for stocking. They consisted of 61 individuals from the Atlantic domestic strain, which was commonly used for stocking practices in France for decades (Bohling et al., 2016), and 41 individuals from a Mediterranean domestic strain, which was initially developed by the Hérault French Fishing Federation in 2004 (Table 1). The Mediterranean domestic strain was founded exclusively with individuals from the Gravezon River (Hérault French Fishing Federation, personal communication, 2014). Additional information on the stocking of Atlantic brown trout in France and sampling is provided in Bohling et al. (2016) and Leitwein et al. (2016), respectively. Sixty individuals (including both farmed and wild) of the 184 analysed in this study were part of a previous study describing genomewide patterns of nucleotide diversity in Atlantic and Mediterranean brown trout lineages using double-digested restriction site-associated DNA (dd-RAD)-seq (Leitwein et al., 2016).

2.2 | DNA extraction and sequencing

Individual genomic DNA was extracted from caudal fin clips using the commercial KingFisher Flex Cell and Tissues DNA Kit. DNA quantity and quality were evaluated using both NanoDrop ND-8000 spectrophotometer (Thermo Fisher Scientific) and Qubit 1.0 fluorometer (Invitrogen, Thermo Fisher Scientific). The dd-RAD library preparation protocol followed Leitwein et al. (2016). Briefly, the two restriction enzymes EcoRI-HF and MspI were used to digest individual genomic DNA, which was subsequently submitted to adapter ligation (one with unique barcodes for each individual and one common to 48 individuals containing Illumina index). Each library consisting of 48 individuals with unique barcodes pooled in equimolar proportions was fragmented and submitted to size selection to retain fragments ranging from 200 to 700 bp using CleanPCR beads. The library was then amplified by PCR and sequenced with Illumina HiSeq2500, producing 125-bp paired-end reads.

2.3 | Genotyping

The bioinformatics pipeline used for SNP calling has been described in Leitwein et al. (2016). Briefly, reads where demultiplexed, cleaned and trimmed to 120 bp using process_radtags.pl implemented in STACKS v1.35 (Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013). Individual genotypes at RAD markers were determined using a reference mapping approach with the Atlantic salmon genome taken as a reference (GenBank Accession no.: GCA_000233375.4, ICSASG_v2; Lien et al., 2016). The BWA_MEM program v. 0.7.9 (Li & Durbin, 2010) was used to align reads along Salmo salar genome before calling SNPs for each individual with the pstacks module (using m = 3 and the bounded error model with α = 0.05). We used a previously established reference RAD catalogue constructed by Leitwein et al. (2016, 2017) (-catalog in the cstacks module) for

| TABLE 1 | Number of individuals sampled for the hatchery strain and wild population (N) along with the number of individuals after filtering for missing data (Nfiltered) and the number of individuals considered as pure domestic or pure wild with ADMIXTURE (Npure) see text for details |
|---------|-----------------|----------|--------|
| Samples | Labels          | N        | Nfiltered | Npure |
| Hatchery strain | Cauteret (Atlantic) | ATL 61 54 | 54 |
|          | Babeau (Mediterranean) | domMED 41 40 | 40 |
| River Orb | Gravezon | Grav 45 45 | 15 |
|          | La Mare | Mare 14 14 | 4 |
|          | Upper Orb | Orb 23 23 | 10 |
|          | Total | 184 176 | 123 |
which the risk of hidden paralogy has been controlled by stringent filtering on both expected Hardy–Weinberg equilibrium proportions and Mendelian segregation ratios in family crosses, but without entirely excluding residual tetrasomic genomic regions (Leitwein et al., 2017). Each of the 184 individuals was then matched against this catalogue with sstacks. Seven domestic Atlantic and one domestic Mediterranean individuals were removed because of high percentage of missing data (>20%), resulting in a final data set of 176 individuals (82 wild Mediterranean individuals, 54 Atlantic and 40 Mediterranean domestic individuals). Finally, the populations module was used to generate a genotype data set in VCF and PLINK formats containing loci that passed the following filters: (a) a minimum stacks depth of 5 reads; (b) a genotype call rate of at least 80% within each of the five populations; (c) a minimum allele frequency of 2%; and (d) a maximum observed heterozygosity of 60%. A single representative of each overlapping site was kept with the option-ordered_export in STACKS.

2.4 | Inference of admixture and hybridization

We used the filtered data set containing 86,175 SNPs to estimate individual cluster membership using ADMIXTURE v1.3 (Alexander, Novembre, & Lange, 2009). This program provides an estimation of individual ancestry proportions from K different source populations (clusters) that are inferred from the data. As we aimed at detecting genetic admixture between wild individuals and two different domestic strains, ADMIXTURE was run separately for each of the three wild populations (Gravezon, Mare and Orb), along with the Atlantic and Mediterranean domestic strains, with a K value of 3. Bar plots of estimated ancestry proportions given by the Q-values estimates by ADMIXTURE were produced with R v. 3.4.3 (R. C. Team, 2015). Individuals with an ancestry assignment greater than 95% were defined as “pure” and used as parental references in the following analyses.

