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Malic enzyme, an "old-new player" in age at maturity in Atlantic salmon

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ABSTRACT

Traditional genetic markers based on allozyme variation had indicated that in Atlantic salmon the genetic variation found for the locus coding for mMEP-2* could be adaptive, with the variation being related to river water temperature, growth, and maturation. More recently, SNP panels used to search for genes related to maturation in Atlantic salmon identified several genes, including the *vgll3* gene related to early maturation. However, none of the analyses performed found an association between maturation and the already known locus coding for mMEP-2*. Recently, a method has been developed to characterize the different alleles of the locus coding for mMEP-2* using molecular techniques. In this work we use this method to study the salmon population of the Bidasoa River, located in the Iberian Peninsula, which is the limit of distribution of the species. We found a relationship between the presence of SNP G, equivalent to the 125* allele, and early maturation of Atlantic salmon in both males and females. It is possible that the relationship between the different alleles of this locus and maturation is not direct but is produced by differential growth during the juvenile and/or adult phase. It is also plausible that this association is exclusive to the salmon populations of the Iberian Peninsula.

1. Introduction

In recent years, population genetics studies have experienced a great improvement with the introduction of more powerful sequencing techniques and the possibility of sequencing complete genomes and performing genome-wide association studies analysis. Nowadays the term population genomics is used to encompass this type of analysis (Allendorf, 2017). Genetic markers based on allozyme, microsatellites and mitochondrial DNA sequencing have made great advances in the knowledge of populations, despite their limited number of markers.

The Atlantic salmon is an anadromous fish that returns to the river of birth to reproduce. This behaviour means that populations in different rivers are isolated and can evolve independently (Webb et al., 2007). Traditional genetic markers have played an important role in the genetic identification of these salmon populations. Initial studies with proteins, starting in 1966, allowed the detection of variation between North American salmon populations and European populations, as well as differences within rivers in both continents (reviewed in Verspoor et al., 2005).

The introduction of DNA techniques, starting with mitochondrial DNA RFLPs studies, allowed a further step in the study of regional differentiation, even allowing the assignment of specific variants to populations (reviewed in Verspoor et al., 2012), as do studies using microsatellites, for example a genetic database that allows for the identification of region of origin of individuals (Griffiths et al., 2010, but also see more recently Gilbey et al., 2018). These studies indicate considerable genetic structuring among European salmon populations. Thus, several clusters can be distinguished including the populations of northern Scotland and northern Ireland, the populations of central Scotland and eastern Ireland, the populations of northern Europe and the edges of Scotland, the populations of southern England and Wales, the populations of southern Ireland, the populations of northern France, and the most differentiated clusters would include the populations of southern England and northern Spain. The latter is located in the extreme south of the species' distribution in Europe. These differences between populations, estimated with neutral markers, are mainly due to the isolation between populations in the different rivers.

A recurring theme in the study of salmon populations is the existence of adaptive variation. A review article by Garcia de Leaniz et al. (2007) extensively reviews the possible sources of adaptive variation and concludes that, in general, the relationship between molecular variation and adaptation is mainly speculative. However, among the above-mentioned

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evidence, the polymorphism of the NAPD-dependent malic enzyme locus encoded by nuclear DNA mMEP-2* stands out. The mMEP-2* are encoded by two loci that combine as tetramers that can be differentiated by starch gel electrophoresis in a reaction containing malate, NADP, MTT, PMS and Mg^{2+}. One of the mMEP-2* loci is polymorphic and the other monomorphic (Cross et al., 1979). The polymorphic locus, has two alleles denominated 100*and 125* which are distinguished by their different electrophoretic mobility. Evidence for adaptive variation at this locus is based on observed differences in allele frequency between populations inhabiting rivers with colder and warmer water, with a north-south clinic where frequencies of the 100* allele range from lower to higher (Verspoor and Jordan, 1989; Jordan et al., 2005). Differences in frequency for this allele have previously been associated with juveniles growth (Gilbey et al., 1999; McCarthy et al., 2003) and the age at maturity (Jordan et al., 1990; Morán et al., 1998; Consuegra et al., 2005). The mMEP-2* is encode in the nuclear genome, there are different isoforms, can be cytosolic or mitochondrial and catalyzes the reversible decarboxylation of Krebs cycle intermediate malate to pyruvate in the presence of the coenzyme and a divalent cation (Mn^{2+} or Mg^{2+}). Malic enzymes are crucial for gluconeogenesis, glycolysis, and fatty acid synthesis (Sarfraz et al., 2018) having a prominent role in bioenergetics and, therefore, it is not surprising to find a possible relationship between the different isoforms and aspects related to growth and cold water performance. Thus, Mommsen (2004) investigating protein degradation in long-distance migration in sockeye salmon (*Oncorhynchus nerka*), points out the crucial role of the malic enzyme in the metabolism during fish migration, maturation, and starvation.

