Spotting genome-wide pigmentation variation in a brown trout admixture context

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ABSTRACT

Colour and pigmentation variation attracted fish biologists for a while, but high-throughput genomic studies investigating the molecular basis of body pigmentation remain still limited to few species and associated conservation biology issues ignored. Using 75,684 SNPs, we explored the genomic basis of pigmentation pattern variation among individuals of the Atlantic and Mediterranean clades of the brown trout (*Salmo trutta*), a polytypic species in which Atlantic hatchery individuals are commonly used to supplement local wild populations. Using redundancy analyses and genome-wide association studies, a set of 337 independent "colour patterning loci" (CPL) significantly associated with pigmentation traits such as the number of red and black spots on flanks, but also the presence of a large black stain on the pre-opercular bone was identified. CPLs map onto 35 out of 40 brown trout linkage groups indicating a polygenic basis to pigmentation patterns. They are mostly located in coding regions (43.4%) of 223 candidate genes, and correspond to GO-terms known to be involved in pigmentation (e.g. calcium and ion-binding, cell adhesion). Annotated candidates include genes with known pigmentation effects (e.g. *SOX10*, *PEML*, *SLC45A2*), but also the Gap-junction Δ2 (*GJD2*) gene that was previously showed be differentially expressed in trout skin. Patterns of admixture were found significantly distinct when using either the full SNP data set or the set of CPLs, indicating that pigmentation patterns accessible to practitioners are not a reliable proxy of genome-wide admixture. Consequences for management are discussed.

Keywords: ddRAD sequencing, admixture mapping, multi-trait phenotype, local ancestry, salmonid, management

1 - INTRODUCTION

The study of pigmentation has a peculiar place in modern biology as colour and its patterning play important roles in natural, artificial and sexual selection (Cieslak, Reissmann, Hofreiter, & Ludwig, 2011; Cuthill et al., 2017). The biology and evolution of animal colouration largely benefited over the past few years in the development of high-throughput sequencing techniques (e.g. San José & Roulin, 2017; Orteu & Jiggins, 2020). While supergenes and large effect mutations have been shown to support many pigmentation patterns in many organisms (Orteu & Jiggins, 2020), genomic studies also generated a huge literature that underscored their polygenic and epistatic nature (e.g. skin and hair colour in human: Crawford et al., 2017; Pavan & Sturm, 2019; structural colour variation or eyespot numbers in butterflies: Brien et al., 2019; Rivera-Colón, Westermann, van Belleghem, Monteiro, & Papa, 2020). Recent research showed that pigmentation variation may result from effects propagated during development by loci belonging to several regulatory networks to hub or key master genes often sufficient to explain most of - but not all - the causal variation with colour expression and/or patterning (e.g. Arnould et al., 2013; Ordway, Hancuch, Johnson, Wiliams, & Rebeiz, 2014; Ding et al., 2020, Fogelholm et al., 2020; Orteu & Jiggins, 2020).

If early genetic and molecular studies were interested in colour and pigmentation patterns in fish - notably as models for sex-linked colour variation (see Kottler & Schartl, 2018) – the issue of body pigmentation principally gained interest with the establishment of zebrafish and medaka as model species (Parichy, 2006; Takeda & Shimada, 2010; Singh & Nüsslein-Völlard, 2015; Nüsslein-Völlard & Singh, 2017) and from comparisons with higher vertebrates (e.g. Kelsh, Harris, Colanesi, & Erickson, 2009). Pigment cells (i.e. chromatophores; mainly melanophores, iridophores, leucophores and xanthophores) are distributed in the hypo- and the epidermis in fish, and mutational or other analyses allowed

for increased understanding in the complex mechanisms of pigment cell fates during development and their resulting distribution (Kelsh et al., 2004, 2009; Kimura et al., 2014; Eom, Bain, Patterson, Grout, & Parichy, 2015; Nüsslein-Völlard & Singh, 2017; Parichy & Spiewak, 2015; Singh & Nüsslein-Völlard, 2015; Salis et al., 2018; Volkening 2020). Studies now extend to other fish species (Maan & Sfec, 2013; Irion & Nüsslein-Völlard, 2019) and to a large array of eco-evolutionary questions regarding the involvement of genes or regulatory pathways in fish pigmentation, its epistatic and pleiotropic nature, its modularity and its control, sexually antagonist selection or its role in speciation as well as the impact of whole-genome duplication that promoted the diversification of pigment cell lineages (Hultman, Bahary, Zon, & Johnson, 2007; Miller et al., 2007; Braasch, Brunet, Volff, & Schartl, 2009; Roberts, Ser & Kocher, 2009; Albertson et al., 2014; Santos et al. 2014; Ceinos, Guillot, Kelsh, Cerdá-Reveter, & Rotlland, 2015; Yong, Peichel, & McKinnon, 2015; Gu & Xia 2017; Kimura, Takehana, & Naruse, 2017; Roberts, Moore, & Kocher, 2017; Sefc et al., 2017; Lorin, Brunet, Laudet, & Volff, 2018; Kratochwil et al., 2018; Nagao et al., 2018; Cal et al., 2019; Lewis et al., 2019; Kon et al., 2020; Liang, Gerwin, Meyer, & Kratochwil, 2020). An increasing number of studies engaged fish research in high-throughput genomic approach of pigmentation variation (guppy: Tripathi et al., 2009; three-spine stickleback: Greenwood et al., 2011; Malek, Boughman, Dworkin, & Peichel, 2012; cichlids: O'Quin, Drilea, Conte, & Kocher, 2013; Henning, Jones, Franchini, & Meyer, 2013; Henning, Lee, Franchini, & Meyer, 2014; Albertson et al., 2014; Zhu et al., 2016; Roberts et al. 2017; koi carp: Xu et al., 2014; arowana: Bian et al., 2016; goldfish: Kon et al., 2020). However, in spite of this impressive research, the genomics of pigmentation variation remain poorly investigated in fish compared to other phenotypic traits (Peichel & Marques, 2017). Furthermore, links between colour patterning and conservation genomics remain virtually absent (but see Boulding et al., 2008) while they begin to be documented in, e.g., birds, notably regarding the impacts of admixture

(Toews et al., 2016; Hanna, Dumbacher, Bowie, Henderson, & Wall, 2018; Billerman, Cicero, Bowie, & Carling, 2019).

Salmonids represent a large fish family with a very diverse and complex body pigmentation going from continuous colour to spotty, marbled, blotchy and striped patterns. If whole body colouration or specific coloured elements have a genetic basis (Yamazaki, 1974; Blanc, Poisson, & Vibert, 1982; Skaala & Jørstad, 1988; Blanc, Chevassus, & Krieg, 1994; Blanc, Poisson, & Quillet, 2006; Boulding et al., 2008; Colihueque, 2010; Nilsson et al., 2016), another part is plastic (Colihueque, 2010; Westley, Stanley, & Fleming, 2013; Jørgensen et al., 2018). Colour patterns support differences in coping styles (Kittilsen et al., 2009; Brännäs et al., 2016) or in the physiological adjustments necessary to avoid predation and match with environmental variability (e.g. Miyamoto, 2016; Jacquin et al., 2017; Zastavniouk, Weir, & Fraser, 2017). In salmonids, pigmentation and colour are also known to interact with social hierarchies, to influence mate choice and affect fitness (e.g. O'Connor, Metcalfe, & Taylor, 2000; Wedekind, Jacob, Evanno, Nusslé, & Müller, 2008; Marie-Orléach et al., 2014; Watt, Swanson, Miller, Chen, & May, 2017, Parolini, Iacobuzio, Bassano, Penatti, & Saino, 2018; Auld, Noakes, & Banks, 2019). However, if the physiological basis of colour changes are known (Leclercq, Taylor, & Migaud, 2010), the genomic basis of their pigmentation patterns remains largely unexplored, with only limited insights coming from quantitative trait loci (QTL) (Boulding et al., 2008) or few gene expression studies (Sivka, Snoj, Palandačić, & Sušnik Bajec, 2013; Djurdjevič, Furmanek, Miyazawa, & Sušnik Bajec, 2019). Furthermore, as pigmentation patterns may reflect admixture in salmonids (e.g. Largiadèr & Scholl, 1996; Mezzera, Largiadèr, & Scholl, 1997; Aparicio, García-Berthou, Araguas, Martinez, & Garcia-Marin, 2005; Miyazawa, Okamoto, & Kondo, 2010; Kirczuk & Domagała; 2012, Kocabaş, Kutluyer, & Başçinar, 2018), it appears necessary to improve knowledge on the genotype-phenotype association supporting observed pigmentation patterns.

Indeed, pigmentation patterns inform on individual origin and use to improve management knowledge, decisions or policies (Ielli & Duchi, 1990; Largiadèr & Scholl, 1996; Poteaux & Berrebi, 1997; Delling, Crivelli, Rubin, & Berrebi, 2000; Aparicio et al., 2005; Kocabaş et al., 2011; Marin, Coon, & Fraser, 2017; Duchi, 2018; Lorenzoni et al., 2019). It thus might be relevant to evaluate if loci supporting phenotypic divergence for body patterning reflect genome-wide admixture.

In this study, we address these issues in the brown trout (*Salmo trutta*), a species submitted to extensive hatchery supplementation within its native range. We take advantage of former genomic knowledge established in this species using double digested restriction site-associated DNA sequencing (ddRADseq) (Leitwein, Gagnaire, Desmarais, Berrebi, & Guinand, 2018) to investigate the loci responsible for variation in body pigmentation among populations of two divergent clades of the brown trout (Atlantic and Mediterranean; Bernatchez, 2001; Sanz, 2018). Then we investigate if those loci could be a relevant proxy to monitor hybridization and genome-wide admixture among individuals of these two clades.

2 - MATERIALS AND METHODS

2 - 1 - BROWN TROUT'S GENOMICS DATA

Dd-RADseq data used in this study are issued from Leitwein et al. (2018). The full data set consists in 75,684 genome-wide SNPs (representing 40,519 RAD-loci or haplotypes) for 112 trout of hatchery and wild caught origins. Eighty-two wild caught individuals were fished in the headwaters of three small rivers of the Mediterranean Orb River catchment (France) (Gravezon, Mare and Upper Orb rivers; Leitwein et al., 2018). This catchment has been

seeded by hatchery fish of both Atlantic and Mediterranean origins for decades, but stopped in 2004. However, unintentional releases due to flooding of private hatcheries are documented (Leitwein et al., 2016, 2018). Analyses have shown that these wild caught individuals consisted in hatchery individuals of both Mediterranean and Atlantic origins, F1's, F2's, backcrossed individuals, and 'pure' natural Mediterranean fish (Leitwein et al., 2018). 'Early' and 'late' backcrossed individuals correspond to Mediterranean wild-caught fish that contained a moderate number and few Atlantic ancestry tracts in their genome, respectively. Thirty individuals of the domestic Atlantic (N = 15) and Mediterranean (N = 15) strains were also included in the analysis. Mediterranean hatchery fish have been randomly sampled in a local strain formerly established using mature adults from the Gravezon River by the Fédération de Pêche de l'Hérault in 2004. Atlantic hatchery fish originated from the Cauterets hatchery that maintains the Atlantic strain which is distributed worldwide (e.g. Bohling, Haffray, & Berrebi, 2016).

In addition to SNP data, all individuals have been sexed using a microsatellite marker targeting a salmonid sex-determining locus (Yano et al., 2013) and one microsatellite locus to correct for possible false negatives. No sex effect was detected and sex will be not considered further.

2 – 2 - PHENOTYPIC DATA

Acquisition of phenotypic data followed a protocol described in Lascaux (1996). Data were recorded from individual photographs of the 112 fish considered for genomic analyses. Photographs were taken at fishing on slightly anesthetized trout with eugenol. A camera Canon® EOS 1000D was used, then fish were released in the wild or in hatchery tanks. The full set of variables originally considered in this study is listed in Table S1 and illustrated on a picture in Fig. S1. Quantitative variables (N =19) and semi-quantitative variables (N =11)

were initially recorded by visual examination of photographs of the left flank of each individual considered in this study. Quantitative variables refer to punctuation patterns of brown trout (e.g. number and diameter of spots) and are recognized important for this species (Blanc et al., 1982, 1994). Semi-quantitative variables refer to 'ornamental' appearance patterns (e.g. parr marks, fringes on caudal or adipose fins, white ring around spots) that are often important to practitioners or anglers. Each quantitative punctuation variable was measured independently. Ornamental variables have been coded by modalities, either present/absent [1, 2] or, e.g., absent/partial/complete [1, 2, 3] (Tables 1 & S1). This set of variables avoided focusing only on peculiar attributes of the pigmentation patterns. Other pigmentation traits that can be also important for trout (e.g. background colour, brightness, hue, or reflectance; Colihueque, 2010) were not considered in this study as standardization between wild and hatchery fish were not possible because of different weather and light conditions at sampling that did not allow for accurate standardization. Phenotypic data and their pictures each individual fish will be deposited on a public repository. Eleven colour patterning variables were found not significantly correlated and retained for subsequent analysis (Table 1). The correlation matrix of variables is reported in Table S2.

2 – 3 - GENOTYPE-PHENOTYPE ASSOCIATION

The evaluation of the genotype-phenotype association between SNPs and pigmentation/colour traits was performed using three distinct approaches. Hereafter, we coined a 'colour patterning locus' (CPL) any SNP or RAD-locus found significantly associated with a colour variable considered in this study. A CPL and its possible associated gene is then not a pigmentation locus (gene) *per se*, but a locus involved in a complex set of features and patterns of trout skin, including pigmentation and colour information.

Redundancy analysis - We first used redundancy analysis (RDA) to investigate any association between genomic and colour trait data. RDA is a constrained (two-table) ordination method in which only the variation of response variables explained by a set of explanatory variables is displayed and analysed (Legendre & Legendre, 2012). RDA was performed with the remaining 11 uncorrelated colour variables as the explanatory and the SNPs as the response variables. Missing genomic data were imputed by the most commonly observed genotype. Missing phenotypic data were imputed by the mean of observed trait for quantitative data, and the most commonly observed phenotype for semi-quantitative data. We classified SNPs as showing statistically significant association with individual pigmentation/ornamental traits when they loaded with more than 2.5 standard deviations (S.D.) from the mean.

A forward model selection was used to select for the most relevant phenotypic variables structuring the RDA (Blanchet, Legendre, & Borcard, 2008). In constrained ordination methods like RDA, increasing the number of explanatory variables becomes similar to unconstrained ordination method (e.g. principal component analysis) as the percentage of variation explained increases when considering more explanatory variables, while some of them add no relevant information. Models were defined, first including the eleven uncorrelated response variables, then reducing this number. The Aikake Information Criterion was computed in each case to select the most appropriate model (i.e. minimizing deviance). Permutation tests (N = 999) were performed by permuting individuals in each model. This procedure was first established for the RDA itself, then for each successive RDA axis to investigate if observed patterns carried significant association between relevant pigmentation traits and SNPs. All analyses were performed with the *vegan* package (https://cran.r-project.org/web/packages/vegan/index.html). Once relevant colour variables

were identified in models, each significant SNP was associated to the pigmentation or ornamental trait it was the most significantly correlated.