We used the NewHYBRIDS v2.0 software (Anderson & Thompson, 2002) to assign individual genotypes to Atlantic and Mediterranean parental lineages and different hybrid pedigrees (including F1 and F2 hybrids and first-generation backcrosses (BC) in each direction) in each of the three rivers. Domestic Atlantic individuals (N = 54) and wild individuals with a Mediterranean ancestry greater than 95% as estimated by ADMIXTURE (N = 29 individuals; \(N_{\text{Gravezon}} = 15, N_{\text{Mare}} = 4, N_{\text{Orb}} = 10\); Table 1) were considered as pure parental references for the Atlantic and Mediterranean lineages, respectively, using parental priors with the z and s options. We only used the most informative SNPs between these parental reference populations to increase the detection efficiency of hybrid categories and to reduce the computational time. In total, 196 SNPs with a Weir and Cockerham’s \(F_{ST} > 0.85\) were retained to run NewHYBRIDS using 50,000 iterations after 100,000 burn-in steps.

2.5 | Inference of local ancestry

The inference of local ancestry was performed with ELAI v1.01 (Guan, 2014), which relies on a two-layer hidden Markov model to detect the structure of haplotypes in unrelated individuals. The program was run separately for each of the 40 linkage groups (LG) of the S. trutta linkage map (Leitwein et al., 2017). We took advantage of the strong collinearity between the S. trutta and S. salar genomes (Leitwein et al., 2017) to anchor the 4,000 mapped RAD loci of the brown trout linkage map onto the reference genome of S. salar. This strategy allowed to determine the relative mapping positions of a large number of additional RAD loci that were not present on the brown trout linkage map, using their relative positions on the Atlantic salmon reference genome. A list of ordered RAD loci was created for each of the 40 brown trout LGs and, using the previously described filters, passed to the STACKS populations module using the whitelist option (−W) to generate an ELAI input file for each brown trout LG. ELAI was run separately for each wild Mediterranean population. In each run, we used the 54 Atlantic and the 40 Mediterranean domestic individuals as source populations and also used wild individuals with a Mediterranean ancestry greater than 95%, according to ADMIXTURE, as a third source population (N = 29; Table 1). The number of upper clusters (−C) was set to 3 (i.e., because wild fish potentially originated from up to three source populations: domestic Atlantic, domestic Mediterranean and wild Mediterranean), the number of lower clusters (−c) to 15 (i.e., 5C, as in Guan, 2014), the number of admixture generations (−mg) to 10 (i.e., approximately corresponding to the beginning of stocking) and the number of expectation–maximization steps (−s) to 20. The ancestral allele dosage from a given source population (see below) at each SNP was finally plotted for each individual along each LG to generate ancestry profiles with R (R. C. Team, 2014).

2.6 | Estimation of tract length and chromosomal ancestry imbalance

The junctions between haplotypes originating from different source populations were identified from the analysis of individual ancestry profiles produced by ELAI, as illustrated in Figure 1. At each variable position, we conservatively considered as evidence for 0, 1 or 2 haplotype copies from a given source when the inferred ancestry dosage was within the range [0, 0.05], [0.95, 1.05] or [1.95, 2], respectively (Figure 1a). Therefore, the junctions between haplotypes from different sources occurred within “uncertainty areas” where the ancestry dosage lays between 0.05 and 0.95, or between 1.05 and 1.95. For each “uncertainty area” within each LG of each individual, the junction between the ending and starting positions of two haplotypes from different sources was determined as the position where the estimated ancestry-dosage curve produced by ELAI crossed the 0.5 or 1.5 values (Figure 1a).

Because the approach implemented here with ELAI does not use phased haplotype data, the delimitation of tract junctions could not be unambiguously resolved in the particular case when the ancestry dosage was found to vary from 1 to 2 and back to 1 haplotype copy (Figure 1a). Such profiles may correspond to two alternative situations: (a) the co-occurrence of a short haplotype inherited from one parent, fully overlapping a long haplotype
inherited from the other parent (case [a] in Figure 1a), and (b) the presence of two medium-sized and partially overlapping haplotypes (case [b] in Figure 1a). Although it is not possible distinguishing between these two alternatives, we systematically resolved these cases by considering the existence of one short and one long haplotype, to avoid oversplitting real long haplotypes in early-generation hybrids, which would artificially downwardly bias our estimates of CAI (see below).

The identification of tract junctions allowed retrieving the number and length of tracts originating from each of the three source populations for each of the 40 LGs of each individual. Haplotypes lengths were then summed per origin across LGs and divided by the diploid genome size to estimate individual ancestry proportions from domestic Atlantic, domestic Mediterranean and wild Mediterranean sources. To evaluate the consistency of admixture proportions estimated using SNP frequencies and haplotype lengths, we tested the correlation between the levels of admixture estimated with ADMIXTURE and ELAI using Spearman test (R. C. Team, 2014). We further tested whether the percentage of individual domestic ancestry from each domestic source correlates with the genomewide averaged heterozygosity computed with VCFTools v0.1.14 (Danecek et al., 2011).