With the advent of more powerful sequencing techniques, the possibility of exploring panels of SNPs has become available. One of the first applications deploying SNP variation in the study of Atlantic salmon is that of Bourret et al. (2013). Candidate genomic regions affected by selection were identified when comparing populations across the entire distribution range of the species. 52 of the outliers potentially under divergent selection in European anadromous populations are correlated with latitude. Since this paper, other papers using different panels of SNPs have been published.

Because of the biological and economic importance of maturation in salmon, much effort has been made to search for associations between genomic regions and maturation traits in Atlantic salmon (review in Mobley et al., 2021). Undoubtedly, most striking has been the association of variation at the vgll3 gene, located on chromosome 25, and age of maturity. The two alleles at the *vgll3* gene are either linked to early maturation (E), which results in a low sea age, or late maturation (L), which results in a greater sea age (Ayllon et al., 2015; Czorlich et al., 2018). In addition, Cauwelier et al. (2018) found, in Scottish salmon, a single locus or a small group of closely linked loci located on chromosome Ssa09, related to returning time. An extensive study by Sinclair-Waters et al. (2020) identified 116 SNPs that were independently associated with maturation and distributed on 22 of the 29 Atlantic salmon chromosomes. They identified 5 SNPs associated with genes that had previously been associated with maturation such as the *vgll3* gene and the *Six6* gene on chromosome 9, plus three other genes, one on chromosome 15, one on chromosome 16, and one on chromosome 22. However, none of the studies performed with SNP panels detect the selective potential of the locus encoding mMEP-2* in relation to maturation, growth, or freshwater temperature. This gene was found previously to be associated with maturation age in salmon population from Spain (Morán et al., 1998; Consuegra et al., 2005). The presence of the 125* allele being related to the probability of maturation as 1SW in these populations.

Taggart et al. (2022) have identified the SNP responsible for the amino acid substitution that alters the charge of the 100* and 125* alleles of locus coding for mMEP-2*. (Gene ID LOC106586750, chromosome ssa25). This is a non-synonymous A_ G substitution in exon 10. This produces a charge-changing amino acid replacement at codon 371 – asparagine (N, no charge; *100 allele) for aspartic acid (D, negative charge; *125 allele). It is thus possible to identify the traditional 100* and 125* allozyme denominated alleles as SNP A and SNP G respectively by RFLPs or using techniques such as KASP assay.

In the present work the RFLPs methodology is used to determine the frequency of the different alleles of the locus coding for mMEP-2* in relationship to maturation age in male and female salmon from the Bidasoa River (Spain).

2. Material and methods

2.1. Study site and sampling

This study uses data of one population of wild Atlantic salmon reproducing in the Bidasoa river. The Bidasoa river (43°22′22″ N, 1°47′31″ W) is the easternmost of the Cantabrian coast rivers, being located near the French river Nive. It is 70 km long, of which 55 km are accessible to salmon (Elso, 2022). Recreational fishing is allowed with annual quotas, in the first 17 Km from the sea, but it is prohibited upstream of this point, and periodic monitoring of adult salmon is carried out using catch stations. This provides reliable statistics on the abundance of adult salmon, which for the period 2010–2021 averaged 456, ranging from 212 to 685 individuals. In the Bidasoa, after hatching, juvenile salmon remain in freshwater for one (about 88% of the population) to two years and then migrate to the sea, maturing after one year at sea (1SW) or two years at sea, with some salmon unusually returning after three years at sea (less that 0.5%). Salmon over one year at sea are grouped in this work as MSW (multi sea winter).