Genome-wide association studies (GWAS) – Despite probable limitations due to, e.g. reduced sample size, single- and multi-trait GWAS were performed to further investigate candidate colour-patterning genes in this species. A single-trait association was first considered by fitting each colour variable with SNPs by linear regression. We used a penalized maximum-likelihood least absolute shrinkage and selection operator (LASSO) model to select for SNPs implied in each trait association, then solving:

$$\min_{(\beta_0,\beta)\in\mathbb{R}^{p+1}} \left[\frac{1}{2N} \sum_{i=1}^{N} (y_i - \beta_0 - x_i\ \beta)^2 + \lambda \sum_{j=1}^{p} |\beta_j| \right] \quad (1)$$

in which $Y \in \mathbb{R}$ represents the response colour variable, $X \in \mathbb{R}^p$ a vector of predictor variables (i.e. SNPs), λ the penalty parameter, β_0 the y-intercept of multiple linear regression, and $\beta \in \mathbb{R}^p$ a vector of β_j coefficients (Friedman, Hastie, & Tibshirani, 2010). This vector of β_j coefficients represents the effect size β_j of the jth SNP conditional on the effects of all other SNPs. The penalized term λ shrinks the regression coefficient towards zero, keeping only a small number of SNPs with large effects in the model. A cyclical coordinate descent procedure was retained for model selection (Friedman et al., 2010). The retained model was determined by cross-validation and log(λ) was estimated by minimizing the mean quadratic error. The number of positive β_j coefficients was estimated from log(λ), with each β_j coefficient associated to a suite of SNPs considered as involved in the association. Analyses were performed with the *glmnet* package (https://cran.r-project.org/package=glmnet).

A multi-trait GWAS was also implemented. We used the MultiPhen package (O'Reilly et al., 2012) to test for the linear combination of phenotypes most associated with the genotypes at each SNP. Such one approach may potentially capture effects hidden to

single phenotype GWAS. It performs a 'reversed regression', with multiple phenotype predictors and genetic variant as outcome (i.e. G SNPs $X_{(i)} = \{X_{(i1)},\ldots, X_{(iG)}\}$ are explained by K pigmentation pattern variables $Y_{(i)} = \{Y_{(i1)},\ldots, Y_{(iK)}\}$). SNPs are encoded by allele count alleles ($X_{(ig)} \in \{0, 1, 2\}$). An ordinal logistic regression (or proportional odds logistic regression) was considered to derive the probability than one SNP is associated to a multi-trait phenotype (O'Reilly & Porter, 2017). Permutation tests were performed to determine one adjusted significance threshold to detect false positives (Dudbridge & Gusnanto, 2008). A probability $P < 5 \times 10^{-8}$ was retained to consider one SNP as being significantly implied in a multi-trait pigmentation association. Only the variables selected by the forward selection model implemented for the RDA were considered in single- and multi-trait GWAS.

In single- and multi-trait GWAS, we controlled for population stratification by using nine distinct trout samples recognized by the length and number of ancestry tracts (Leitwein et al., 2018). Local ancestry tracts may integrate linkage disequilibrium patterns which are crucial in association studies (e.g. Shriner, 2017; Li, Kemppainen, Rastas, & Merilä, 2018). The nine groups include the two hatchery samples (Atlantic, Mediterranean), then seven distinct samples of wild-caught trout. Following Leitwein et al. (2018), wild-caught trout were grouped as follows: F1's, F2's, 'early' and 'late' backcrossed individuals, then samples of 'pure' wild individuals assigned to each of the three local populations. As some wild caught trout were identified as 'pure' hatchery individuals (Leitwein et al., 2018), they have been grouped with individuals sampled in the hatchery type they have been previously assigned.

2 – 4 - MAPPING AND ANNOTATION

CPLs found by RDA and the different GWAS have been mapped on the Atlantic salmon genome (*Salmo salar*; Lien et al. 2016; Genbank assembly: GCA_000233375.4) and on the

high density linkage map of *S. trutta* (Leitwein et al., 2017). We searched for genes located in a 25kb window upstream or downstream of each colour-associated marker (arbitrary range). When one association with a gene was detected, markers were assigned to coding or non–coding (intron, upstream or downstream sequences) regions of genes, or other specific entities (transposable elements, pseudogenes). Using *S. salar* annotations as inputs, gene ontologies (GO) for biological processes and molecular functions were derived for genes associated to CPLs using QuickGO (https://www.ebi.ac.uk/QuickGO/). When annotations were unavailable on the salmon genome, a research was launched on UniProtKB (https://www.uniprot.org/uniprot/) for the protein sequence encoded by the gene. Only annotations with similarity >90% were retained. All were found to match teleost species.

Because many genes implied in melanocyte development are also implied in melanoma development when mutated (e.g., Patton, Mitchell, & Naim, 2010; Uong & Zon, 2010), we further performed a literature search in order to know if genes detected within the 25kb windows have been already mentioned in former studies. Queries were made using the gene ID (or aliases taken from GeneCards, https://www.genecards.org/) and a specific keyword. The retained keywords were: skin, melanocyte, melanophore, melanosome, melanoma (squamous, cutaneous and uveal), pigment/-ation, keratinocyte, chromatophore, iridiophore, follicle (hair or skin), nevus/-i, (epi)dermis, vitiligo, erythema/-tous, and sebocyte. This search was updated until the beginning of June, 2020, each time using the Web of Science, PubMed and Google Scholar.

2 - 5 - GENOMIC DIFFERENTIATION

Distributions of F_{ST} values were established using all SNPs, then for the subset of CPLs found significant in the RDA and GWAS. Single-locus F_{ST} values were estimated considering five

distinct trout samples: the two samples of hatchery fish, then trout caught in each of the three rivers within the Orb catchment.

2 – 6 – Comparison of individual assignment

In order to compare if individual assignment (probability of membership) ranked individuals similarly or not using all loci or CPLs only, we first established the patterns of admixture using independent CPLs for individuals used in this study. Individual admixture proportions were computed with the LEA package (Frichot & François, 2015), using $K = 2$ (Atlantic and Mediterranean clades). We then used the genome-wide admixture established by Leitwein et al. (2018) that were based on individuals used in this study, using the same set of 40,519 RAD-loci, also considering $K = 2$. A Wilcoxon signed-ranked test between each assignment probability distribution was performed. Similar rankings of individuals in assignment will indicate that CPLs may represent a proxy of genome-wide admixture, while a significant difference will be indicative of the reverse.

3 - RESULTS

3 – 1 - Redundancy analysis

Forward model selection showed that a model based on eight colour variables minimized the deviance in RDA *(Macrost, N.PR.Tot, Zeb, Fr.An, L.Lat, Fr.P, Diam.PN, N.PN.Tot*) (Table S3). Hereafter, results are reported for this model. Patterns of variation explained by the RDA were found to significantly structure the association between SNPs and pigmentation

variables ($P < 0.001$). The first axis of RDA was found to represent 29.81% of the total inertia of the data set (Fig. 1), and was also significant ($P < 0.001$). RDA axis 2 was found to explain only a tiny fraction of observed variation for pigmentation variables (2.10%; Fig. 1), and be only marginally significant ($P \approx 0.051$). As RDA is a constrained ordination method, constrained inertia (the percentage of variation explained by the set of uncorrelated pigmentation variables) was found to represent 35.50% of the total inertia. The remaining portion of the variation might be considered as not explained by observed genomic variation. Axis 1 represented approx. 84% (ratio: 29.81/35.50) of constrained inertia, while axis 2 explained only approx. 6% (2.10/35.50) of inertia.

Ordination results in RDA are represented as a triplot in which individuals are positioned according to the relationship established between response and explanatory variables (Fig. 1). The ornamental trait *Macrost* (hereby a large pre-opercular black stain/spot) and the pigmentation trait *N.PR.Tot* (total number of red spots) explained 23.6% and 22.7%, respectively, of the total loading score of phenotypic variables onto RDA axis 1. In Fig. 1A, trout were labelled in reference to the nine clusters defined from Leitwein et al. (2018). Results showed a clear distinction between colour patterns of Mediterranean and Atlantic hatchery fish. Atlantic hatchery fish were preliminary characterized by the total number of red spots, while the macrostigma spots, but also zebra marks (*Zeb*) and total number of black spots (*N.PN.Tot*) characterized Mediterranean hatchery fish along the first axis of the RDA. Results confirmed that few wild caught individuals from the Gravezon River were released Atlantic hatchery fish, as showed by Leitwein et al. (2018). Other individuals from the Gravezon River and ‘late backcrossed’ individual have colour patterns more characteristic from hatchery Mediterranean fish (Fig. 1A; the hatchery was seeded by individuals caught in this river [see Materials and Methods section]). Other wild caught individuals are distributed between these extremes, with individuals identified as F1’s

presenting one intermediary position between wild-caught and wild Mediterranean fish on one side, and Atlantic fish on the other side (Fig. 1A). The position of the wild caught Orb River individuals referred to the marginally significant second axis of the RDA and to specific variables (*L. Lat*, continuity of the lateral line; *Fr.P.*, a coloured fringe on the pelvic fin) that were mostly unobserved in other fish excepted some F2 and early backcrossed individuals (Fig. 1A). F2 individuals presenting these characters were effectively fished in the Upper Orb River, suggesting they possibly inherited colour characters present in this local population.

The ordination of SNPs by RDA is further detailed in Fig.1B. A total of 1,130 distinct loci (1.49% of 75,684 SNPs) were found significantly associated to the first two RDA axes (i.e. > 2.5 S.D. from the mean). Only 22 loci (0.03%) were significantly associated to each of the two axes for distinct phenotypic variables. Different numbers of SNPs were found associated with pigmentation variables: 299 with *Fr.P*, 269 with *N.PR.Tot*, 225 with *N.PN.Tot*, and 213 with *Macrost*. *Macrost* showed significance for loci associated to both first and second axis of RDA (yellow circles on Fig.1B), while some variables showed no association (*Fr.An*, presence/absence of a fringe on the anal fin). In this latter case, this does not mean that no association exists, but as loci were associated to a single phenotypic variable based on the strength of the association, no loci appeared to be significantly associated to *Fr.An*. As axis 2 was found marginally significant and substantially affected by individuals from mainly one single population and the few F2's, only SNPs associated to the first RDA axis will be considered further. This discarded loci associated to *Fr.P* that explained most of the variation for the second RDA axis. Three hundred and twenty SNPs were found associated to the main RDA axis, all of them being associated to *Macrost* and *N.PR.Tot*, these variables being negatively correlated (Fig. 1B).

As a complement to this analysis and because the rule of ± 2.5 S.D. from the mean is somewhat arbitrary, we looked back at specific genes known to be involved in colour

patterning in vertebrates and fish and searched for their position within the distribution of loci over the first axis of the RDA. We detected eight genes representing 30 SNPs for which sequencing reads were available (Fig. S2). One SNP from the *Mitf1* (microphthalmia-associated transcription factor 1) gene was close to the ± 2.5 S.D. threshold considered in this study. The *Mitf1* gene is a master gene for melanocyte differentiation (e.g. Levy, Khaled, & Fisher, 2006; Cheli, Ohanna, Ballotti, & Bertolotto, 2010) and with a negative value on the first axis of the RDA, this SNP is associated to *Macrost*, thus the expression of the black colour.

3 – 2 - Single and multi-trait GWAS

Over the eight colour variables retained by forward selection for RDA, only two allowed for relevant LASSO model construction after selection of the penalized term using the cyclical descent procedure (Fig. S3). Single trait GWAS provided two variables with significant association with some SNPs: *N.PN.Tot* (total number of black spots: 17 candidate SNPs) and *N.PR.Tot* (total number of red spots: 9 candidate SNPs) (Table S4). These two variables were found significant in RDA, and negatively correlated (Fig. 1). Candidate SNPs from single GWAS models are reported in Fig. 2A.

Two multi-trait GWAS models were retained (Fig. 2). As the single-trait GWAS, the first one jointly retained *N.PN.Tot* and *N.PR.Tot* as variables for which some SNPs were found associated. The second selected model considered seven of the eight variables formerly used in the RDA (except *Zeb*). Seven and thirty-one SNPs were considered as significant in these multi-trait GWAS, respectively (Table S4).

Despite some similarities for the pigmentation and colour variables put forward by the different genotype-phenotype associations, only one single SNP (RAD-locus) was found

in common between RDA and the single-trait GWAS for number of black spots (Fig. 2A). No other SNP was commonly detected by distinct genotype-phenotype association methods, and especially no SNP shared among GWAS models. It results in a total of 384 SNPs considered as CPLs putatively implied in pigmentation variation in brown trout (~0.51% of the total number of SNPs; 320 coming from RDA and 64 from the single- and multi-trait GWAS models). These CPLs corresponds to 337 independent RAD-loci (0.83% of the total number of RAD-loci).

3 – 3 - GENOMIC DIFFERENTIATION

The distributions of F_{ST} values for the 40,519 RAD-loci and the 320 CPLs associated to axis 1 of the RDA are reported in Fig. 3. The mean F_{ST} was estimated to F_{ST} = 0.286 [95% CI: 0.284, 0.287] for the full set of SNPs, while this estimate was F_{ST} = 0.575 [95% CI: 0.570-0.583] for the 320 CPLs detected with the RDA. Observed mean F_{ST} values were F_{ST} = 0.176 (min. 0.02 – max: 0.38) and F_{ST} = 0.025 (min.: 0.00; max. 0.20) for the single- and the multi-trait GWAS, respectively. Min/max values are reported rather than 95% CI because the low number of SNPs/CPLs associated to phenotypic variables in each GWAS. As distributions do not overlap, the mean F_{ST} value of the 384 CPLs has no real meaning, but its estimate is F_{ST} = 0.547 [95% CI: 0.538 - 0.555].

3 – 4 - MAPPING ON *S. TRUTTA* LINKAGE GROUPS AND ANNOTATION

We considered only the 337 independent CPLs as linked SNPs provide redundant information. Three hundred independent CPLs were mapped onto 35 of the forty brown trout LGs defined in Leitwein et al. (2017) (Fig. 4), while 37 of them could not be adequately positioned on the high density linkage map of Leitwein et al. (2017). Their position relative to

other RAD-loci suffered ambiguity. The distribution of these 300 CPLs on each LG varied from zero (LG13, LG14, LG15, LG20, LG39) to 86 (LG31). The 337 CPLs have been mapped on the Atlantic salmon genome and their annotations are given in Table S4. Among the 337 independent CPLs, ~75.9% were found located in coding (43.4%) or so-called regulatory regions (32.5%; 17.8% upstream and 14.7% downstream genes) within the 25kb window retained around genes. This represents 245 loci associated to one gene (223 distinct genes; Table S4). Approximately 22.0% of the CPLs did not match any gene in the 25kb window, and ~2% were found associated to pseudogenes or long non-coding (lnc) RNA (Fig. S4). LncRNAs might be important in fish colour patterning (Luo et al., 2019), but will be not considered further. Overall 89.86% (200 out of 223) of the genes in close vicinity of the CPLs detected in this study have been mentioned in the literature dealing with colour, or affecting integument patterning, differentiation or structure. Table S4 provides details on gene names, their position and the location of associated CPL; references mentioning each of these 200 CPLs are reported.

Twenty-four of these 200 genes (12.0%) that suffer very few ambiguities for their implication in pigmentation variation are given in Table 2 to summarize data. Among these genes, nine candidate genes were detected using GWAS (four by single-, and five by multi-trait GWAS) (Table 2). The RAD-locus detected by both RDA and single-trait GWAS for black spotting is located ~21kb downstream of *GJD2* (Gap-junction protein Δ2 gene; located on LG9 of *S. trutta*; Table S4). This gene was found differentially expressed in trout skin (marble *vs* fario/Atlantic morph) by Djurdjevič et al. (2019).