To characterize the dilution of Atlantic alleles within Mediterranean genomes over time, we defined the CAI (Figure 1b). For a given chromosome, the CAI represents the difference between the cumulated lengths of Atlantic haplotypes from each of the two parental homologues, divided by the haploid chromosome length. Therefore, the CAI ranges from 0 to 1 and can be averaged across the 40 LGs to provide a genomewide measure for each individual. The CAI theoretically reaches its maximal value of 1 for F1 hybrids obtained by crossing two pure (i.e., nonadmixed) parental individuals and is progressively reduced at every generation postadmixture due to recombination and random transmission of homologues from one generation to the next. Therefore, the CAI converges to 0 after a sufficient number of generations postadmixture. The value of 0 also characterizes pure individuals (e.g., F0_ATL and F0_MED; Figure 1b).

2.7 Quantifying time since hybridization

The length distribution of tracts originating from the two domestic sources was used to estimate the timing of hybridization in each of the three wild populations. Following (Racimo et al., 2015), the
relationship between the time since admixture ($T$ in generations) and the mean length of introgressed tracts ($l$) is given by:

$$T = 1 + \frac{l(1 - m)}{r}$$

(1)

where $m$ is the proportion of domestic ancestry within the recipient population and $r$ is the recombination rate (in Morgan per base pair per generation). We used the genomewide average recombination rate estimated in Leitwein et al. (2017), taking into account its variation across the genome to account for uncertainty. The mean tract length ($l$) in Equation 1 was replaced by the mode of the length distribution of domestic tracts (either of Atlantic or Mediterranean origin). Because the stocking history which has been going on for many years have not been rigorously recorded, and because intraspecific hybridization might have been continuous over years (i.e., stocking was made on a regular annual basis by Fish Federations, first with the Atlantic strain, then with the locally derived Mediterranean strain) instead of having a unique pulse of hybridization, using the mode of the distribution will certainly better reflect the average time at which the intensity of stocking was stronger. Alternatively, the mean of the distribution would instead capture the presence of long tracts in early hybrid generations (Figure S1). Thus, using the mode of the distribution for estimating the timing of hybridization was considered more realistic to obtain more relevant estimates for conservation in this study. However, a detailed picture of continuous introgression events cannot be provided and conditions for detection have to be investigated further. Individuals identified as “pure” F0 individuals were discarded prior to building the length distributions of Atlantic and Mediterranean domestic tracts. The mode of the distribution has been estimated by computing kernel density estimates in R with default bandwidth, and 10,000 replicates to obtain a 97.5% confidence interval (Figure S1; Table S4).

3 | RESULTS

3.1 | SNP calling

A total of 1.5 billion of demultiplexed raw reads resulting in an average of 8.76 million reads per individuals were retained for the STACKS analysis (Table S1). The previously established RAD catalogue (Leitwein et al., 2016) based on 64 individuals was updated using the 176 individuals considered in this study. After applying quality and population filters, we finally retained for subsequent analyses a total of 86,175 SNPs from 45,435 RAD loci, with a mean coverage depth of $47.7 \pm 1.79 \times$ per SNP per individuals and a percentage of missing genotypes of $4.20 \pm 2.9\%$ per individuals (Table S1).

3.2 | Inference of admixture and hybridization

The admixture program identified the three expected groups ($K = 3$) for each run performed separately for each wild population, including the domestic Atlantic and Mediterranean strains along with the wild populations considered in each run (either Gravezon, Mare or Orb; Figure 2). In the Gravezon River, six individuals were assigned as pure domestic Atlantic fish, and several individuals found with a 50% domestic Atlantic and a 50% wild Mediterranean ancestry were probably F1 hybrids. In the Mare River, four individuals had more than 50% of Mediterranean domestic ancestry, and no putative Atlantic/ Mediterranean F1 individual was detected. As well as in the Orb River, low proportions of domestic ancestries from either Atlantic or Mediterranean strains were detected. Moreover, each of the three admixture runs revealed that the Atlantic domestic strain displays a low proportion of Mediterranean ancestry, with consistent estimates.

**FIGURE 2** Plots of the individual ancestry inference for the 86,175 SNPs present in wild-caught brown trout. Analyses were separately run ($K = 3$) for individuals of the three wild populations (from top to bottom: Gravezon; Orb and Mare populations) using admixture v1.3. Each individual is represented by a single vertical line, with colours indicating the estimated ancestry in each of the $K = 3$ groups: Atlantic domestic strain (blue), Mediterranean domestic strain (red) or wild Mediterranean (green) [Colour figure can be viewed at wileyonlinelibrary.com]
of individual admixture proportions among runs (Figure 2). This might reflect a possible admixture with Mediterranean individuals during the development of the Atlantic strain, or ADMIXTURE artefacts.

The numbers of pure, F1, F2 and first-generation BC between Atlantic and Mediterranean lineages present in each wild population were estimated with NEWHYBRIDS. Despite setting priors on parental samples, two Atlantic individuals from the hatchery were not assigned to the parental Atlantic lineage, indicating possible admixture in their recent ancestry (Table S2). Similarly, two individuals from the Orb, one from the Gravezon and four from the Mare River were not assigned as “pure” wild individuals despite being defined as parental individuals in the prior settings (Table S2). According to NEWHYBRIDS, hybrid genotypes were present in all of the three wild populations, including seven F1s in the Gravezon River, and four, three and five F2s in the Orb, Gravezon and Mare rivers, respectively (all with \( \geq 95\% \) probability of assignment; Table S2). The first generation of backcrossed genotypes (BC between F1 and pure wild Mediterranean individuals) were mostly detected in the Orb River with NEWHYBRIDS (Table S2).