A total of 809 salmon from the Bidasoa River caught between 2014 and 21 by the anglers or in the monitoring station, were analyzed. For each of these salmon we have the following data: date of capture, length, weight, and from all of them scales were collected to determine the age, and a sample of adipose fin, or in its absence scales, to carry out genetic analysis. The scales were used for age determination, both river and sea, following the indications of Baglinière et al. (1985).

2.2. Genotyping

DNA was isolated from fin clips or scales using Chelex (Estoup et al., 1996). Genotypic sex determination was performed following the methods described by Quéméré et al. (2014) and King and Stevens (2020). mMEP-2* gene genotyping was achieved by using the RFLP-based detection described in Taggart et al. (2022). The correspondence between the nomenclature used for the allozyme and the SNPs detected by this method is as follows: the 100* allele is A and the 125* allele is G. Therefore, homozygous salmon are either AA or GG and AG heterozygotes.

2.3. Data analysis

A logit link function built using the GAMLj in the package jamovi statistical software (The jamovi project, 2021), was used to investigate the effects of the genotype of locus coding for mMEP-2* on maturation odds. The response variable, maturation, was coded as 0 for individuals returning to the river after one year at sea (1SW), and as 1 for individuals returning to the river after two or more years at sea (MSW). The independent variables considered were sex (male, female) and genotype for the locus coding for mMEP-2* (AA, AG and GG). In addition, year was included as a random effect, since the data series covers a total of eight years. We hypothesize that salmon with at least one G allele (GG homozygotes or AG heterozygotes) mature earlier than AA homozygotes.

An initial omnibus test was performed to check that the two independent variables have a significant effect on the dependent variable. Finally, a post hoc test was performed using Holm's correction. Odd ratios were calculated by applying the exponential function to the parameter estimates in post hoc pair comparisons in jamovi.

3. Results

A total of 809 salmon were analyzed, 54.57% of the salmon were females, and of these, 33.03% were 1SW. Males represented 45.43% of the population, 80.98% of the males being 1SW. Genetic variation for the mMEP-2* gene is shown in Fig. 1. Detailed data by year of capture are shown in supplementary Table 1S.

The generalized mixed model (maturation ~ sex * MEP-2 + (1 | year) reveals that sex (X^2 22; $P < 0.01$) and genotype of locus coding for mMEP-2* ($X^2 = 10.97$; $P = 0.004$) have a significant effect on age at maturation. There was no interaction between sex and genotype of the locus coding for mMEP-2* ($X^2 = 3.29$; $P = 1.193$). The results are independent of the year of return (2014–2021) of the salmon to the river (ICC = 0.445).

The interaction was removed from the model and the results of the generalized mixed model (maturation ~ sex + MEP-2 + (1 | year) considering sex and genotypes of the gene encoding the malic enzyme are shown in Table 1.

As could already be expected from the percentage of 1SW in males and females the model indicates that males had 8.4 odds of returning as 1SW than females. The GG and GA genotypes increased the log (odds ratio) of early maturing relative to the AA genotype by 4.7 and 1.7 respectively.

4. Discussion

A well-known and very intriguing illustration of an evolutionary trait trade-off is the timing of maturation in Atlantic salmon. Larger individuals that spend more time at sea before returning to freshwater to spawn have potentially better reproductive rates i.e. multi-sea winter females have more eggs than 1SW females, but also have a higher risk of dying at sea (Mobley et al., 2021). For this reason, the genetic basis of maturation has been the subject of intense study in recent years. Due to substantially reduced genotyping costs, the possibility of performing GWAS, together with the extensive knowledge that exists of salmon populations, has allowed the identification of genes, or regions close to genes that are related to the age of maturation in Atlantic salmon. The vgll3 gene on chromosome 25 is presently the best known primary candidate locus for influencing early maturation (Sinclair-Waters et al., 2022). The effects of this gene appear to be more pronounced in females than in males, and there is also this variation between populations (Kusche et al., 2017). In some populations vgll3 variation has been found to explain 39% of the variation in maturation (Barson et al., 2015). However, this level of effect on maturation is not universal across the species range (Boulding et al., 2019). Salmon maturation is polygenetic in nature, with genes of major effect as such and other genes of minor effect. Other genes have been shown to be positively associated with maturation (reviewed in Mobley et al., 2021) but again, as with vgll3, their affect across the species' range is not universal. In addition, maturation is also influenced by the environment, and conditioned by the early stages of salmon development (Tréhin et al., 2021). It is therefore a complex trait, and due to the genetic structure of the populations, there may be variation which would explain the differences found among different populations (Boulding et al., 2019).