3 – 5 - GO TERMS

GO terms for molecular functions and biological processes of the 337 CPLs are reported in Fig. S5. Calcium and metal ion binding were found to be the most representative molecular functions. GO-terms for biological functions highlighted cellular adhesion processes (Fig. S5A), signal transduction and translation, and transmembrane transport (Fig. S5B).

3 – 6 – WILCOXON SIGNED-RANKED TEST ON INDIVIDUAL ASSIGNMENT

As expected because of large differences in mean F_{ST} values, individual rankings in probability of membership were found to significantly differ when using the whole RAD-loci established in Leitwein et al. (2018) and the independent CPL (N = 337) data sets defined in this study (Wilcoxon signed-rank test, $P < 10^{-5}$). As CPLs were mainly associated to red and black spots (*N.PN.Tot*, *N.PR.Tot*, *Macrost*) in the GWAS and the RDA, this suggests that such patterns are not reliable indicators of genome-wide admixture, while frequently, but intuitively used by managers.

4 - DISCUSSION

Because of the importance of visual information for exchanges between conspecifics or camouflage, we aimed to investigate the genomic basis of body colour patterning between trout from the Mediterranean and Atlantic clades recognized in this species. The second one is heavily used for stocking practices and has been introduced worldwide, including Mediterranean watersheds (Elliott, 1989; Budy et al., 2013; Bohling et al., 2016). Based on findings by Leitwein et al. (2018), admixture (ancestry) patterns were included in our study and improved the detection of significant associations as shown in other species (e.g. human:

Shriver et al., 2003; Daya et al., 2014; rodent: Pallares, Harr, Turner, & Tautz, 2014; bird: Brelsford, Toews, & Irwin, 2017), but also fish (Malek et al., 2012). This information allowed for the detection of CPLs associated to candidate genes formerly identified in former pigmentation studies. These CPLs complement gene expression studies by Sivka et al. (2013) and Djurdjevič et al (2019) and improve knowledge on the genomic basis of pigmentation in the brown trout. While pigmentation patterns are important to evaluate the impact of hatchery individuals into wild trout populations (Mezzera et al., 1997; Aparicio et al., 2005), we showed that it is premature to rely on phenotypic colour patterns as a proxy of genome-wide admixture to promote management decisions.

4 – 1 - ASSOCIATIONS PROMOTE SPOTTING TRAITS…

Genotype-phenotype associations used in this study principally put forward the numbers of red and black spots and the presence/absence of the black pre-opercular spot as the main pigmentation variables that aimed to distinguish Mediterranean and Atlantic trout. This was more or less expected as these variables have been repeatedly shown to characterize pigmentation differences between Atlantic or Mediterranean trout in studies previously conducted in France (Poteaux & Berrebi, 1997) and Spain (Aparicio et al., 2005), but, while some spotting traits are important, they are not always found in Italy depending on the local populations analyzed and associated to other not found consistent in our study (e.g. patterns of the adipose fin) (see Lorenzoni et al., 2019). While focusing on few local French populations, results presented in this study reflect former results obtained for these two clades at a broader scale. The heritability of these traits was sometimes estimated ($h^2 \approx 0.40$ and $h^2 \approx 0.70$ for black and red spotting; Blanc et al., 1982, 1994, respectively). Nevertheless, model selection showed that potentially more variables might be considered at the local population level, and spotting traits do not uncover the full panel of colour variation present in a polytypic species

like brown trout. For example, as it might be explained by mathematical modelling (Miyazawa et al., 2010), spotting is replaced by irregular lines in the marbled trout, *S. t. marmoratus*, that represents another recognized clade within the brown trout complex (Bernatchez, 2001). It means that further works participating to establish the genotype-phenotype association for colour pattern in brown trout should integrate other tools and variables than used in these studies to analyze such a diversity of patterns. Recent software developments may participate to improve this issue (Endler, Cole, & Kranz, 2018; Van Bellenghem et al., 2018; Chan, Stevens, & Todd, 2019).

4 – 2 - … BUT DISTINCT LOCI INVOLVED IN PIGMENTATION VARIATION

If the different genotype-phenotype association methods used in our study retrieved the same pigmentation variables, CPLs detected by RDA and GWAS appeared clearly distinct with exception of *GJD2* formerly studied in trout (Djurdjevič et al., 2019). Indeed, the identity of loci considered associated to a single and/or multi-trait phenotype, their number, and their respective levels of genomic differentiation did not overlap. This is expected as models behind methods are different and may direct the analyses towards specific findings. For example, we already mentioned that the shrinkage procedure in LASSO aimed to keep only SNPs with large effects while discarding others, also explaining why far less CPLs come from the distinct GWAS. It is also widely known that results from single-trait GWAS lack power when few individuals or SNPs are considered (e.g. Schielzeth & Husby, 2014), especially when considering wild populations (Kardos, Husby, McFarlane, Qvanström, & Ellegren, 2018; Santure & Garant, 2018) and polygenic variation (Berg & Coop, 2014). These drawbacks were moderated by consideration of population stratification based on ancestry patterns. Discarding population stratification led to detection of no GWAS-related CPL (not shown). In the opposite, GWAS retrieved loci with low and sometimes null F_{ST} values that

RDA ignored while balancing selection is crucial to explain pigmentation variation in many case studies (e.g. Croucher, Oxford, Lam, & Gillespie, 2011; Lindtke et al., 2017; Schweizer et al., 2018). We do not expand further, but a sub-section below is devoted to RDA that, despite caveats, remains potentially neglected in association studies.

Nevertheless, if we adopt a pragmatic view, RDA and GWAS detected a large proportion of markers that were formerly mentioned in the literature on skin and pigmentation. Over the 337 independent CPLs retained overall, 245 loci mapped within a 25kb window downstream or upstream 223 distinct genes. Two hundreds of them were formerly mentioned in our literature survey regarding issues and processes dealing with colour patterning or skin-related issues. The GO terms highlighted by our study for both molecular functions and biological processes are known important for pigmentation patterning. Calcium binding is effectively known to be important for diverse processes during melanogenesis (Bush & Simon, 2007; Bellono & Oancea, 2014; Jia et al., 2020). For example, the *SLC45A2* gene detected in this study that encodes for a transporter protein involved in the production and regulation of melanin is regulated by calcium binding levels (Ginger et al., 2008; Bellono & Oancea, 2014). Melanin is also known to deeply interact with metal ions at various steps of melanogenesis (Hong & Simon, 2007). Metal ion binding is necessary in the integrin-mediated signaling pathways whose importance in colour patterning is well-known (Kelsh et al., 2009; Klotz et al., 2018). Zinc binding activity is critical to control melanocyte migration to the epidermis (Denecker et al., 2014) and microtubule activity for melanosome trafficking within melanocytes (e.g. Aspengren, Hedberg, Sköld, & Wallin, 2009). Other molecular functions such ATP- or DNA- binding activities are also known be crucial in melanosome/melanocyte biology and regulation (Levy et al., 2006; Heimerl, Bosserhof, Langmann, Ecker, & Schmitz, 2007). DNA-binding especially concerns transcription factors that have been shown to promote growth, survival and differentiation of melanocytes. The

main biological functions highlighted in this study (cellular adhesion processes, signal transduction and translation, transmembrane transport) are involved in chromatophore or melanocyte development, interactions among chromatophores or between melanocytes and other cell types (fibroblasts, keratinocytes) (Yamaguchi, Brenner, & Hearing, 2007; Raposo & Marks, 2007; Kelsh et al., 2009; Singh & Nüsslein-Völlard, 2015; Mahalwar, Singh, Fadeev, Nüsslein-Vollard, & Irion, 2016; Nüsslein-Völlard & Singh, 2017). Cell adhesion processes are illustrated by the numerous calcium-dependent (proto)cadherin genes reported in this study (*CDH4*, *FAT1*, and *PCDH* genes), as well as integrins (*ITGAV*) (Tables 2 and S4). Protocadherins like *PCDH10A* and *PCDHAC2* are essential to fish skin patterning (e.g. Kelsh et al., 2009, Williams, Hsu, Rossi, & Artinger, 2018; Du et al., 2019).

Each genotype-phenotype association method especially reported classical candidate genes associated to pigmentation. The RDA reported the above-mentioned (proto)cadherins, but also the *ZIC4* or *SLC7A11* genes. *ZIC4* was shown to participate the dorso-ventral patterning of medaka (Ohtsuka et al., 2004). The observed trait association with *SLC7A11* – a gene controlling pheomelanin production and red/yellow pigmentation in mammals (Chintala et al., 2005) - is another report in fish after carp (*Cyprinus carpio*; Xu et al., 2014; Jiang et al., 2014) and the hybrid ‘red’ tilapia (Zhu et al., 2016; Wang et al., 2019). The GWAS detected some classical players of pigmentation and body colouration in vertebrates (e.g. *SOX10*, *PEML*, *SLC45A2*), including fish (e.g. Schonthaler et al., 2005; Hou, Arnheiter, & Pavan, 2006; Kelsh et al., 2009; Singh & Nüsslein-Vollard, 2015). Indeed, *SOX10* is implied in neural crest development and influences colour patterning during zebrafish development (e.g. Elworthy, Lister, Carney, Raible, & Kelsh, 2003; Greenhill, Rocco, Vibert, Nikaido, & Kelsh, 2011; Nüsslein-Vollard & Singh, 2017), and *SLC45A2* in proton-transport and osmoregulation of melanosomes resulting in colour dilution as observed in medaka (Fukamachi, Shimada, & Shima, 2001) and other organisms (e.g. tiger: Xu et al., 2013;

human: Adhikari et al., 2019; horse: Sevane, Sanz, & Dunner, 2019). *PEML* (premelanosome protein) also contributes to eumelanin deposition and colour dilution in tetrapods (Raposo & Marks, 2007; Ishishita et al., 2018). *PEML* was recently found to be involved in colouration in the koi carp (Luo et al., 2019) and the cichlid *Neolamprologus meeli* (Ahi & Sfec, 2017).

Finally, few CPLs are associated to genes that are involved in background colour adaptation (*GABRA2*, gamma-aminobutyric acid receptor subunit alpha-2-like; Bertolesi, Vazhappill, Hehr, & McFarlane, 2016), or light-induced colour change (*MTRR*, methionine synthase reductase-like; Jones et al., 2018; *FGFR1*, fibroblast growth factor receptor-like 1; Czyz, 2019). *FGF* signaling is essential to the developmental patterning of skin and scales in fish (Aman, Fulbright, & Parichy, 2018), but also eyespots on butterfly wings (Özsu & Monteiro, 2017). This raises interesting questions regarding the conservation of mechanisms involved in spot formation across animals and in different developmental contexts.

Overall, results established for the brown trout identified both key genes and an array of far less-known and less-reported genes involved in biological and molecular processes related to the regulation of colour and skin pigmentation patterning.

4 – 3 - A HIGHLY POLYGENIC ARCHITECTURE

With 337 independent CPLs distributed over 35 LGs and a fraction of them with well-established relationships in modulating colour and pigmentation patterns, a polygenic architecture of body colour patterning is satisfied in the case of brown trout. Informative CPLs participating to genomic architecture of pigmentation in trout are shared in both coding and regulatory regions. This partitioning is now often reported in the literature (e.g. She & Jarosz, 2018), but still remains relatively poorly investigated in fish (Peichel & Marques, 2017, but see Jones et al., 2012). Within pigmentation studies, literature emphasizes both the

relative roles of coding (Protas & Patel, 2008; Uy et al., 2016) and regulatory variation to limit pleiotropic costs during colour pattern establishment and evolution (Larter, Dunbar-Wallis, Berardi, & Smith, 2018; Toomey et al., 2018). More coding changes than loci dispersed in regions that might be the basis of regulatory changes were detected in trout, but, due to the reduced representation of the genome provided by ddRAD sequencing, it is difficult to appreciate the relevance of this observation. It might be simply due to the choice of restriction enzymes that returned a peculiar genomic distribution of CPLs. Furthermore, we provided only a loose definition of upstream or downstream regulatory regions (a 25kb window) that, undoubtedly, has to be refined in further investigations.

A highly polygenic architecture may somewhat contrast with former studies that often proposed character inheritance based on Mendelian oligogenic models or few major QTLs in trout and salmonids (Blanc et al., 1982, 1994; Boulding et al., 2008; Colihueque, 2010). More generally, oligogenic models or low numbers of pigmentation-related QTLs located on few LGs per investigated trait were generally reported in genomic studies interested in fish pigmentation ($n < 8$; Borowsky & Wilkens, 2002; Miller et al., 2007; Magalhaes & Seehausen, 2010; Greenwood et al., 2011; Malek et al., 2012; O'Quin et al., 2013; Yong et al., 2015). However, the role of few QTLs has been considered not sufficient to explain all causal variation for pigmentation differences (Greenwood et al., 2011) and comparisons with our study be carefully considered. First, we did not use fish from controlled crossing experiments and our ability to detect any QTL is null. Second, we did not focus on a given body part (e.g. fin) or a pigmentation character (stripe, bars, belly spots) as done in other studies (e.g. Ahi & Sfec, 2017; Roberts et al., 2017), including trout (red spots in Blanc et al., 1994). We explored at a genome-wide level the complexity in trout pigmentation in order to detect the most important body pattern-related variables relevant to our samples. This automatically increased the panel of loci involved in body colour pigmentation, and, as in this

study, when more pigmentation traits were considered, the estimated number of pigmentation-related QTLs was found far higher and distributed over more LGs than when studying a lower number of traits ($n > 20$; Tripathi et al., 2009; Albertson et al., 2014).

Nevertheless, one increased genomic basis to pigmentation patterns was also reported when drift was present (Protas, Conrad, Gross, Tabin, & Borowsky, 2007). This last issue is fundamental in trout in which drift is very likely because of small local effective population sizes (N_e) (e.g. Palm, Laikre, Jorde, & Ryman, 2003; Charlier, Laikre, & Ryman, 2012). If this study certainly reflects associations that are valid for Mediterranean and Atlantic trout (above), it also reported local variation in color patterning as exemplified by, e.g., the SNPs associated to the second axis of the RDA that seemed population specific (notably in the Upper Orb River), associated to peculiar traits *(L. Lat*, *Fr.P*), and be likely promoted by demographic processes like drift. As loci associated to RDA axis 2 were not used in this study, they did not participate to define the set of CPLs and were not mapped on LGs. If they were, they will still extend the distribution of loci associated to body colour patterning. Rather than considering them a mess (some of them are significantly associated to a phenotypic variable in RDA), it might be more interesting to consider that the genomic basis for body colour patterning in trout results in the distribution of numerous loci with various – but actually unknown - effect sizes that segregate in many regions and may translate to a genomically diffuse information to specific phenotypes at the local population level. Rationales may rely on the classical contrast between the quantitative trait nucleotide (QTNs) hereby represented by some master genes (Sub-section 4.2), and the infinitesimal model of trait variation represented by additional loci of smaller effects whose role results in increased phenotypic variation for body pigmentation, especially when stochastic processes like drift associated to small N_e and reduced gene flow are of prime importance as in trout (Rockman, 2012). The highly polytypic brown trout (e.g. dozens of pigmentation phenotypes might be defined in the

Rhône County rivers [France] that, as the Orb River naturally hosts only the Mediterranean trout clade; Faure, 2012) may illustrate one intermediate situation in which some master genes are detected at the clade level by both GWAS and RDA and are associated to recognised spotting traits, while 'small players' mostly detected by RDA illustrate patterns specific to local populations, more locally expressed phenotypic traits *(L. Lat, Fr.P*). If the big pictures provided by master genes should be investigated further, small players cannot be *a priori* totally ignored in trout, but rather be integrated as a raw material that might be recruited to better figure out the mechanisms and programmes of pigmentation variation in trout.