### 3.3 Inference of local ancestry

Local ancestry inference was performed in ELAI using 75,684 mapped SNPs distributed along the forty *S. trutta* LGs for 53 admixed individuals captured in the wild (30 from the Gravezon, 10 from the Mare and 13 from the Orb; Table S3), using individuals identified from ADMIXTURE analyses as pure domestic (ATL-Dom or MED-Dom) or wild Mediterranean (MED-Wild) as reference samples. For clarity, Figure 3 provides four examples illustrating the inference of local ancestry profiles along a LG in individuals with different ancestries. It first shows an introgressed Atlantic haplotype of about 3 Mb (ATL-Dom ancestry dosage = 1) within a wild Mediterranean fish (MED-Wild ancestry dosage = 2) (Figure 3a). Then, a panel describes a typical profile of a F1 hybrid resulting from the first generation of crossing between a pure domestic Atlantic and a pure wild Mediterranean individual, both contributing to an ancestry dosage of 1 all along the LG (Figure 3b). A F1 hybrid resulting from the crossing of a pure domestic Atlantic and a wild Mediterranean parent introgressed by the Atlantic domestic strain (i.e., resulting in homozygous Atlantic positions with an ATL-Dom ancestry dosage = 2) is reported in Figure 3c. The last panel illustrates the typical profile of a pure domestic Atlantic individual (ATL-Dom ancestry dosage = 2) (Figure 3d). In these four selected examples, no signature of domestic Mediterranean introgression was observed (i.e., the MED-Dom ancestry dosage is expected to be equal to 0).

Junctions between adjacent ancestry tracts were generally represented by sharp transitions in chromosomal ancestry profiles. On average, regions of uncertain ancestry were estimated to be

![FIGURE 3] Examples of four individual plots of the local ancestry inference run established with ELAI (Guan, 2014). The ancestry dosage (y-axis; see Figure 1a) is represented along linkage groups 18, 3 and 2 (x-axis), for four distinct Gravezon individuals (18, 9, 1 and 7). In green: the wild Mediterranean ancestry, in red: the domestic Mediterranean ancestry and in blue: the Atlantic domestic ancestry [Colour figure can be viewed at wileyonlinelibrary.com]
6.54 ± 5.75% of the genome around domestic Atlantic tracts, and 12.15 ± 8.15% around domestic Mediterranean tracts. This result indicates a reasonably high precision to identify junctions between adjacent ancestry tracts, with precision increasing with genetic divergence between strain lineages and wild-caught fish.

3.4 Analysis of hybridization and admixture from local ancestry tracts

To provide an estimate of the genomewide ancestry from each source population, local ancestry patterns were summarized across LGs to determine the total number and the cumulative length of introgressed haplotypes along the genome of each individual. The number and the mean length of introgressed haplotypes of domestic Atlantic and Mediterranean ancestry were found to considerably vary among the 53 admixed individuals considered in this study (Table S3). Within individual, the length of ancestry tracts was also highly variable (Table S3), partly because chromosome length (which varies among chromosomes) should influence the length of tracts during the first generation of admixture. Individual admixture proportions calculated as the percentages of Atlantic and Mediterranean domestic tracts relative to the total length of chromosomes were found to be significantly positively correlated with the percentages of domestic ancestry computed with ADMIXTURE (ATL-Dom: ρSpearman = 0.95, p < 2.2e-16; MED-Dom: ρSpearman = 0.88, p < 2.2e-16; Figure 4a,b). This result confirms that the genomewide average ancestry estimated with ELAI is highly consistent with the estimates obtained using the more classical ADMIXTURE approach, despite the existence of a small fraction of “uncertainty areas” where local ancestry could not be determined. However, ADMIXTURE reported the existence of individuals with no domestic ancestry—from either Atlantic or Mediterranean strains—that were found with small, but nonnegligible domestic ancestries in ELAI (Figure 4). This discrepancy in estimating the presence/absence of nonadmixed individuals in wild populations may be explained by a tendency of ADMIXTURE to minimize the estimated admixture fraction of the least introgressed samples.

We then compared local ancestry patterns among wild individuals to characterize the diversity of admixed genotypes between wild Mediterranean populations and the more evolutionary distant Atlantic strain. The estimated CAI was expressed as a function of the percentage of Atlantic ancestry estimated with ELAI in a triangle plot, together with the number of Atlantic haplotypes (Figure 5). These estimates were compared to the theoretical expectations that the maximum CAI = 1 for F1 hybrids, and CAI = 0 for pure Atlantic (ATL-Dom) and Mediterranean (MED-Wild) individuals. Moreover, the expected number of Atlantic haplotype tracts in pure Atlantic individuals corresponds to the diploid chromosome number (2n = 80), whereas it is equal to 0 in pure Mediterranean fish. These theoretical expectations were not totally met by “real” F1 hybrids and parental samples (Figure 5), indicating that introgression has occurred in both parental populations (ATL-Dom and Med-Wild). This was further supported by the results obtained with NEWHYBRIDS.