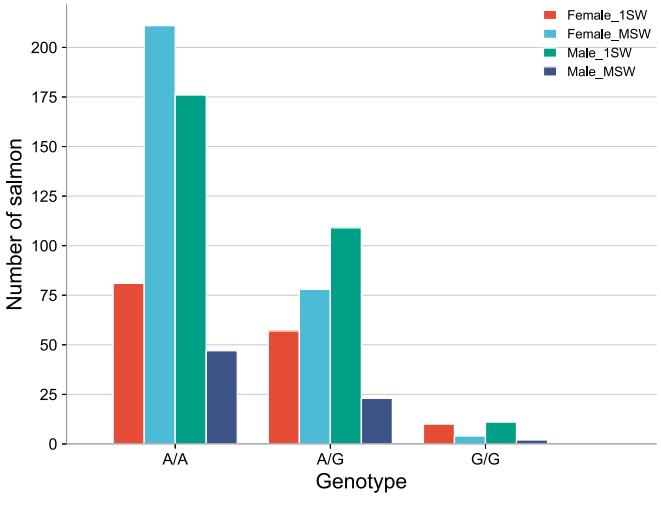

Fig. 1. Number of salmon according to sex and maturity for each of the three genotypes.

In this work carried out with salmon from the Bidasoa river, we demonstrated that the presence of the SNP G in locus coding for mMEP-2* (equivalent to the mMEP-2*, 125* allele) is related to the early maturation of Atlantic salmon. Salmon carrying this allele, especially homozygous individuals (GG), are more likely to mature early as 1WS. The results of this new SNP based study are in agreement with the work carried out in the past using isoenzymatic loci (Morán et al., 1998; Consuegra et al., 2005) but with the difference that, in our study, the sex of the salmon is taken into account. Although the percentage of 1SW is much higher in males than in females, the model applied shows that this gene has an effect in both males and females. The odds of maturation are similar to those reported for the *vgll3* gene where the *vgll3* EE and EL genotypes increased the log(odds ratio) of maturing relative to the LL genotype by 4.21 and 1.79, respectively (Sinclair-Waters et al., 2020). It is interesting to note that both genes are on chromosome ssa25 although far apart (*vgll3* gene position 28,995,238–28,999,269; LOC106586750 (mMEP-2*) position 40,604,600–40,635,550). Tight linkage between the two genes can therefore be ruled out.

With results derived from our work It is not possible to hypothesize about the universality of this locus coding for mMEP-2* and its relationship with maturation. It is striking, however that the analyses performed with high density panels (Gutierrez et al., 2015; Barson et al., 2015; Cauwelier et al., 2018; Sinclair-Waters et al., 2020) did not find a relationship between this gene and maturation although Jordan et al. (1990) found that in the Girnock River (Scotland) there were more 1SW salmon with the 125* allele than MSW salmon.

In a recent study using a panel of high density SNPs specific for Atlantic salmon we have found, comparing populations from southern Europe with Scottish populations, that the locus coding for mMEP-2* is a strong candidate for local adaptation in the Iberian Peninsula (Gabián et al., 2022). The result was obtained after a modification of the HacDivSel method, a computer program designed to find signals of natural selection in the genome of populations that share polymorphisms (Carvajal-Rodríguez, 2017), to avoid the loss of statistical power due to multitest corrections. Therefore, it cannot be ruled out that this association is specific to the populations of the Iberian Peninsula. The population analyzed, river Bidasoa, is characterized by large numbers of 1SW salmon. 'The percentage of 1SW salmon in the Bidasoa river was higher in males than in females, at 80% and 33% respectively, with an overall population average of 54.9%. In the Sinclair-Waters et al. (2022) study the average of 1SW fish in their population was 18.9%. The high number of 1SW individuals in the Bidasoa River is not particularly high when compared to other European populations, since in Norway the proportion of 1SW is in some rivers higher than 95% with lower limits of approximately 30% (L'Abée-Lund et al., 2004). In the Teno river in Finland, also the percentage of 1SW presents a great variation with percentages varying between 20% and 90% depending on the different parts of this river (Erkinaro et al., 2019). The most common age combinations in the Teno river are 4 year old river-1SW (35%), and 5 year old river 5-1SW(17%) having also 3–5 SW salmon. The large differences in the age structure may influence the results found, since in the Bidasoa river, as in the rest of the rivers of the Iberian Peninsula, MSW salmon and repeat spawning fish are rare with the exception of the populations found in the rivers Miño and Ulla (Álvarez et al., 2010) and river age is mostly 1 year old. In addition, the presence of repeat spawners is residual.