4 – 4 - RDA: A NEGLECTED METHOD TO SCREEN FOR GENOTYPE-PHENOTYPE ASSOCIATIONS

Our findings illustrate the ability to investigate genotype-phenotype associations with a constrained ordination method like RDA and accurate modeling of phenotypic variables. Jombart, Pontier and Dufour (2009) already mentioned that RDA was somewhat neglected in association studies despite now recognized desirable properties to limit false positives (Capblanc, Luu, Blum, & Bazin, 2018; Forester et al., 2018) and robustness to recombination rate variation (Lotterhos, 2019). Effectively, the use of RDA to investigate genotype-phenotype associations remains seldom (Talbot et al., 2017; Vangestel, Eckert, Wegrzyn, St. Clair, & Neale, 2018; Carvalho et al., 2020), while it became a standard in genotype-environment association studies (Forester, Lasky, Wagner, & Urban, 2018), including trout (Bekkevold et al., 2019). Basically, RDA allowed for distinguishing the part of variation collectively explained by RAD-loci and independent pigmentation variables, and hereby estimated to ~35% of observed trait variation. A large portion of observed pigmentation is then explained by other variables or is environment-dependent as already suggested in salmon (Jørgensen et al. 2018) and trout (Westley et al., 2013). While it discards markers under balancing selection, a beneficial feature of RDA is to possibly focus on SNPs that may

explain for causal variation with at least one phenotypic variable, and not only using outlier loci with the highest observed F_{ST} values but without demonstrated association to a locus. For example, in a study dealing with pigmentation variation, Neethiraj, Hornett, Hill, and Wheat (2017) used genome scans tools to identify outlier loci, then *a posteriori* searched for association of these loci with pigmentation. If RDA remains certainly affected by some false positives as are genome scans, it certainly targets more relevant loci as illustrated by the high number of CPLs retrieved from the literature in this study. It should be further mentioned that Gautier (2015) proposed a modified model based on Bayesian outlier detection model that may use a color variable as a covariate, but it is limited so far to a single covariate when model-based selection prior to RDA might consider several ones concurrently. Adding on Jombart et al. (2009), RDA or related approaches (e.g canonical correlation analysis that showed similar performance and power than multi-trait GWAS in association search; Porter & O'Reilly, 2017) deserve more attention to investigate genotype-phenotype associations.

4 – 5 - IMPLICATIONS FOR MANAGEMENT

Contrary to ,e.g., birds (Hanna et al., 2018; Billerman et al., 2019) or mammals (e.g. Anderson et al., 2009; Fulgione et al., 2016), how differences in body pigmentation are related to differentiation and admixture was poorly studied in fish and was not related to conservation and management issues (Malek et al., 2012; Meier et al., 2018, but see Boulding et al., 2008). Using mitochondrial and allozymic markers, Aparicio et al. (2005) paved the way to use pigmentation as a proxy of admixture between Atlantic and Mediterranean trout and to address such issues. Indeed, body pigmentation patterns are often important to anglers and managers to characterize local trout populations and their "integrity". Earlier studies addressing this issue using old-generation markers indicated that pigmentation might represent such a proxy (Mezzera et al., 1997; Aparicio et al., 2005; see Delling et al., 2000 for

S. t. marmoratus), but this issue was not addressed with modern markers like SNPs. In this study, RDA showed that 'late backcrossed' individuals clustered very close from the local Mediterranean hatchery strain and wild individuals of the Gravezon River that were used to seed this strain. This suggests that the pigmentation pattern of these latter individuals came back close to the local Mediterranean patterns and that counter selection of Atlantic pigmentation alleles or other changes (e.g. Endler, Betancourt, Nolte, & Schlötterer, 2016) occurred. Counter selection of Atlantic alleles in the Orb River's trout was also postulated by Poteaux, Bonhomme and Berrebi (1998) using allozymes. However, different rankings in assignment between this study for CPLs and the full RAD-loci data set (Leitwein et al., 2018) also suggest that this set of CPLs is not a proxy of genome-wide admixture in this catchment. Managers could build a policy based on wild-caught individuals that exhibit the 'local pigmentation' whereas trout remain largely admixed with foreign alleles. If this mismatch between rankings is mainly due to (near) neutral alleles, admixture would hopefully not constrain the adaptive potential of populations. In such a case, investigating pigmentation differences at the phenotypic level may contain enough information to drive management policies and limit the genotyping costs for managers. On the contrary, if a mismatch occurs and that CPLs do not mirror admixture at other locally-adapted fitness effect loci, management using pigmentation as a proxy of local genetic integrity alone might be at risk despite apparent counter-selection. Distinguishing between these situations is a difficult task, but, at that time, heavy reliance on the '*local is best*' paradigm (i.e. considering that individuals presenting the 'local' phenotype are more adapted to local conditions) might be harmful if its application reduces population fitness (Broadhurst et al., 2008; Kronenberger et al., 2018). We are aware that the list of CPLs we propose at that time is a list of candidate markers that has to be refine in many ways for further testing against genome-wide admixture,

but, in the meantime, managers have to be precautionary when evaluating population 'integrity' based on body pigmentation.

4 – 6 - LIMITS AND FUTURE DIRECTIONS

While providing interesting results, comparisons using individuals from more natural populations, clades and/or subspecies within the *S. trutta* complex remain necessary in order to better investigate and elucidate the evolutionary history of trout regarding body colour patterns and to refine the CPL list. DdRADseq used in this study is however a reduced representation sequencing technique targeting only a tiny portion of the genome (~1%) that is considered either to successfully target (Belsford et al., 2017) or miss important loci responsible for colour and pigmentation patterns (Gauthier et al., 2020). Thus, RNA sequencing or gene expression studies promoted by Djurdjevič et al. (2019), together with more standard association studies in control settings that, as for other traits, may provide better estimates of QTL number (if any), variation in effect sizes among loci and more accurately pointing key master genes (e.g. Sinclair-Waters et al., 2020) seems necessary to improve findings. As one ornamental trait, gaining insights into the body pigmentation genomics in trout can have economically valuable consequences (Colihueque, 2010), but the particular settings of most natural populations (low population size, high genetic drift and reduced gene flow) should be taken into account to explain pigmentation diversity observed in this study. Pigmentation issues that represents one outstanding case to investigate genotype-phenotype-environment relationships, and increasing knowledge on such relationships is crucial in salmonid conservation (Waples et al., 2020). As thousands of trout pictures are taken each year and tissue samples might be also collected, investigating new methods to acquire phenotypic data may provide new opportunities to develop conservation phenogenomics programmes involving scientists and practitioners.

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DATA ACCESSIBILITY

Genomic data are from Leitwein et al. (2018) and available at NCBI Short Read Archive under the study accession SRP136716. The final VCF and the PLINK files for *S. trutta* LGs are available under the study accessions SRZ187687 and SRZ187688. Upon acceptance of the paper, trout pictures and phenotypic data will be deposited on Dryad or on a similar repository.

REFERENCES

Adhikari, K., Mendoza-Revilla, J., Sohail A., Fuentes-Guajardo, M., Lampert, J., Chacon-Duque, J. C., … Ruiz-Linares, A. (2019). A GWAS in Latin Americans highlights the convergent evolution of lighter skin pigmentation in Eurasia. *Nature Communications*, 10(1), *358*. doi: 10.1038/s41467-018-08147-0

Ahi, E. P., & Sefc. K. M. (2017). A gene expression study of dorso-ventrally restricted pigment pattern in adult fins of *Neolamprologus meeli*, an African cichlid species. *PeerJ, 5*, e2843. doi: 10.7717/peerj.2843

Albertson, R. C., Powder, K. E., Hu, Y., Coyle, K. P., Roberts, R. B., & Parsons K. J. (2014). Genetic basis of continuous variation in the levels and modular inheritance of pigmentation in cichlid fishes. *Molecular Ecology, 23(21),* 5135-5150. doi: 10.1111/mec.12900

Aman, A. J., Fulbright, A. N., & Parichy D. M. (2018). Wnt/β-catenin regulates an ancient signaling network during zebrafish scale development. *Elife, 7*, e37001, doi: 10.7554/eLife.37001

Amsen, E. M., Pham, N., Pak, Y., & Rotin, D. (2006). The guanine nucleotide exchange factor CNrasGEF regulates melanogenesis and cell survival in melanoma cells. *Journal of Biological Chemistry, 281(1),* 121-128. doi: 10.1074/jbc.M507595200

Anderson, T. M., vonHoldt, B. M., Candille, S. I., Musiani, M., Greco, C., Stahler, D. R., … Barsh, G. S. (2009). Molecular and evolutionary history of melanism in North American gray wolves. *Science, 323,* 1339-1343. doi: 10.1126/science.1165448

Aparicio, E., García-Berthou, E., Araguas, R. M., Martinez, P., & Garcia-Marin, J. L. (2005). Body pigmentation pattern to assess introgression by hatchery stocks in native *Salmo trutta* from Mediterranean streams. *Journal of Fish Biology, 67(4),* 937-949. doi: 10.1111/j.0022-1112.2005.00794.x

Arnoult, L., Su, K. F., Manoel, D., Minervino, C., Magriña, J., Gompel, J., & Prud'Homme, B. (2013). Emergence and diversification of fly pigmentation through evolution of a gene regulatory module. *Science, 339,* 1423-1426. doi: 10.1126/science.1233749

Aspengren, S., Hedberg, D., Sköld, H. N., & Wallin, M. (2009). New insights into melanosome transport in vertebrate pigment cells. *International Review of Cell and Molecular Biology, 272,* 245-302. doi: 10.1016/S1937-6448(08)01606-7

Auld, H.L., Noakes, D.L.G., & Banks, M.A. (2019). Advancing mate choice studies in salmonids. *Reviews in Fish Biology and Fisheries, 29,* 249–276. doi: 10.1007/s11160-019-09551-5

Beauvais., A, Erickson, C. A., Goins, T., Craig, S. E., Humphries, M. J., Thiery, J-P., & Dufour, S. (1995). Changes in the fibronectin-specific integrin expression pattern modify the migratory behavior of sarcoma S180 cells in vitro and in the embryonic environment. *Journal of Cell Biology, 128(4),* 699-713. doi: 10.1083/jcb.128.4.699

Bekkevold, D., Höjesjö, J, Nielsen, E. E., Aldvén, D., Damm Als, T., Sodeland, M., … Hansen, M. M. (2019). Northern European *Salmo trutta* (L.) populations are genetically divergent across geographical regions and environmental gradients. *Evolutionary Applications, 13(2),* 400-416. doi: 10.1111/eva.12877

Bellono, N. W., & Oancea, E. V. (2014). Ion transport in pigmentation. *Archives of Biochemistry and Biophysics, 563,* 35-41. doi: 10.1016/j.abb.2014.06.020

Berg, J. J., & Coop, G. 2014. A population genetic signal of polygenic adaptation. *PLoS Genetics, 10(8),* e1004412. doi: 10.1371/journal.pgen.1004412

Bernatchez, L. (2001). The evolutionary history of brown trout (*Salmo trutta* L.) inferred from phylogeographic, nested clade, and mismatch analyses of mitochondrial DNA variation. *Evolution,* 55, 351-379. doi: 10.1111/j.0014-3820.2001.tb01300.x

Bertolesi, G. E., Vazhappilly, S. T., Hehr, C. L., & McFarlane, S. (2016). Pharmacological induction of skin pigmentation unveils the neuroendocrine circuit regulated by light. *Pigment Cell and Melanoma Research, 29,* 186-198. doi: 10.1111/pcmr.12442

Blanc, J-M., Poisson, H., & Vibert R. (1982). Variabilité génétique de la ponctuation noire sur la truitelle Fario (*Salmo trutta* L.). *Annales de Génétique et de Sélection Animale,* 14, 225-236

Blanc, J-M., Chevassus, B., & Krieg, F. (1994). Inheritance of the number of red spots on the skin of the brown trout. *Aquatic Living Resources, 7,* 133-136. doi: 10.1051/alr:1994016

Blanc, J-M., Poisson, H., & Quillet, E. (2006). A blue variant in the rainbow trout, *Oncorhynchus mykiss* Walbaum. *Journal of Heredity, 97,* 89–93. doi: 10.1093/jhered/esj010

Bian, C., Hu, Y., Ravi, V., Kuznetsova , I. S., Shen, X., Mu, X.,… Shi, Q. (2016). The Asian arowana (*Scleropages formosus*) genome provides new insights into the evolution of an early lineage of teleosts. *Scientific Reports,* 6, 24501. doi:10.1038/srep24501

Billerman, S. M., Cicero, C., Bowie, R. C. K., & Carling, M. D. (2019). Phenotypic and genetic introgression across a moving woodpecker hybrid zone. *Molecular Ecology, 28,* 1692-1708. doi: 10.1111/mec.15043

Blanchet, F. G., Legendre, P., & Borcard, D. (2008). Forward selection of explanatory variables. *Ecology, 89,* 2623-2632. doi: 10.1890/07-0986.1

Bohling, J., Haffray, P., & Berrebi, P. (2016). Genetic diversity and population structure of domestic brown trout (*Salmo trutta*) in France. *Aquaculture* 462: 1-9. doi: 10.1016/j.aquaculture.2016.04.013

Borowsky, R., & Wilkens, H. 2002. Mapping a cave fish genome: polygenic systems and regressive evolution. *Journal of Heredity, 93(1),* 19-21. doi: 10.1093/jhered/93.1.19

Boulding, E. G., Culling, M., Glebe, B., Berg, P.R., Lien, S., & Moen T. (2008). Conservation genomics of Atlantic salmon: SNPs associated with QTLs for adaptive traits in parr from four trans-Atlantic backcrosses. *Heredity, 101,* 381-391. doi: 10.1038/hdy.2008.67

Braasch, I., Brunet, F., Volff, J-N., & Schartl, M. (2009). Pigmentation pathway evolution after whole-genome duplication in fish. *Genome Biology and Evolution, 1,* 479-493. doi: 10.1093/gbe/evp050

Brännäs, E., Backström, T., Nilsson, J., Carlberg, H., Stien, L. H., & Magnhagen, C. (2016). Distinguishing Arctic charr with different stress coping styles by visual screening of spottiness – reliability and consistency over time. *Journal of Zoology, 300,* 213-220. doi: 10.1111/jzo.12374

Brelsford, A., Toews, D. P. L., & Irwin D. E. (2017). Admixture mapping in a hybrid zone reveals loci associated with avian feather coloration. *Proceedings: Biological Sciences, 284,* 20171106. doi: 10.1098/rspb.2017.1106

Brien, M. N., Enciso-Romero, J., Parnell, A. J., Salazar, P. A., Morochz, C., Chala, D., … Nadeau, N. J. (2019). Phenotypic variation in *Heliconius erato* crosses shows that iridescent structural colour is sex-linked and controlled by multiple genes. *Interface Focus, 9,* 20180047. doi:10.1098/rsfs.2018.0047

Broadhurst, L. M., Lowe, A., Coates, D. J., Cunningham, S. A., McDonald, M., Vesk, P. A., & Yates, C. (2008). Seed supply for broadscale restoration: maximizing evolutionary potential. *Evolutionary Applications, 1:* 587–597. doi: 10.1111/j.1752-4571.2008.00045.x