![Figure 4](https://wileyonlineibrary.com)
based on 196 SNPs with $F_{ST} > 0.85$. Indeed, the parental Atlantic samples identified by NEWHYBRIDS clustered close to the theoretical position of pure Atlantic parents in the triangle plot, although they contained more than 80 Atlantic tracts and displayed small fractions of Mediterranean ancestry (Figure 5). Likewise, fish identified as F1 showed a reduced CAI and an increased number of Atlantic ancestry tracts compared to what was expected for F1 hybrids produced between nonintrogressed parental populations. Discrepancies were also found to occur for some individuals beyond the first hybrid generation. For example, F2 individuals should have similar Atlantic ancestry to F1 hybrids, but an estimated CAI of −0.5 vs. −1.0. Four individuals were found to meet these expectations in Figure 5 (i.e., individuals with 72, 87, 97 and 98 Atlantic haplotypes located in the centre of the triangle plot), three of which have been assigned as F2 with NEWHYBRIDS. The NEWHYBRIDS analysis further identified several F2 in the Mare and the Orb rivers (Figure 5), which did not present either a number of Atlantic haplotypes or an Atlantic ancestry compatible with the expectation of the F2 category (Figure 5, Table S3).

The observation of reduced Atlantic tract numbers and Atlantic ancestries (down to 55% and 25%, respectively) suggested that these individuals were more likely backcross produced between a F2 and a backcross parent. Moreover, two individuals were assigned as pure wild Mediterranean fish with NEWHYBRIDS (Table S3), whereas they appeared to be more likely BC produced between a F1 and a Mediterranean parent. Indeed, one of them displayed a CAI of 0.49 together with Atlantic ancestry of 25.3%, which corresponds to what is expected for a first-generation backcross. Therefore, our results probably illustrate some limitation of NEWHYBRIDS to assign genotypes to predefined hybrid classes in the presence of complex admixtures that go beyond the categories that are specified prior to the analysis. However, both methods produced mostly similar results for parental, F1 and F2 classes.

The analysis of CAI, estimated percentage of Atlantic ancestry and estimated number of Atlantic tracts in wild individuals showed a complete absence of backcross individuals in the Atlantic genetic background (Figure 5). On the contrary, we found extensive backcrossing in the opposite direction, with individuals showing CAI and Mediterranean ancestry values compatible with a range of backcross pedigree beyond the first backcross generation (Figure 5). This was illustrated by a decreasing gradient in CAI, Atlantic ancestry and estimated number of Atlantic tracts between F2 hybrids and the least introgressed Mediterranean individuals.

The percentage of domestic ancestry was also compared to the individual heterozygosity to evaluate the consequences of admixture and introgression on the level of polymorphism.
domestic Atlantic ancestry and individual heterozygosity (Figure 6a; \( \rho_{\text{Spearman}} = 0.88, p < 2.2e\text{-}16 \)). Conversely, a significantly negative correlation was found between the percentage of domestic Mediterranean ancestry and the individual heterozygosity (\( \rho_{\text{Spearman}} = -0.44, p < 2.2e\text{-}16 \)).

3.5 Estimation of time since admixture

To estimate the time in generations since the introduction of Atlantic and Mediterranean domestic haplotypes within each wild Mediterranean population, the mode of the length distribution of domestic haplotypes was used within each population. The individuals formerly recognized as “pure” domestic Atlantic (Figure 5; Gravezon’s individuals at the right corner) were removed from data before estimating \( T \), as well as four individuals with more than 80% of domestic Mediterranean ancestry identified in the Mare River (Table S3). The proportions of Atlantic ancestry in each wild population were relatively close from each other (0.244, 0.323 and 0.299 for the Gravezon, the Mare and the Orb rivers, respectively; Table S4). The modes of the estimated length of Atlantic haplotypes were heterogeneous across populations (6,533,308 bp, 7,878,364 bp and 5,421,128 bp for the Gravezon, the Mare and the Orb rivers, respectively) (Figure S1; Table S4). The mean recombination rate was estimated to 0.88 cM/Mb in brown trout (Leitwein et al., 2017), and to take into account the recombination rate variation, we used the first and the third quartile estimates of the recombination rate (0.37 cM/Mb and 1.13 cM/Mb, respectively; derived from Leitwein et al., 2017). The time \( T \) was estimated to 24.01 generations ([18.92–55.73] generations using the first and third quartiles of the estimated recombination rate) for Gravezon, 22.31 generations ([15.55–45.46] generations) for the Mare and 30.90 generations ([24.28–72.10] generations) for the Orb (Figure 7; Table S4).

Similarly, we estimated \( T \) for domestic Mediterranean admixture into the wild populations. The proportions of Mediterranean ancestry in the populations were 0.155, 0.381 and 0.242 for the Gravezon, the Mare and the Orb rivers, respectively. The mean lengths of domestic Mediterranean haplotypes were 12,786,692 bp, 7,633,631 bp and 6,740,239 bp for the Gravezon, the Mare and the Orb rivers, respectively (Figure S1, Table S4). \( T \) was estimated to 11.51 generations ([9.19–26.01] generations) for Gravezon, 25.07 generations ([19.75–58.25] generations) for the Mare and 23.23 generations ([18.31–53.87] generations) for the Orb (Figure 7; Table S4).