Table 1
results of the generalized linear mixed-effects model predicting the relationship between maturity, polymorphism locus coding for mMEP-2* and sex as fixed effects and year of capture as random effects.

				95% Exp(B) CI			
Effect	Estimate	SE	exp(B)	Lower	Upper	z	p
Intercept	−0.888	0.236	0.412	0.2591	0.654	−3.76	<0.001
Male-female	−1.546	0.517	0.213	0.0774	0.588	−2.99	0.003
GG-AA	−0.513	0.178	0.598	0.4218	0.849	−2.88	0.004
GA-AA	−2.109	0.173	0.121	0.084	0.171	−12.17	<0.001

The influence of the locus coding for mMEP-2*. may be on size rather than age at maturity as suggested for other genes that have also been linked to maturation such as those located on chromosome 9, including the *six6* locus (Barson et al., 2015, Sinclair-Waters et al., 2020). According to Tréhin et al. (2021), there is a probabilistic reaction norm related to sex, with an individual's likelihood of returning after a year at sea rising as growth rates increases. In order to reach their maturation threshold, females may need to grow more slowly than males. These authors predict that whether an individual matures after one year or later will depend largely on the body length reached in the first summer at sea, which is the result of the accumulated growth during the freshwater phase and the first summer at sea. In addition, Debes et al. (2020) demonstrated that body length in late summer predicted the probability of sea age migration.

The enzyme malic is involved in the formation of pyruvate in the citric acid cycle, the study by Gilbey et al. (1999) shows that 0+ Atlantic salmon parr with the 125*for locus coding for mMEP-2* allele grow more than non-carriers, while for the *vgll3* gene known to regulate adiposity in humans, a study shows that juvenile salmon of different *vgll3* genotypes have similar maintenance energy requirements under experimental conditions (Asheim et al., 2022). It is likely, therefore, that different genotypes of the *vgll3* gene have a direct impact on maturation whereas, the effect of different genotypes for locus coding for mMEP-2*influences maturation from the earliest stages of the salmon life cycle, impacting freshwater growth, sea migration, and growth during the sea phase that results in maturation as 1SW or as MSW.

5. Conclusions

Regardless of whether the effect of locus coding for mMEP-2* is indirect through differential growth throughout the salmon life cycle or whether the effect is direct on maturation, in this study we demonstrate a relationship between the presence of the different genotypes of locus coding for mMEP-2* and maturation. The presence of 1SW in the Bidasoa river population has a great impact on the conservation of the populations, since this is a very small population, subject to angling pressure resulting in a reduction of 1.9 mm in body length per generation (Saura et al., 2010). The possibility that through the supportive breeding program carried out in this river the number of MSW females can be increased, by the elimination, at the hatchery, of salmon with allele 125* (G SNP) could have a positive impact on the conservation of this population. However, there are studies that advise against these practices because the selection of certain breeders could have unknown effects on other genes, especially those that are closely linked to the selected single-locus (Kuparinen and Hutchings, 2017). In addition using simulations Oomen et al. (2020) have demonstrated that hypothetical single-locus control of a life history trait produces highly variable and unpredictable harvesting-induced evolution relative to the classically applied multilocus model.

Our investigation was made possible by the identification of the polymorphism of the locus encoding mMEP-2* by Taggart et al. (2022), which makes it straightforward to answer the question of the relationship between this enzyme and the maturation and growth in salmon. The simplicity of the method to identify the different alleles of the locus coding for mMEP-2* offers multiple possibilities to perform experiments to demonstrate the influence of the different alleles on salmon populations from the juvenile stage onwards which can also have an important impact on aquaculture since early maturation is undesirable.

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Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

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