Budy, P., Thiede G. P., Lobón-Cerviá, J., Gonzales, G., McHugh, P., McIntosh, A., … Jellyman, P. (2013). Limitation and facilitation of one of the world‘s most invasive fish: an intercontinental comparison. *Ecology, 94,* 356–367. doi: 10.2307/23435983

Bush, W. D., & Simon, J. D. (2007). Quantification of Ca^{2+} binding to melanin supports the hypothesis that melanosomes serve a functional role in regulating calcium homeostasis. *Pigment Cell Research, 20,* 134-139. doi: 10.1111/j.1600-0749.2007.00362.x

Cal, L., Suarez-Bregua, P., Braasch, I., Irion, U., Kelsh, R., Cerda-Reveter, M., & Rotllant, J. (2019). Loss-of-function mutations in the melanocortin 1 receptor cause disruption of dorso-ventral countershading in teleost fish. *Pigment Cell and Melanoma Research, 32,* 817-828. doi: 10.1111/pcmr.12806

Capblancq, T., Luu, K., Blum, M. G. B., & Bazin, E. (2018). Evaluation of redundancy analysis to identify signatures of local adaptation. *Molecular Ecology Resources, 18,* 1223-1233. doi:10.1111/1755-0998.12906

Carvalho, C. S., Forester, B. R., Mitre, S. K., Alves, A., Imperatriz-Fonseca, V. L., Ramos, S. J., … Jaffé, R. (2020). Combining genotype, phenotype, and environmental data to delineate site-adjusted provenance strategies for ecological restoration. *Molecular Ecology Resources* , doi:10.1111/1755-0998.13191

Ceinos, R. M., Guillot, R., Kelsh, R. N., Cerdá-Reverter, J. M., & Rotllant, J. (2015). Pigment patterns in adult fish result from superimposition of two largely independent pigmentation mechanisms. *Pigment Cell and Melanoma Research, 28(2),* 196-209. doi: 10.1111/pcmr.12335

Chan, I. Z. W., Stevens, M., & Tood, P. A. (2019). PAT-GEOM: a software package for analysis of animal colour patterns. *Methods in Ecology and Evolution, 10(4),* 591-600. doi: 10.1111/2041-210X.13131

Charlier, J., Laikre, L., & Ryman, N. (2012). Genetic monitoring reveals temporal stability over 30 years in a small, lake-resident brown trout population. *Heredity, 109(4),* 246-253. doi:10.1038/hdy.2012.36

Cheli, Y., Ohanna, M., Ballotti, R., & Bertolotto, C. (2010). Fifteen-year quest for microphthalmia-associated transcription factor target genes. *Pigment Cell and Melanoma Research, 23(1),* 27-40. doi: 10.1111/j.1755-148X.2009.00653.x

Chintala, S., Li, W., Lamoreux, M. L., Ito, S., Wakamatsu, K., Sviderskaya, E. V., … Swank, R. T. (2005). *Slc7a11* gene controls production of pheomelanin pigment and proliferation of cultured cells. *Proceedings of the National Academy of Sciences of the USA, 102(31),* 10964-10969. doi:10.1073/pnas.0502856102

Cichorek, M., Wachulska, M., Stasiewicz, A., & Tymińska, A. (2013). Skin melanocytes: biology and development. *Postepy Dermatology and Alergology, 30(1),* 30-41. doi: 10.5114/pdia.2013.33376

Cieslak, M., Reissmann, M., Hofreiter, M., & Ludwig, A. (2011). Colours of domestication. *Biological Reviews of the Cambridge Philosophical Society, 86(4),* 885-899. doi: 10.1111/j.1469-185X.2011.00177.x

Colihueque, N. (2010). Genetics of salmonid skin pigmentation: clues and prospects for improving the external appearance of farmed salmonids. *Reviews in Fish Biology and Fisheries, 20,* 71–86. doi: 10.1007/s11160-009-9121-6

Crawford, N. G., Kelly, D. E., Hansen M. E. B., Beltrame, M. H. Fan, S., Bowman, S. L., … Tishkoff, S. A. (2017). Loci associated with skin pigmentation identified in African populations. *Science, 358,* eaan8433. doi:10.1126/science.aan8433

Croucher, P. J., Oxford, G. S., Lam, A., & Gillespie, R. G. 2011. Stabilizing selection maintains exuberant colour polymorphism in the spider *Theridion californicum* (Araneae, Theridiidae). *Molecular Ecology, 20(2),* 206-218. doi:10.1111/j.1365-294X.2010.04941.x

Cuthill, I. C., Allen, W. L., Arbuckle, K., Caspers, B., Chaplin, G., Hauber, M. E., … Caro, T. (2017). The biology of color. *Science, 357,* ean0221. doi:10.1126/science.aan0221

Czyz, M. (2019). Fibroblast growth factor receptor signaling in skin cancers. *Cells, 8(6),* 540. doi: 10.3390/cells8060540

da Silva Romero, A. R., Siquera, F., Santiago, G. G., Correia de Almeida Regitano, L., Dorta de Souza Junior, M., de Ameida Torres Junior, R. A., … Barufatti Grisolia, A. (2018). Prospecting genes associated with navel length, coat and scrotal circumference traits in Canchim cattle. *Livestock Science, 210,* 33-38. doi: 10.1016/j.livsci.2018.02.004

Daya, M., van der Merwe, L., Gignoux, C. R., van Helden, P. D., Möller, M., & Hoal, E. G. (2014). Using multi-way admixture mapping to elucidate TB susceptibility in the South African coloured population. *BMC Genomics, 15(1),* 1021. doi: 10.1186/1471-2164-15-1021

Delling, B., Crivelli, A. J., Rubin, J-F., & Berrebi, P. (2000). Morphological variation in hybrids between *Salmo marmoratus* and alien *Salmo* species in the Volarja stream, Soca River basin, Slovenia. *Journal of Fish Biology, 57,* 1199-1212. doi: 10.1006/jfbi.2000.1383

Denecker, G., Vandamme, N., Akay, O., Koludrovic, D., Taminau, J., Lemaire, K., … Berx, G. (2014). Identification of a *ZEB2-MITF-ZEB1* transcriptional network that controls melanogenesis and melanoma progression. *Cell Death and Differentiation, 21(8),* 1250-1261. doi:10.1038/cdd.2014.44

Ding, B., Patterson, E. L., Holalu, S. V., Li, J., Johnson, G. A., Stanley, L. E., … Yuan, Y-W. (2020). Two MYB proteins in a self-organizing activator-inhibitor system produce spotted pigmentation patterns. *Current Biology, 30(5),* 802-814.e8. doi:10.1016/j.cub.2019.12.067

Djurdjevič, I., Furmanek, T., Miyazawa, S., & Sušnik Bajec, S. (2019). Comparative transcriptome analysis of trout skin pigment cells. *BMC Genomics, 20(1),* 359. doi:10.1186/s12864-019-5714-1

Du, J., Chen, X., Wang, J., Chen, H., Yue, W. Lu, G., & Wang, C. (2019). Comparative skin transcriptome of two Oujiang color common carp (*Cyprinus carpio* var. color) varieties. *Fish Physiology and Biochemistry, 45(1),* 177-185. doi: 10.1007/s10695-018-0551-8

Dudbridge, F., & Gusnanto, A. (2008). Estimation of significance thresholds for genomewide association scans. *Genetic Epidemiology, 32(3),* 227-234. doi: 10.1002/gepi.20297

Duchi, A. (2018). Flank spot number and its significance for systematics, taxonomy and conservation of the near-threatened Mediterranean trout *Salmo cettii*: evidence from a genetically pure population. *Journal of Fish Biology, 92,* 254-260. doi:10.1111/jfb.13492

Elworthy, S., Lister, J.A., Carney, T. J., Raible, D. W., & Kelsh, R. N. (2003). Transcriptional regulation of *mitfa* accounts for the *sox10* requirement in zebrafish melanophore development. *Development, 130(12),* 2809-2818. doi: 10.1242/dev.00461

Elliott, J. M. (1989). Wild brown trout *Salmo trutta*: an important national and international resource. *Freshwater Biology, 21,* 1-5. doi: 10.1111/j.1365-2427.1989.tb01343.x

Endler, J. A., Cole, G. L., & Kranz, A. M. (2018). Boundary strength analysis: combining colour pattern geometry and coloured patch visual properties for use in predicting behaviour and fitness. *Methods in Ecology and Evolution, 9,* 2334-2448. doi: 10.1111/2041-210X.13073

Endler, L., Betancourt, A. J., Nolte, V., & Schlötterer, C. (2016). Reconciling differences in pool-GWAS between populations: A case study of female abdominal pigmentation in *Drosophila melanogaster*. *Genetics, 202,* 843–855.

Eom, D. S., Bain, E. J., Patterson, L. B., Grout, M. E., & Parichy, D. M. (2015). Long-distance communication by specialized cellular projections during pigment pattern development and evolution. *Elife,* 4, e12401. doi:10.7554/eLife.12401

Faure, J-P. (2012). *Etude génétique des populations de truites fario du département du Rhône – Rapport final*. Fédération du Rhône pour la Pêche et la Protection du Milieu Aquatique. La Tour de Salvagny : France.

Fogelholm, J., Henriksen, R., Höglund, A., Huq, K., Johnson, M., Lenz, R., … Wright, D. (2020). *CREBBP* and *WDR 24* identified as candidate genes for quantitative variation in red-brown plumage colouration in the chicken. *Scientifc Reports, 10(1),* 1161. doi: 10.1038/s41598-020-57710-7

Forester, B. R., Lasky, J. R., Wagner, H. H., & Urban, D. L. (2018). Comparing methods for detecting multilocus adaptation with multivariate genotype-environment associations. *Molecular Ecology, 27(9),* 2215-2233. doi: 10.1111/mec.14584

Frichot, E., & Francois, O. (2015). LEA: an R package for Landscape and Ecological Association studies. *Methods in Ecology and Evolution, 6(8),* 925-929. doi: 10.1111/2041-210X.12382

Friedman, J., Hastie, T., & Tibshirani, R. (2010). Regularization paths for generalized linear dodels via coordinate descent. *Journal of Statistical Softwares, 33(1),* 1-22. PMID: 20808728

Fukamachi, S., Shimada, A., & Shima, A. (2001). Mutations in the gene encoding B, a novel transporter protein, reduce melanin content in medaka. *Nature Genetics, 28(4),* 381-385. doi: 10.1038/ng584

Fulgione, D., Rippa, D., Buglione, M., Trapanese, M., Petrelli, S., & Maselli, V. (2016). Unexpected but welcome. Artificially selected traits may increase fitness in wild boar. *Evolutionary Applications, 9(6),* 769-776. doi: 10.1111/eva.12383

Gallagher, S. J., Rambow, F., Kumasaka, M., Champeval, D., Bellacosa, A., Delmas, M., & Larue, L. (2013). Beta-catenin inhibits melanocyte migration but induces melanoma metastasis. *Oncogene, 32(17),* 2230-2238. doi: 10.1038/onc.2012.229

Gautier, M. (2015). Genome-wide scan for adaptive divergence and association with population-specific covariates. *Genetics, 201(4),* 1555-1579. doi: 10.1534/genetics.115.181453

Gauthier, J., de Silva, D. L., Gompert, Z., Whibley, A., Houssin, C., Le Poul, Y., ... Elias, M. 2020. Contrasting genomic and phenotypic outcomes of hybridization between pairs of mimetic butterfly taxa across a suture zone. *Molecular Ecology, 29(7),* 1328-1343. doi: 10.1111/mec.15403

Ginger, R. S., Askew, S. E., Ogborne, R. M., Wilson, S., Fernadino, D., Dadd, T., … Green, M. R. (2008). *SLC24A5* encodes a trans-Golgi network protein with potassium-dependent sodium-calcium exchange activity that regulates human epidermal melanogenesis. *Journal of Biological Chemistry, 283(9),* 5486-5495. doi:10.1074/jbc.M707521200

Gramann, A. K., Venkatesan, A. M., Guerin, M., & Ceol, C. J. (2019). Regulation of zebrafish melanocyte development by ligand-dependent BMP signaling. *Elife, 8,* e50047. doi: 10.7554/eLife.50047

Greenhill, E. R., Rocco, A., Vibert, L., Nikaido, M., & Kelsh, R. N. (2011). An iterative genetic and dynamical modelling approach identifies novel features of the gene regulatory network underlying melanocyte development. *PLoS Genetics, 7(9),* e1002265. doi: 10.1371/journal.pgen.1002265

Greenwood, A. K., Jones, F. C., Chan, Y. F., Brady, S. D., Absher, D. M., Grimwood, J., … Peichel, C. L. (2011). The genetic basis of divergent pigment patterns in juvenile threespine sticklebacks. *Heredity, 107(2),* 155-166. doi: 10.1038/hdy.2011.1

Gu, L., & Xia, C. (2017). Revelation of the genetic basis for convergent innovative anal fin pigmentation patterns in cichlid fishes. *bioRxiv*. doi: 10.1101/165217.

Hanna, Z. R., Dumbacher, J. P., Bowie, R. C. K., Henderson, J. B., & Wall, J. D. (2018). Whole-genome analysis of introgression between the spotted owl and barred owl (*Strix occidentalis* and *Strix varia*, respectively; Aves: Strigidae) in western North America. *G3, 8(12),* 3945-3952. doi: 10.1534/g3.118.200754

Heimerl, S., Bosserhoff, A. K., Langmann, T., Ecker, J., & Schmitz, G. (2007). Mapping ATP-binding cassette transporter gene expression profiles in melanocytes and melanoma cells. *Melanoma Research, 17(5),* 265-273. doi: 10.1097/CMR.0b013e3282a7e0b9

Hellström, A. R., Watt, B., Fard, S. S., Tenza, D., Mannström, P., Narfström, K., ... Andersson, L. (2011). Inactivation of *Pmel* alters melanosome shape but has only a subtle effect on visible pigmentation. *PLoS Genetics, 7(9),* e1002285. doi: 10.1371/journal.pgen.1002285

Henning, F., Jones, J. C., Franchini, P., & Meyer, A. (2013). Transcriptomics of morphological color change in polychromatic Midas cichlids. *BMC Genomics, 14,* 171. doi: 10.1186/1471-2164-14-171

Henning, F., Lee, H. J., Franchini, P., & Meyer, A. (2014). Genetic mapping of horizontal stripes in Lake Victoria cichlid fishes: benefits and pitfalls of using RAD markers for dense linkage mapping. *Molecular Ecology, 23(21),* 5224-5240. doi: 10.1111/mec.12860

Hirobe, T. (2011). How are proliferation and differentiation of melanocytes regulated? *Pigment Cell and Melanoma Research, 24(3),* 462-478. doi: 10.1111/j.1755-148X.2011.00845.x

Hong, L., & Simon, J. D. (2007). Current understanding of the binding sites, capacity, affinity, and biological significance of metals in melanin. *Journal of Physical Chemistry, B111(28),* 7938-7947. doi: 10.1021/jp071439h

Hou, L., Arnheiter, H., & Pavan, W. J. (2006). Interspecies difference in the regulation of melanocyte development by *SOX10* and *MITF*. *Proceedings of the national Academy of Sciences of the USA, 103(24),* 9081-9085. doi: 10.1073/pnas.0603114103

Huang, J. M., & Hornyak, T. J. (2015). Polycomb group proteins - epigenetic repressors with emerging roles in melanocytes and melanoma. *Pigment Cell and Melanoma Research, 28(3),* 330-339. doi: 10.1111/pcmr.12341

Hultman, K. A., Bahary, N., Zon, L. I., & Johnson, S. L. (2007). Gene duplication of the zebrafish *kit ligand* and partitioning of melanocyte development functions to *kit ligand a*. *PLoS Genetics, 3(1),* e17. doi: 10.1371/journal.pgen.0030017

Ielli, F., & Duchi, A. (1990). Indagini preliminari sulle caratteristiche morfologiche di alcune trote *Salmo trutta marmoratus* Cuv., pure ed ibride del torrente Avisio (Val di Fiemme, Trento). *Rivista di Idrobiologia, 29,* 275–280.