4 DISCUSSION

By combining Anderson and Stebbins’ (1954) “genic blocks” with population genomic approaches, we investigated local ancestry...
patterns and introduced a new metric—the CAI—to evaluate the consequences of admixture in a context of intraspecific HMH in the brown trout. First of all, we show that genomewide ancestries estimated with a method considering linkage information (ELAI) strongly correlate with the ones obtained with a classical method based on allele frequencies alone (ADMIXTURE). However, our results support that the inference of local ancestry at the chromosome level is more powerful to detect small proportions of domestic introgression. Most importantly, some individuals assigned as pure wild individuals with ADMIXTURE were most probably introgressed. This suggests that some management decisions could be inappropriate using only classical tools such as ADMIXTURE; however, this has to be investigated further.

Using admixture proportion and the CAI, we then provide a detailed picture of recent intraspecific hybridization in wild-caught individuals, without restricting our analyses to predefined hybrid categories. Individuals were found to present variable amounts of admixture characterized by a gradient of CAI and introgressed haplotype number, opening the way for more detailed analyses of interindividual patterns of hybridization and introgression. The length distribution of introgressed domestic haplotypes was also used to estimate the time since the maximum rate of hybridization in wild-caught individuals. Finally, we show that admixture had dramatically different consequences on the level of polymorphism depending on the domestic source population used for stocking, thus providing important information to future conservation actions. This study suggests that using SNP linkage information in local ancestry inference methods might provide a powerful approach to study HMH in the future.

4.1 Three-way admixture inference

The use of a large panel of SNPs allowed detecting admixture with the locally derived Mediterranean strain, which was not possible with the small number of microsatellite markers traditionally used in brown trout (see also Leitwein et al., 2016). We hereby show that the individual proportions of domestic and wild ancestry assessed with ELAI (i.e., using the haplotype information) are consistent with the proportions inferred with ADMIXTURE (i.e., an allele frequency method) for both the Atlantic and Mediterranean domestic strain. This finding reinforces the value of the local ancestry inferences performed with ELAI, which has not yet been evaluated for its performance on similar data.

Both methods found six individuals sampled in the Gravezon River to be assigned as domestic Atlantic trout. Because the Hérault French Fishing Federation has stopped supplementing with domestic Atlantic fish in this region, these individuals were probably escapes from one or several small private hatchery that are frequently flooded in Mediterranean streams with poorly predictable discharge events. Besides these six Atlantic fish, a low to moderate percentage (from 10% to 20%) of domestic Atlantic ancestry was detected in each of the three wild populations. This likely results from the past introgression of Atlantic alleles starting since the beginning of supplementation practices. Domestic Mediterranean ancestry was also detected in all three local populations, especially in the Mare River where four of the 14 individuals had a high percentage of domestic Mediterranean ancestry (>60%), probably due to recent stocking in this river (personal communication from the Hérault Fishing Federation).
Our analyses also revealed discrepancies between methods, especially for estimating the lowest proportions of admixture in wild individuals. Indeed, ADMIXTURE assigned several individuals as pure (i.e., zero per cent of domestic ancestry), whereas ELAI detected low to moderate proportions of admixture (i.e., 0%–20%) (Figure 4). This might reflect some limitations in the inference of ancestry proportions with ADMIXTURE, which tends to minimize the level of admixture in the least introgressed genotypes. This discrepancy is also possibly explained by the fact that ADMIXTURE estimates ancestry proportions globally across the genome, whereas ELAI performs local ancestry inference. Our results suggest that, by taking advantage of the information contained in allelic association at linked markers, ELAI provides a finer scale picture of the ancestry mosaic within each individual. This is expected to improve the estimation of admixture proportions when introgression occurs over a small fraction of the genome. This interpretation, however, will need to be confirmed by a dedicated simulation study to more deeply evaluate the performance of ELAI under different introgression scenarios and generations of recombination.

4.2 Detection of hybridization

Compared to the hybrid index (Anderson, 1953; Buerkle, 2005) and the interspecific heterozygosity, which are both classically used to characterize hybridization at an individual level (Gompert & Buerkle, 2016; Larson, Andrés, Bogdanowicz, & Harrison, 2013; Wielstra, Burke, Butlin, & Arntzen, 2017), the joint use of local ancestry tracts and the CAI allowed characterizing admixture between populations exhibiting low population differentiation (i.e., domestic and wild Mediterranean). Moreover, as the number and the length distribution of introgressed tracts reflect the number of recombination events since hybridization (Gravel, 2012; Harris & Nielsen, 2013; Racimo et al., 2015), these haplotype statistics are more informative than the interspecific heterozygosity, which is not dependent on the number of generations since admixture beyond the first hybrid generation. For illustration, more abundant and longer Atlantic tracts were found in Mediterranean BC compared to introgressed individuals displaying a low percentage of Atlantic ancestry with less abundant and shorter Atlantic tracts (Figure 5). This result is consistent with a dilution of the Atlantic tracts over generations since the beginning of the supplementation history (i.e., supplementation with the domestic Atlantic strain has been recently stopped in this region). Moreover, the six “pure” domestic Atlantic individuals found in Gravezon River displayed a number of Atlantic haplotypes greater than the 80 expected from the diploid number of chromosomes (Leitwein et al., 2017; Phillips & Ráb, 2001). Along with an estimated percentage of Atlantic ancestry lower than one hundred per cent and a CAI greater than zero, this result indicates a possible admixture with Mediterranean individuals during the development of the Atlantic domestic strain. This hypothesis is further comforted by the low percentage of Mediterranean ancestry detected in the Atlantic domestic population with ADMIXTURE (Figure 2).