Irion, U., & Nüsslein-Volhard, C. (2019). The identification of genes involved in the evolution of color patterns in fish. *Current Opinion in Genetics and Development, 57,* 31-38. doi: 10.1016/j.gde.2019.07.002

Ishishita, S., Takahashi, M., Yamaguchi, K., Kinoshita, K., Nakano, M., Nunome, M., … Matsuda, Y. (2018). Nonsense mutation in *PMEL* is associated with yellowish plumage colour phenotype in Japanese quail. *Scientific Reports, 8(1),* 16732. doi: 10.1038/s41598-018-34827-4

Jacquin, L., Gauthey, Z., Roussille, V., Le Hénaff, M., Tentelier, C., & Labonne, J. (2017). Melanin in a changing world: brown trout coloration reflects alternative reproductive strategies in variable environments. *Behavioral Ecology, 28(6),* 1423–1434. doi: 10.1093/beheco/arx102

Jia, Q., Hu, S., Jiao, D., Li, X., Qi, S., & Fan, R. (2020). Synaptotagmin-4 promotes dendrite extension and melanogenesis in alpaca melanocytes by regulating Ca^{2+} influx via *TRPM1* channels. *Cell Biochemistry and Functions, 38(3),* 275-282. doi: 10.1002/cbf.3465

Jiang., Y, Zhang, S., Xu, J., Feng, J. Mahboob, S., Al-Ghanim, K.A.,… Xu, P. (2014). Comparative transcriptome analysis reveals the genetic basis of skin color variation in common carp. *PLoS One, 9(9),* e108200. doi: 10.1371/journal.pone.0108200

Jin, Y., Andersen, G., Yorgov, D., Ferrara, T. M., Ben, S., Brownson, K. M., … Spritz, R. A. (2016). Genome-wide association studies of autoimmune vitiligo identify 23 new risk loci and highlight key pathways and regulatory variants. *Nature Genetics, 48(11),* 1418-1424. doi: 10.1038/ng.3680

Jombart, T., Pontier, D., & Dufour, A. B. (2009). Genetic markers in the playground of multivariate analysis. *Heredity, 102(4),* 330-341. doi: 10.1038/hdy.2008.130

Jones, F. C., Grabherr, M. G., Chan, Y. F., Russell, P., Mauceli, E., Johnson, J., … Kingsley, D. M. (2012). The genomic basis of adaptive evolution in threespine sticklebacks. *Nature, 484,* 55-61. doi: 10.1038/nature10944

Jones, P., Lucock, M., Veysey, M., Jablonski, N., Chaplin, G., & Beckett, E. (2018). Frequency of folate-related polymorphisms varies by skin pigmentation. *American Journal of Human Biology, 30(2),* 10.1002/ajhb.23079. doi: 10.1002/ajhb.23079

Jørgensen, K. M., Solberg, M. F., Besnier, F., Thorsen, A., Fjelldal, P. J., Skaala, Ø., ..., Glover, K. A. (2018). Judging a salmon by its spots: environmental variation is the primary determinant of spot patterns in *Salmo salar*. *BMC Ecology, 18(1),* 14. doi: 10.1186/s12898-018-0170-3

Kang, X., Liu, G., Liu, Y., Xu, Q., Zhang, M., & Fang, M. (2013). Transcriptome profile at different physiological stages reveals potential mode for curly fleece in Chinese tan sheep. *PLoS One, 8(8),* e71763. doi: 10.1371/journal.pone.0071763

Kardos, M., Husby, A., McFarlane, S. E., Qvarnström, A., & Ellegren, H. (2016). Whole-genome resequencing of extreme phenotypes in collared flycatchers highlights the difficulty of detecting quantitative trait loci in natural populations. *Molecular Ecology Resources, 16(3),* 727-741. doi: 10.1111/1755-0998.12498

Kelsh, R. N., Inoue, C., Momoi, A., Kondo, H., Furutani-Seiki, M., Ozato, K., & Wakamatsu, Y. (2004). The Tomita collection of medaka pigmentation mutants as a resource for understanding neural crest cell development. *Mechanisms of Development, 121(7-8),* 841-859. doi: 10.1016/j.mod.2004.01.004

Kelsh, R. N., Harris, M. L., Colanesi, S., & Erickson, C. A. (2009). Stripes and belly-spots -- a review of pigment cell morphogenesis in vertebrates. *Seminars in Cell and Developmental Biology, 20(1),* 90-104. doi: 10.1016/j.semcdb.2008.10.001

Kido, K., Sumimoto, H., Asada, S., Okada, S. M., Yaguchi, T., Kawamura, N., … Kaxakami, Y. (2009). Simultaneous suppression of MITF and $BRAF^{V600E}$ enhanced inhibition of melanoma cell proliferation. *Cancer Science, 100(10),* 1863-1869. doi: 10.1111/j.1349-7006.2009.01266.x

Kimura, T., Nagao, Y., Hashimoto, H., Yamamoto-Shiraishi, Y., Yamamoto, S., Yabe, T., … Naruse, K. (2014). Leucophores are similar to xanthophores in their specification and differentiation processes in medaka. *Proceedings of National Academy of Sciences of the USA, 111(20),* 7343-7348. doi: 10.1073/pnas.1311254111

Kimura, T., Takehana, Y., & Naruse, K. (2017). *pnp4a* is the causal gene of the medaka iridophore mutant *guanineless*. *G3* 7(4): 1357-1363. doi:10.1534/g3.117.040675

Kirczuk, L., & Domagała, J. (2012). Colouration of reciprocal hybrids of salmon (*Salmo salar* L., 1758) and sea trout (*Salmo trutta m.* trutta L, 1758) aged 0+ and 1+ in the natural conditions. *Electronic Journal of Polish Agricultural University, 15(1),* 02. available online: http://www.ejpau.media.pl/volume15/issue1/art-02.html

Kittilsen, S., Schjolden, J., Beitnes-Johansen, I., Shaw, J. C., Pottinger, T. G., Sørensen, C., … Øverli Ø. (2009). Melanin-based skin spots reflect stress responsiveness in salmonid fish. *Hormones and Behavior, 56,* 292–298. doi: 10.1016/j.yhbeh.2009.06.006

Klotz, B., Kneitz, S., Regensburger, M., Hahn, L., Dannemann, M., Kelso, J., … Schartl, M. (2018). Expression signatures of early-stage and advanced medaka melanomas. *Comparative Biochemistry and Physiology C: Toxicology and Pharmacology, 208,* 20-28. doi: 10.1016/j.cbpc.2017.11.005

Kocabaş, M., Kayim, M., Can, E., Ateş, M., Kutluyer F., & Aksu, Ö. (2011). Spotting pattern features in the brown trout (*Salmo trutta macrostigma,* T., 1954) population. *Scientific Research and Essays, 6(23),* 5021-5024.

Kocabaş, M., Kutluyer F., & Başçinar N. (2018). Phenotypic differentiation analysis: A case study in hybridizing Çoruh trout (*Salmo coruhensis*), Rize trout (*Salmo rizeensis*) and brown trout (*Salmo trutta fario*). *Acta Zoologica, 99(3),* 274-280. doi: 10.1111/azo.12211

Kon, T., Omori, Y., Fukuta, K., Wada, H., Watanabe, M., Chen, Z., … Furukawa, T. (2020). The genetic basis of morphological diversity in domesticated goldfish. *Current Biology, 30(12),* 2260-2274.e6. doi:10.1016/j.cub.2020.04.034

Kottler, V. A., & Schartl, M. (2018). The colorful sex chromosomes of teleost fish. *Genes, 9(5),* 233. doi: 10.3390/genes9050233

Kratochwil, C. F., Liang, Y., Gerwin, J., Woltering, J. M., Urban, S., Henning, F., … Meyer, A. (2018). Agouti-related peptide 2 facilitates convergent evolution of stripe patterns across cichlid fish radiations. *Science, 362,* 457-460. doi: 10.1126/science.aao6809

Kronenberger, J. A., Gerberich, J. C., Fitzpatrick, S. W., Broder, E. D., Angeloni, L. M., & Funk, W. C. (2018). An experimental test of alternative population augmentation scenarios. *Conservation Biology, 32(4),* 838-848. doi: 10.1111/cobi.13076

Lamason, R. L., Mohideen, M. A., Mest, J. R., Wong, A. C., Norton, H. R., Aros, M. C., … Cheng, K. C. (2005). *SLC24A5,* a putative cation exchanger, affects pigmentation in zebrafish and humans. *Science, 310,* 1782-1786. doi: 10.1126/science.1116238

Largiadèr, C. R., & Scholl, A. (1996). Genetic introgression between native and introduced brown trout *Salmo trutta* L. populations in the Rhône River Basin. *Molecular Ecology, 5,* 417–426. doi: 10.1046/j.1365-294X.1996.00099.x

Larter, M., Dunbar-Wallis, A., Berardi, A .E., & Smith, S. D. (2018). Convergent evolution at the pathway level: predictable regulatory changes during flower color transitions. *Molecular Biology and Evolution, 35(9),* 2159-2169. doi: 10.1093/molbev/msy117

Lascaux, J-M. (1996). *Analyse de la variabilité morphologique de la truite commune (Salmo trutta L.) dans les cours d'eau du bassin pyrénéen méditerranéen* (Unpublished doctoral dissertation). Ecole Nationale Supérieure Agronomique de Toulouse, France.

Leclercq, E., Taylor, J. F., & Migaud, H. (2010). Morphological skin colour changes in teleosts. *Fish and Fisheries, 11,* 159–193. doi: 10.1111/j.1467-2979.2009.00346.x

Lee, J. L., & Streuli, C. H. (2014). Integrins and epithelial cell polarity. *Journal of Cell Science, 127(Pt 15),* 3217-3225. doi: 10.1242/jcs.146142

Legendre, P., & Legendre, L. (2012). *Numerical Ecology, 3rd Edition*. Amsterdam: Elsevier.

Leitwein, M., Gagnaire, P-A., Desmarais, E., Guendouz, S., Rohmer, M., Berrebi, P., & Guinand B. (2016). Genome-wide nucleotide diversity of hatchery-reared Atlantic and Mediterranean strains of brown trout *Salmo trutta* compared to wild Mediterranean populations. *Journal of Fish Biology, 89(6),* 2717-2734. doi: 10.1111/jfb.13131

Leitwein, M., Guinand, B., Pouzadoux, J., Desmarais, E., Berrebi, P., Gagnaire, P-A. (2017). A dense brown trout (*Salmo trutta*) linkage map reveals recent chromosomal rearrangements in the *Salmo* genus and the impact of selection on linked neutral diversity. *G3, 7(4),* 1365-1376. doi:10.1534/g3.116.038497

Leitwein, M., Gagnaire, P-A., Desmarais, E., Berrebi, P., & Guinand B. (2018). Genomic consequences of a recent three-way admixture in supplemented wild brown trout populations revealed by local ancestry tracts. *Molecular Ecology* 27(17): 3466-3483. doi: 10.1111/mec.14816

Levy, C., Khaled, M., & Fisher, D. E. (2006). *MITF*: master regulator of melanocyte development and melanoma oncogene. *Trends in Molecular Medicine, 12(9),* 406-414. doi: 10.1016/j.molmed.2006.07.008

Lewis, V. M., Saunders, L. M., Larson, T. A., Bain, E. J., Sturiale, S. L., Gur, D., … Parichy, D. M.. (2019). Fate plasticity and reprogramming in genetically distinct populations of *Danio* leucophores. *Proceedings of the National Academy of Sciences of the USA, 116(24),* 11806-11811. doi: 10.1073/pnas.1901021116

Li, Z., Kemppainen, P., Rastas, P., & Merilä, J. (2018). Linkage disequilibrium clustering-based approach for association mapping with tightly linked genomewide data. *Molecular Ecology Resources, 18(4),* 809-824. doi: 10.1111/1755-0998.12893

Liang, Y., Gerwin, J., Meyer, A., & Kratochwil, C. F. (2020). Developmental and cellular basis of vertical bar color patterns in the East African cichlid fish *Haplochromis latifasciatus*. *Frontiers in Cell and Developmental Biology, 8,* 62. doi: 10.3389/fcell.2020.00062

Lien, S., Koop, B. F., Sandve, S. R., Miller, J. R., Kent, M. P., Nome, T., ... Davidson, W. S. (2016). The Atlantic salmon genome provides insights into rediploidization. *Nature, 533,* 200-205. doi: 10.1038/nature17164

Lindtke, D., Lucek, K., Soria-Carrasco, V., Villoutreix, R., Farkas, T. E., Riesch, R., … Nosil, P. (2017). Long-term balancing selection on chromosomal variants associated with crypsis in a stick insect. *Molecular Ecol*ogy, *26(22),* 6189-6205. doi:10.1111/mec.14280

Lorenzoni, M., Carosi, A., Giovannotti, M., La Porta, G., Splendiani, A., & Caputo Barucchi, V. (2019). Morphological survey as powerful detection tool of pure and local phenotypes in *Salmo trutta* complex. *Knowledge and Management of Aquatic Ecosystems, 420,* 48. doi: 10.1051/kmae/2019041

Lorin, T., Brunet, F. G., Laudet, V., & Volff, J-N. (2018). Teleost fish-specific preferential retention of pigmentation gene-containing families after whole genome duplications in vertebrates. *G3, 8(5),* 1795-1806. doi: 10.1534/g3.118.200201

Lotterhos, K. E. (2019). The effect of neutral recombination variation on genome scans for selection. *G3, 9(6),* 1851-1867. doi: 10.1534/g3.119.400088

Luo, M., Wang, L., Yin, H., Zhu, W., Fu, J., & Dong, Z. (2019). Integrated analysis of long non-coding RNA and mRNA expression in different colored skin of koi carp. *BMC Genomics, 20(1),* 515. doi: 10.1186/s12864-019-5894-8

Maan, M. E., & Sefc, K. M. (2013). Colour variation in cichlid fish: developmental mechanisms, selective pressures and evolutionary consequences. *Seminars in Cell and Developmental Biology, 24(6-7),* 516-528. doi: 10.1016/j.semcdb.2013.05.003

Magalhaes, I. S., & Seehausen, O. (2010). Genetics of male nuptial colour divergence between sympatric sister species of a Lake Victoria cichlid fish. *Journal of Evolutionary Biology, 23(5),* 914-924. doi: 10.1111/j.1420-9101.2010.01960.x

Mahalwar, P., Walderich, B., Singh, A. P., & Nüsslein-Volhard, C. (2014). Local reorganization of xanthophores fine-tunes and colors the striped pattern of zebrafish. *Science, 345,* 1362-1364. doi: 10.1126/science.1254837

Mahalwar, P., Singh, A. P., Fadeev, A., Nüsslein-Volhard, C., & Irion, U. (2016). Heterotypic interactions regulate cell shape and density during color pattern formation in zebrafish. *Biology Open, 5(11),* 1680-1690. doi: 10.1242/bio.022251

Malek, T. B., Boughman, J. W., Dworkin, I., & Peichel, C. L. (2012). Admixture mapping of male nuptial colour and body shape in a recently formed hybrid population of threespine stickleback. *Molecular Ecology, 21(21),* 5265-5279. doi: 10.1111/j.1365-294X.2012.05660.x

Marie-Orléach, L., Roussel, J-M., Bugeon, J., Tremblay, J., Ombredane, D., & Evanno, G. (2014). Melanin-based coloration of sneaker male Atlantic salmon is linked to viability and emergence timing of their offspring. *Biological Journal of the Linnean Society, 111(1),* 126-135. doi: 10.1111/bij.12187

Marin, K., Coon, A., & Fraser, D. J. (2017). Traditional ecological knowledge reveals the extent of sympatric lake trout diversity and habitat preferences. *Ecology and Society, 22(2),* 20. doi:10.5751/ES-09345-220220

Meier, J. I., Marques, D. A., Wagner, C. E., Excoffier, L., Seehausen, O. (2018). Genomics of parallel ecological speciation in Lake Victoria cichlids. *Molecular Biology and Evolution, 35(6),* 1489-1506. doi: 10.1093/molbev/msy051

Mezzera, M., Largiadèr, C. R., & Scholl A. (1997). Discrimination of native and introduced brown trout in the River Doubs (Rhône drainage) by number and shape of parr marks. *Journal of Fish Biology, 50,* 672-677. doi: 10.1111/j.1095-8649.1997.tb01959.x

Miller, C. T., Beleza, S., Pollen, A. A., Schluter, D., Kittles, R. A., Shriver, M. D., & Kingsley, D. M. (2007). *Cis*-regulatory changes in *Kit ligand* expression and parallel evolution of pigmentation in sticklebacks and humans. *Cell, 131(6),* 1179-1189. doi: 10.1016/j.cell.2007.10.055

Miyamoto, K. (2016). Effects of body color luminance and behavioral characteristics on predation risk in salmonid fishes. *Hydrobiologia, 783,* 249–256. doi: 10.1007/s10750-015-2573-x

Miyazawa, S., Okamoto, M., & Kondo, S. (2010). Blending of animal colour patterns by hybridization. *Nature Communications, 1(6),* 66. doi: 10.1038/ncomms1071

Nagao, Y., Takada, H., Miyadai, M., Adachi, T., Seki, R., Kamei, Y., … Hashimoyo, H. (2018). Distinct interactions of *Sox5* and *Sox10* in fate specification of pigment cells in medaka and zebrafish. *PLoS Genetics, 14(4),* e1007260. doi: 10.1371/journal.pgen.1007260

Nazari-Ghadikolaei, A., Mehrabani-Yeganeh, H., Miarei-Aashtiani, S.R., Staiger, E.A., Rashidi, A., & Huson H. J. (2018). Genome-wide association studies identify candidate genes for coat color and mohair traits in the Iranian Markhoz goat. *Frontiers in Genetics, 9,* 105. doi: 10.3389/fgene.2018.00105

Neethiraj, R., Hornett, E.A., Hill, J.A., & Wheat, C.W. (2017). Investigating the genomic basis of discrete phenotypes using a Pool-Seq-only approach: new insights into the genetics underlying colour variation in diverse taxa. *Molecular Ecology, 26(19),* 4990-5002. doi: 10.1111/mec.14205

Nilsson, J., Backström, T., Stien, L. H., Carlberg, H. Jeuthe, H., Magnhagen, C., & Brännäs, E. (2016). Effects of age and rearing environment on genetic parameters of growth and body weight and heritability of skin pigmentation in Arctic charr (*Salvelinus alpinus* L.). *Aquaculture, 453,* 67-72. doi: 10.1016/j.aquaculture.2015.11.035

Nüsslein-Volhard, C., & Singh, A. P. (2017). How fish color their skin: A paradigm for development and evolution of adult patterns: multipotency, plasticity, and cell competition regulate proliferation and spreading of pigment cells in zebrafish coloration. *Bioessays, 39(3),* 10.1002/bies.201600231. doi:10.1002/bies.201600231

O'Connor, K. I., Metcalfe, N. B., & Taylor, A. C. (2000). Familiarity influences body darkening in territorial disputes between juvenile salmon. *Animal Behaviour, 59(6),* 1095-1101. doi: 10.1006/anbe.2000.1401

Ohtsuka, M., Kikuchi, N., Yokoi, H., Kinoshita, M., Wakamatsu, Y., Ozato, K., … Kimura, M. (2004). Possible roles of *zic1* and *zic4*, identified within the medaka Double anal fin (Da) locus, in dorsoventral patterning of the trunk-tail region (related to phenotypes of the Da mutant). *Mechanisms of Development, 121(7-8),* 873-882. doi: 10.1016/j.mod.2004.04.006

Ordway, A.J., Hancuch, K. N., Johnson, W., Wiliams, T. M., & Rebeiz, M. (2014). The expansion of body coloration involves coordinated evolution in *cis* and *trans* within the pigmentation regulatory network of *Drosophila prostipennis*. *Developmental Biology, 392,* 431-440. doi: 10.1016/j.ydbio.2014.05.023

O'Quin, C. T., Drilea, A. C., Conte, M. A., & Kocher T. D. (2013). Mapping of pigmentation QTL on an anchored genome assembly of the cichlid fish, *Metriaclima zebra*. *BMC Genomics, 14,* 287. doi: 10.1186/1471-2164-14-287

O'Reilly, P. F., Hoggart, C. J., Pomyen, Y., Calboli, F. C. F., Elliott, P., Jarvelin, M-R., & Coin, L. J. M. (2012). MultiPhen: joint model of multiple phenotypes can increase discovery in GWAS. *PLoS One, 7(5),* e34861. doi: 10.1371/journal.pone.0034861

Orteu, A., & Jiggins, C. D. (2020). The genomics of coloration provides insights into adaptive evolution. *Nature Reviews Genetics, 21,* 461-475. doi:10.1038/s41576-020-0234-z

Özsu, N., & Monteiro, A. (2017). Wound healing, calcium signaling, and other novel pathways are associated with the formation of butterfly eyespots. *BMC Genomics, 18(1),* 788. doi: 10.1186/s12864-017-4175-7

Pallares, L. F., Harr, B., Turner, L. M., & Tautz, D. (2014). Use of a natural hybrid zone for genomewide association mapping of craniofacial traits in the house mouse. *Molecular Ecology, 23(23),* 5756-5770. doi: 10.1111/mec.12968

Palm, S., Laikre, L., Jorde, P.E., & Ryman, N. (2003). Effective population size and temporal genetic change in resident brown trout (*Salmo trutta* L.). *Conservation Genetics, 4(3),* 249-264. doi: 10.1023/A:102406913094

Parichy, D. M. 2006. Evolution of danio pigment pattern development. *Heredity, 97(3),* 200-210. doi: 10.1038/sj.hdy.6800867

Parichy, D. M., & Spiewak, J. E. (2015). Origins of adult pigmentation: diversity in pigment stem cell lineages and implications for pattern evolution. *Pigment Cell and Melanoma Research, 28(1),* 31-50. doi: 10.1111/pcmr.12332

Parolini, M., Iacobuzio, R., Bassano, B., Pennati, R., & Saino, N. (2018). Melanin-based skin coloration predicts antioxidant capacity in the brown trout (*Salmo trutta*). *Physiological and Biochemical Zoology, 91(5),*1026-1035. doi:10.1086/699522

Patton, E.E., Mitchell, D.L, & Nairn, R.S. (2010). Genetic and environmental melanoma models in fish. *Pigment Cell and Melanoma Research, 23(3),* 314-337. doi: 10.1111/j.1755-148X.2010.00693.x

Pavan, W.J., & Sturm, R.A. (2019). The genetics of human skin and hair pigmentation. *Annual Review in Genomics and Human Genetics, 20,* 41-72. doi: 10.1146/annurev-genom-083118-015230

Peichel, C. L., & Marques, D. A. (2017). The genetic and molecular architecture of phenotypic diversity in sticklebacks. *Philosophical Transactions of the Royal Society of London B: Biological Sciences, 372(1713),* 20150486. doi: 10.1098/rstb.2015.0486

Porter, H. F., & O'Reilly, P. F. (2017). Multivariate simulation framework reveals performance of multi-trait GWAS methods. *Scientific Reports, 7,* 38837. doi: 10.1038/srep38837

Poteaux, C., Berrebi, P. (1997). Intégrité génomique et repeuplements chez la truite commune du bassin méditerranéen. *Bulletin Français de la Pêche et de la Pisciculture, 344/345,* 309–322. doi : 10.1051/kmae:1997031

Poteaux, C., Bonhomme, F., & Berrebi, P. (1998). Differences between nuclear and mitochondrial introgressions of brown trout populations from a restocked main river and its unrestocked tributary. *Biological Journal of the Linnean Society, 63,* 379–392. doi: 10.1111/j.1095-8312.1998.tb01524.x

Protas, M., Conrad, M., Gross, J. B., Tabin, C., & Borowsky, R. (2007). Regressive evolution in the Mexican cave tetra, *Astyanax mexicanus*. *Current Biology, 17(5),* 452-454. doi: 10.1016/j.cub.2007.01.05

Protas, M. E., Patel, N. H. (2008). Evolution of coloration patterns. *Annual Review in Cell and Developmental Biology, 24,* 425-446. doi: 10.1146/annurev.cellbio.24.110707.175302

Rammah, C., Gadin, S., Cormier, S., Souilhol, C., Cohen-Tannoudji, M., Panthier, J-J., & Aubin-Houzelstein, G. (2009). Implication of notchless and strawberry notch homolog 2 genes in melanocyte stem cells homeostasis. *Pigment Cell and Melanoma Research, 22,* 671. doi: 10.1111/j.1755-148X.2009.00612.x 652 a

Raposo, G., & Marks, M. S. (2007). Melanosomes - dark organelles enlighten endosomal membrane transport. *Nature Reviews in Molecular and Cell Biology, 8(10),* 786-797. doi: 10.1038/nrm2258

Rivera-Colón, A. G., Westerman, E. L., Van Belleghem, S. M., Monteiro, A., & Papa, R. (2020). Multiple loci control eyespot number variation on the hindwings of *Bicyclus anynana* butterflies. *Genetics, 214(4),* 1059-1078. doi: 10.1534/genetics.120.303059

Roberts, R. B., Moore, E. C., & Kocher, T. D. (2017). An allelic series at *pax7a* is associated with colour polymorphism diversity in Lake Malawi cichlid fish. *Molecular Ecology, 26(10),* 2625-2639. doi: 10.1111/mec.13975

Roberts, R. B., Ser, J. R., & Kocher, T. D. (2009). Sexual conflict resolved by invasion of a novel sex determiner in Lake Malawi cichlid fishes. *Science, 326,* 998-1001. doi: 10.1126/science.1174705

Rockman, M. V. (2012). The QTN program and the alleles that matter for evolution: all that's gold does not glitter. *Evolution*. *66(1),* 1-17. doi:10.1111/j.1558-5646.2011.01486.x

Salis, P., Roux, N., Soulat, O., Lecchini, D., Laudet, V., & Frédérich, B. (2018). Ontogenetic and phylogenetic simplification during white stripe evolution in clownfishes. *BMC Biology, 16(1),* 90. doi:10.1186/s12915-018-0559-7

San-José, L. M., & Roulin, A. (2017). Genomics of coloration in natural animal populations. *Philosophical Transactions of the Royal Society of London B: Biological Sciences, 372(1724),* 20160337. doi: 10.1098/rstb.2016.0337

Santos, M. E., Braasch, I., Boileau, N., Meyer, B. S., Sauteur, L., Böhne, A., … Salzburger,W. (2014). The evolution of cichlid fish egg-spots is linked with a *cis*-regulatory change. *Nature Communications, 5,* 5149. doi: 10.1038/ncomms6149

Santure, A. W., & Garant, D. (2018). Wild GWAS-association mapping in natural populations. *Molecular Ecology Resources, 18(4),* 729-738. doi: 10.1111/1755-0998.12901

Sanz, N. (2018). Phylogeographic history of brown trout: a review. In: J. Lobón-Cerviá & N. Sanz (Eds.), *Brown Trout: Biology, Ecology and Management.* Pp. 17-63. Oxford: Wiley Ltd.

Schielzeth, H., & Husby, A. (2014). Challenges and prospects in genome-wide quantitative trait loci mapping of standing genetic variation in natural populations. *Annals of the New York Academy of Sciences, 1320,* 35-57. doi: 10.1111/nyas.12397

Schonthaler, H. B., Lampert, J. M., von Lintig, J., Schwarz, H., Geisler, R., & Neuhauss, S. C. (2005). A mutation in the silver gene leads to defects in melanosome biogenesis and alterations in the visual system in the zebrafish mutant fading vision. *Developmental Biology, 284(2),* 421-436. doi: 10.1016/j.ydbio.2005.06.001

Schweizer, R.M., Durvasula, A., Smith, J., Vohr, S.H., Stahler, D.R, Galaverni, M., … Wayne, R.K. (2018). Natural selection and origin of a melanistic allele in North American gray wolves. *Molecular Biology and Evolution, 35(5),* 1190-1209. doi:10.1093/molbev/msy031

Sefc, K. M., Mattersdorfer, K., Ziegelbecker, A., Neuhüttler, N., Steiner, O., Goessler, W., & Koblmüller, S. (2017). Shifting barriers and phenotypic diversification by hybridisation. *Ecology Letters, 20(5),* 651-662. doi: 10.1111/ele.12766

Sevane, N., Sanz, C.R., & Dunner, S. (2019). Explicit evidence for a missense mutation in exon 4 of *SLC45A2* gene causing the pearl coat dilution in horses. *Animal Genetics, 50(3),* 275-278. doi: 10.1111/age.12784

She, R., & Jarosz, D.F. (2018). Mapping causal variants with single-nucleotide resolution reveals biochemical drivers of phenotypic change. *Cell, 172(3),* 478-490.e15. doi: 10.1016/j.cell.2017.12.015

Schouwey, K., & Beermann, F. (2008). The Notch pathway: hair graying and pigment cell homeostasis. *Histology and Histopathology, 23(5),* 609-619. doi: 10.14670/HH-23.609

Shriner, D. (2017). Overview of admixture mapping. *Current Protocols in Human Genetics, 94,* 1.23.1-1.23.8. doi: 10.1002/cphg.44

Shriver, M. D., Parra, E.J., Dios, S., Bonilla, C., Norton, H., Jovel, C., ... Kittles, R. A. (2003). Skin pigmentation, biogeographical ancestry and admixture mapping. *Human Genetics, 112(4),* 387-399. doi: 10.1007/s00439-002-0896-y

Sinclair-Waters, M., Ødegård, J., Korsvoll, S. A., Moen, T., Lien, S., Primmer, C. R., & Barson, N. J. (2020). Beyond large-effect loci: large-scale GWAS reveals a mixed large-effect and polygenic architecture for age at maturity of Atlantic salmon. *Genetics Selection Evolution, 52(1),* 9 doi: 10.1186/s12711-020-0529-8

Singh, A. P., & Nüsslein-Volhard, C. (2015). Zebrafish stripes as a model for vertebrate colour pattern formation. *Current Biology, 25(2),* R81-R92. doi: 10.1016/j.cub.2014.11.013

Singh, S. K., Abbas, W. A., & Tobin, D. J. (2012). Bone morphogenetic proteins differentially regulate pigmentation in human skin cells. *Journal of Cell Science, 125(18),* 4306-4319. doi10.1242/jcs.102038

Sivka, U., Snoj, A., Palandačić, A., & Sušnik Bajec, S. (2013). Identification of candidate genes involved in marble color pattern formation in genus *Salmo*. *Comparative Biochemistry and Physiology D: Genomics and Proteomics, 8(3),* 244-249. doi: 10.1016/j.cbd.2013.06.003

Skaala, Ø., & Jørstad, K. E. (1988). Inheritance of the fine-spotted pigmentation pattern of brown trout. *Polskie Archiwum Hydrobiologii, 35,* 295–304.