The use of haplotype information also provides a more detailed picture of recent hybridization and its consequences on admixture and introgression than NEWHYBRIDS (Anderson & Thompson, 2002), which can only assign individual genotypes to discrete predefined hybrid classes (e.g., typically parents, F1, F2 and first-generation BC). For example, several individuals in Gravezon, Mare and Orb rivers were assigned as pure Mediterranean by NEWHYBRIDS (F0med; Figure 5), whereas their percentage of Atlantic ancestry ranged from 8% to 32%, with a number of Atlantic tracts ranging from 11 to 63 (Figure 5, Table S3). These individuals were unlikely to be pure Mediterranean, but rather introgressed individuals resulting from the past hybridization with the domestic Atlantic strain at the beginning of supplementation. Several individuals were also assigned as F2 with NEWHYBRIDS, whereas they were most likely a result of backcrossing, as their percentage of domestic Atlantic ancestry and their CAI were lower than expected for F2 genotypes (Figure 5). This suggests that coupling haplotype information to probabilistic model-based methods such as NEWHYBRIDS would greatly improve the detection of complex hybrid pedigrees in a multicomponent system impacted by HMH.

4.3 Timing of gene flow

The use of haplotype information can also allow modelling historical hybridization to estimate the timing of introgression events (Allen-dorf et al., 2010; Duranton et al., 2018; Gravel, 2012; Homburger et al., 2015). Indeed, the length distribution of introgressed haplotypes within a population is a proxy of the time since hybridization (Liang & Nielsen, 2014; Racimo et al., 2015). Because introgressed haplotypes are progressively broken down into shorter fragments at each generation by recombination, long introgressed tracts denote recent hybridization, whereas short haplotypes denote more ancient hybridization events (Racimo et al., 2015). For estimating the number of generations since the most intensive introgression of domestic strain into each river, we used the mode of the length distribution of introgressed tracts instead of the mean. This estimation was predominantly based on shorter fragments reflecting the past supplementation. A bias towards the detection of short fragments might have occurred in our data set due to the method used for the delimitation of tract junctions that could not be unambiguously resolved (see methods), thus potentially increasing the density of the shorter fragments. The recombination rate is also highly variable along the genome of the brown trout (Leitwein et al., 2017). Although we accounted for such variation using the first and third quartiles of the observed distribution of recombination rate, the estimated time in generation is not expected to be precise and remain an approximation that should be interpreted with caution. Moreover, the method used here (Equation 1; Racimo et al., 2015) was designed to detect single pulses of hybridization which are unlikely to occur in our system shaped by iterative hybridization events due to annual stocking practices. Despite these limitations, the goal here was to approximately estimate the time interval that occurred between the most intensive pulse of domestic Atlantic and Mediterranean hybridization events, which is likely captured with our approach. In addition, the length and the number of introgressed tracts is largely independent
of the demographic history of the population and only depend of the time of hybridization and the number of initial migrants, if selection is not strong. In the Gravezon and the Orb rivers, we found that the number of generations since the most extensive events of hybridization was greater for the domestic Atlantic strain than for the domestic Mediterranean strain, revealing a more ancient introgression of domestic Atlantic alleles which was expected from the known history of hybridization. Conversely, in the Mare River, the introgression of domestic Atlantic alleles was found to be more recent than the introgression from the Mediterranean strain. This could denote a more recent undocumented introgression of domestic Atlantic strain into the Mare river, but uncertainty remains as the detailed (i.e., river-based) history of supplementation in the Orb watershed is poorly documented.

4.4 | Implications in conservation genomics

Genotype data at unlinked markers have been commonly used to assess hybridization, genomewide levels of admixture and introgression in conservation genomics studies (Bradbury et al., 2015; Hassain, 2015; Hohenlohe et al., 2013; King, Stelkens, Webster, Smith, & Brockhurst, 2015; Lamaze, Sauvage, Marie, Garant, & Bernatchez, 2012; Le Moan, Gagnaire, & Bonhomme, 2016; Rougemont et al., 2017). Nevertheless, the use of a linkage map enabling to explicitly consider the relative order and linkage disequilibrium among loci, and the increasing availability of references genome allow to access local ancestry information at the haplotype level, which has the potential to increase the sensitivity of admixture estimates even for low sample size studies (Allendorf et al., 2010; Duranton et al., 2018). Here, despite the low sample sizes in admixed populations, we were able to quantify admixture and assess the history of hybridization in three populations of the Orb watershed. This is because the oldest tracts that are shared across individuals in the entire population provide information on the most ancient admixture events. However, characterizing recent and/or a local hybridization event might benefit of increased sample sizes to improve the reliability of the inferences. In addition to the estimation of the timing since hybridization, our approach seems particularly relevant to identify allochthonous and autochthonous sources of supplementation for a conservation biology perspective, especially when local and domestic stains are genetically close to each other. In particular, when the development and prioritization of conservation actions is ruled by the presence of “pure” wild individuals increasing the value of a stream or a watershed (Hansen & Mensberg, 2009), it is often required to prioritize the identification of pure individuals in the wild. Nonetheless, we show in this study that the identification of pure individuals was sensitive to the method used and that, certainly, no pure individuals likely remains in the studied populations, which is not surprising considering the history of supplementation. An inference relying on ADMIXTURE alone would have led to the inadequate conclusion that domestic alleles can be totally purged through the time, which is not consistent with the finding of short residual domestic tracts in introgressed wild individuals. This suggests that numerous studies might benefit from haplotype-based genomic inferences to improve the detection of pure wild individuals and adjust management programmes.

Finally, our results also show that the consequences of supplementation on the polymorphism of admixed populations were dramatically different depending on the hatchery strain used. The mosaic of individual haplotype ancestries resulting from the past and present management programmes has to be evaluated and described to understand how hybridization/introggression have impacted the genomic make-up and the fitness of wild populations. For example in our study, an increase in heterozygosity was associated with an increase in domestic Atlantic ancestry, as expected when mixing two distinct evolutionary lineages (Atlantic and Mediterranean), especially during the first generation of hybridization (Allendorf, Luikart, & Aitken, 2012). This effect is here amplified by the fact that the Atlantic domestic strain is more genetically variable than the wild Mediterranean populations (Leitwein et al., 2016). On the contrary, an increase in domestic Mediterranean ancestry was associated with a decrease in heterozygosity (Figure 6), potentially indicating negative consequences associated with a loss of diversity and/or increased genetic load, because of the low polymorphism observed in the Mediterranean domestic strain, due to the very low number of individuals used to seed this strain (Leitwein et al., 2016).

5 | CONCLUSION

Using extensive individual genomic data and the brown trout as a case study, we hereby showed that the information contained in local ancestry blocks might improve the detection of admixture patterns at both the individual and population levels. Few others studies (Galaverni et al., 2017; Nelson et al., 2017; VonHoldt et al., 2016) also explored this issue, but this certainly has to be expanded further. For example, an in-depth analysis of the genomewide landscape of introgression would be useful to further identify genomic regions where introgression departs from neutral expectations and is potentially adaptive or maladaptive (Duranton et al., 2018; Edmands, 2007; Hedrick, 2013; Racimo et al., 2015; Sankararaman et al., 2014; Steinrücken, Spence, Kamn, Wieczorek, & Song, 2018; reviewed in Dannemann & Racimo, 2018). This is of prime importance for future conservation and management actions (Allendorf, Leary, Spruell, & Wenburg, 2001; Allendorf et al., 2010; Frankham, 2010; Garner et al., 2016; Stronen & Paquet, 2013), and more generally, the haplotype-based approach associated with recent theoretical findings will provide new ways to explore genomic data over a broad scope of both applied and fundamental evolutionary topics (Dannemann & Racimo, 2018; Hvala, Frayer, & Payseur, 2018; Jansen, Nolte, & Traulsen, 2018; Ni et al., 2018).

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DATA ACCESSIBILITY

Supporting Information Table S1 displays the number of reads per individuals before and after the mapping, the number of reads properly paired, the percentage of singleton, the number of SNPs in the STACKS catalogue and the number of SNPs after filtering in the VCF and PLINK files. Table S2 contains NEWHYBRIDS results with sample ID and priors. Table S3 contains sample ID, the corresponding population, the mean percentage of Atlantic ancestry retrieved with ELAI and ADMIXTURE, the mean number and the mean length of introgressed Atlantic and domestic Mediterranean haplotypes as well as the pedigree inferred with NEWHYBRIDS. Table S4 contains for each domestic strains and populations the estimated time in generation along with the first and third quartile; as well as the mean percentage of domestic ancestry and the estimated mode of the tracts length distribution along with the 97.5% confidence interval. Figure S1 represents the mode and the 97.5% confidence interval of the length distribution of both domestics Atlantic (in blue) and Mediterranean (in red) tracts for each wild rivers.

Raw demultiplexed sequence reads by individuals (fastq) are available at NCBI Short Read Archive under the study accession SRP136716 with one file per paired-end reads Sample.1.fq and Sample.2.fq and one file per unpaired reads Sample.rem.fq. Moreover, the final VCF and the PLINK files for all 40 Salmo trutta LG are available under the study accessions SRZ187687 and SRZ187688.

AUTHOR CONTRIBUTIONS

M.L and P.A.G. conceived and designed the analyses. M.L and E.D. performed the laboratory work. M.L does the analyses and wrote the manuscript. B.G. and P.B. supervised the research. All coauthors critically revised the manuscript and approved the final version to be published.

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**SUPPORTING INFORMATION**

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

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