Soong, J., & Scott, G. (2013). *Plexin B1* inhibits MET through direct association and regulates *Shp2* expression in melanocytes. *Journal of Cell Science, 126(Pt 2),* 688-695. doi: 10.1242/jcs.119487

Soong, J., Chen, Y., Shustef, E. M., & Scott, G. A. (2012). *Sema4D,* the ligand for *Plexin B1,* suppresses c-Met activation and migration and promotes melanocyte survival and growth. *Journal of Investigative Dermatology, 132(4),* 1230-1238. doi: 10.1038/jid.2011.414

Talbot, B., Chen, T. W., Zimmerman, S., Joost, S., Eckert, E. J., Crow, T. M., … Manel, S. (2017). Combining genotype, phenotype, and environment to infer potential candidate genes. *Journal of Heredity, 108(2),* 207-216. doi: 10.1093/jhered/esw077

Takeda, H., & Shimada, A. (2010). The art of medaka genetics and genomics: what makes them so unique? *Annual Review of Genetics, 44,* 217-241. doi: 10.1146/annurev-genet-051710-151001

Toews, D. P. L., Taylor, S. A., Vallender, R., Brelsford, A., Butcher, B. G., Messer, P. W., & Lovette I. J. (2016). Plumage genes and little else distinguish the genomes of hybridizing warblers. *Current Biology, 26,* 2313-2318. doi: 10.1016/j.cub.2016.06.034

Toomey, M. B., Marques, C. I., Andrade, P., Araujo, P. M., Sabatino, S., Gazda, M. A., … Carneiro, M. (2018). A non-coding region near *Follistatin* controls head colour polymorphism in the Gouldian finch. *Proceedings: Biological Sciences, 285(1888),* 20181788. doi: 10.1098/rspb.2018.1788

Tripathi, N., Hoffmann, M., Willing, E. M., Lanz, C., Weigel, D., & Dreyer, C. (2009). Genetic linkage map of the guppy, *Poecilia reticulata*, and quantitative trait loci analysis of male size and colour variation. *Proceedings: Biological Sciences, 276(1665),* 2195-2208. doi: 10.1098/rspb.2008.1930

Uong, A., & Zon, L. I. (2010). Melanocytes in development and cancer. *Journal of Cell Physiology, 222(1),* 38-41. doi: 10.1002/jcp.21935

Uy, J. A., Cooper, E. A., Cutie, S., Concannon, M. R., Poelstra, J. W., Moyle R. G., & Filardi, C. E. (2016). Mutations in different pigmentation genes are associated with parallel melanism in island flycatchers. *Proceedings: Biological Sciences, 283(1834),* 20160731. doi: 10.1098/rspb.2016.0731

Van Belleghem, S.M., Papa, R., Ortiz-Zuazaga, H., Hendrickx, K., Jiggins, C.D., McMillan, W.O., & Counterman, B.A. (2018). patternize: An R package for quantifying colour pattern variation. *Methods in Ecology and Evolution* 9(2): 390-398. doi: 10.1111/2041-210X.12853

Vangestel, C., Eckert, A. J., Wegrzyn, J. L., St.Clair, J. B., & Neale, D. B. (2018). Linking phenotype, genotype and environment to unravel genetic components underlying cold hardiness in coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*). *Tree Genetics and Genomes, 14,* 10. doi: 10.1007/s11295-017-1225-x

Volkening, A. (2020). Linking genotype, cell behavior, and phenotype: multidisciplinary perspectives with a basis in zebrafish patterns. *Current Opinion in Genetics and Development, 63,* 78-85. doi:10.1016/j.gde.2020.05.010

Wade, N., Bryant, N.J., Connolly, L. M., Simpson R. J., Luzio, J. P., … James, D. E.. (2001). *Syntaxin 7* complexes with mouse *Vps10p tail interactor 1b, syntaxin 6, vesicle-associated membrane protein (VAMP)8,*

and *VAMP7* in b16 melanoma cells. *Journal of Biological Chemistry, 276(23),* 19820-19827. doi: 10.1074/jbc.M010838200

Wang, L. M., Bu, H. Y., Song, F. B., Zhu, W. B., Fu, J. J., & Dong, Z. J. (2019). Characterization and functional analysis of *slc7a11* gene, involved in skin color differentiation in the red tilapia. *Comparative Biochemistry and Physiology A: Molecular and Integrative Physiology, 236,* 110529. doi: 10.1016/j.cbpa.2019.110529

Wang, Z., Jinnin, M., Kobayashi, Y., Kudo, H., Inoue, K., Nakayama, W., … Ihn, H. (2015). Mice overexpressing integrin *αv* in fibroblasts exhibit dermal thinning of the skin. *Journal of Dermatological Science, 79(3),* 268-278. doi: 10.1016/j.jdermsci.2015.06.008

Waples, R. S., Naish, K. A., & Primmer, C. R. (2020). Conservation and management of salmon in the age of genomics. *Annual Review of Animal Biosciences, 8,* 117-143. doi: 10.1146/annurev-animal-021419-083617

Watt, C., Swanson, C., Miller, D., Chen, M. & May, C. (2017). Social hierarchies override environmental conditions in determining body coloration of brook trout. *Journal of Freshwater Ecology, 32(1),* 575-580. doi: 10.1080/02705060.2017.1360218

Wedekind, C., Jacob, A., Evanno, G., Nusslé, S, & Müller, R. (2008). Viability of brown trout embryos positively linked to melanin-based but negatively to carotenoid-based colours of their fathers. *Proceedings: Biological Sciences, 275(1644),* 1737-1744. doi: 10.1098/rspb.2008.0072

Westley, P. A., Stanley, R., & Fleming, I. A. (2013). Experimental tests for heritable morphological color plasticity in non-native brown trout (*Salmo trutta*) populations. *PLoS One, 8(11),* e80401. doi: 10.1371/journal.pone.0080401

Williams, J. S., Hsu, J. Y., Rossi, C. C., & Artinger, K. B. (2018). Requirement of zebrafish *pcdh10a* and *pcdh10b* in melanocyte precursor migration. *Developmental Biology, 444(Suppl. 1),* S274-S286. doi: 10.1016/j.ydbio.2018.03.022

Xu, P., Zhang, X., Wang, X., Li, J., Liu, G., Kuang, Y., … Sun, X. (2014). Genome sequence and genetic diversity of the common carp, *Cyprinus carpio*. *Nature Genetics, 46(11),* 1212-1219. doi: 10.1038/ng.3098

Xu, X., Dong, G. X., Hu, X.S., Miao, L. Zhang, X. L., Zhang D. L., … Luo, S. J. (2013). The genetic basis of white tigers. *Current Biology, 23(11),* 1031-1035. doi: 10.1016/j.cub.2013.04.054

Yamaguchi, Y., Brenner, M., & Hearing, V. J. (2007). The regulation of skin pigmentation. *Journal of Biological Chemistry,* 282(38), 27557-27561. doi: 10.1074/jbc.R700026200

Yamazaki, F. (1974). On the so-called "cobalt" variant of rainbow trout. *Bulletin of the Japanese Society of Sciences and Fisheries, 40,* 17–25. doi: 10.2331/suisan.40.17

Yano, A., Nicol, B., Jouanno, E., Quillet E., Fostier A. Guyomard R., & Guiguen, Y. (2013). The sexually dimorphic on the Y-chromosome gene (*sdY*) is a conserved male-specific Y-chromosome sequence in many salmonids. *Evolutionary Applications, 6(3),* 486-496. doi: 10.1111/eva.12032

Yong, L., Peichel, C. L., & McKinnon, J. S. (2015). Genetic architecture of conspicuous red ornaments in female threespine stickleback. *G3, 6(3),* 579-588. doi: 10.1534/g3.115.024505

Zastavniouk, C., Weir, L. K., & Fraser, D. J. (2017). The evolutionary consequences of habitat fragmentation: body morphology and coloration differentiation among brook trout populations of varying size. *Ecology and Evolution, 7(17),* 6850-6862. doi: 10.1002/ece3.3229

Zhu, W., Wang, L., Dong, Z., Chen, X., Song, F., Liu, N., … Fu, J. (2016). Comparative transcriptome analysis identifies candidate genes related to skin color differentiation in red tilapia. *Scientific Reports, 6,* 31347. doi: 10.1038/srep31347

Captions of the Tables

Table 1: Uncorrelated pigmentation variables retained to build the forward selection model in RDA, and also used as a basis to multi-GWAS modelling. The full list of variables is reported in Table S1; correlation among variables in Table S2.

Table 2: List of the most indicative pigmentation- or colour-related genes associated to some CPLs (*N* = 24; 30 distinct loci) found in this study. The method of association that detected them is reported, together with their locations in the Atlantic salmon (*Salmo salar*) genome (Lien et al., 2016) and brown trout (*S. trutta*) high density linkage map of Leitwein et al. (2017). Their position in coding sequence or in a 25kb window downstream or upstream the gene is reported. Significant GO-terms are reported. For each gene, relevant references with link to colour and/or skin features and pigmentation are provided. The full list of CPLs and other relevant information is given in Table S4.

Captions of the Figures

Fig. 1: RDA triplots for significant or nearly significant canonical axes 1 and 2, respectively. Pigmentation-related variables retained by forward modelling (Blanchet et al., 2008) are represented by arrows; description of the retained model in Table S4. Length of arrows is proportional to the strength of correlation of each variable with individual axis. Arrows pointing to different direction indicate negatively correlated variables (*e.g. N.PR.Tot* and the large pre-opercular black stain *Macrost* for RDA axis 1). The percentage of variance associated to each axis is reported, as well as the eigenvalue graph for constrained axis. **(A):** Individual trout are positioned on the map with positioning of SNPs as a grey block to the center of the factor map. Individuals have been coloured according to the nine clusters defined by Leitwein, Gagnaire, Desmarais, Berrebi, & Guinand (2018) (top left); Clusters reflect the origin (hatchery *vs* wild) of trout, but also the degree of admixture of each of them as measured by co-ancestry tracts. **(B):** Zoom on the centre of the map to illustrate the position of SNPs. SNPs departing ± 2.5 standard deviates from the mean either on RDA axis 1 or RDA axis 2 have been coloured; a colour being associated to each pigmentation-related variable included in the model (insert). These SNPs are coloured with the variables they have been found the most significantly associated in the model. SNPs given as grey spots are within the ± 2.5 S.D. interval and not considered in this study. Percentages of variation explained by each RDA axis are reported. Fish are representatives of hatchery Mediterranean (left) and Atlantic (right) individuals used in this study.

Figure 2: Manhattan plots of multi-trait GWAS models selected using the MultiPhen package (O'Reilly et al., 2012), plus results associated to the single-trait GWAS model used in this study. The abscissa axis represents the forty brown trout LGs (Leitwein et al., 2017), the

ordinate axis reports values of $-log_{10}(P)$, with P being the probability of one association between a variable and a SNP (adjusted significance threshold based on permutation, Dudbridge & Gusnanto, 2008). The red line indicates the 5×10^{-8} significance threshold retained in this study. **A:** Results for the multi-trait GWAS for the model using two variables: *N. PN.Tot* and *N.PR.Tot.* The seven SNPs found significant in this model are represented by circles. The results from single trait GWAS have been added to this panel to provide a full summary of results. In this case, approximate position of the RAD-loci associated to the *N. PN.Tot* (17 loci) and *N.PR.Tot* (9 loci) variables are indicated by black and red arrows, respectively. One asterisk indicates two loci to close of each other to be indicated by separate arrows. The locus associated to the *GJD2* gene on LG9 and also detected by the RDA is indicated by a longer arrow. **B:** Multi-trait GWAS based on the variables retained with the RDA except *Zeb* (31 candidate SNPs). The significance with each variable of the model is indicated by a different symbol. Each vertical line of symbols points to a single SNP. Some overlapping is possible. The full list of candidate SNPs detected using single and multi-trait GWAS are reported in Table S4. Variables labelled as in Table 1.

Figure 3: Distribution of F_{ST} values for the 40,519 RAD-loci (blue) and for CPLs found significantly associated with RDA axis 1 (N = 320 loci, reported in red in the insert). For each data set, mean F_{ST} values and confidence intervals are provided as full and hatched lines, respectively. Mean values for the single- and multi-trait GWAS approaches are illustrated by the green and the orange lines, respectively. No confidence intervals are reported for GWAS because of the low number of CPLs detected with these approaches. Details in the main text.

Figure 4: Positioning of the 300 mapped CPLs detected in this study on the forty LGs of the brown trout. Loci found associated at least one single phenotypic trait are indicted by green,

red and yellow circles when detected with the RDA, single- or multi-GWAS analyses, respectively. Horizontal bars indicate the distribution of the full set of SNPs over LGs. The number of CPLs detected on each LG is indicated. As numerous loci are close to each other, circles may overlap.

Table 1

Phenotypic variables	Description	Nature* (coding)
N. PR. Tot	Total number of red spots over flanks	Q
N. PN. Tot.	Total number of black spots over flanks	Q
Diam. PN	Mean diameter of black spots	Q
Oc. PN	White ring around black spots	SQ (1= no ring; 2 = attenuated/thin ring, 3 = marked/thick ring)
Fr. D	Fringe on the dorsal fin	SQ (1= no fringe; 2 = white fringe, 3 = black & white fringe)
Fr. An	Fringe on the anal fin	SQ (1= no fringe; 2 = white fringe, 3 = black & white fringe)
Fr. P	Fringe on the pelvic fins	SQ (1= no fringe; 2 = white fringe, 3 = black & white fringe)
L. Lat	Visibility of the lateral line	SQ (1 = not visible; 2 = partially visible; 3 = fully visible)
Parr	Presence of parr marks	SQ (1 = absence; 2 = presence)
Macrost.	Macrostigma spot (large black dot on the pre-opercular bone)	SQ (1 = absence; 2 = partially visible/diluted; 3 = fully visible)
Zeb	Zebras on flank	SQ (1 = absence; 2 = presence)

* : Q = quantitative variable; SQ = semi-quantitative variable (coding in brackets)

Table 2

Method of Association	LG - *S. trutta*	LG - *S. salar*	Position on *S. salar* genome (pb)	Position of the 'color-associated' locus (coding vs upstream/downstream) and distance to the nearest gene (in bp).	Protein Name (or lncRNA or pseudogene)	Gene Id	GO terms - Molecular Functions	GO terms - Biological processes	Associated reference(s)	Note
RDA	1	29	16654071	Downstream 1947 bp	notchless protein homolog 1	*NLE1*			Schouwey and Beermann (2008) Rammah et al (2009)	Notch signalling is essential in some pigmentation and epidermal differentiation (e.g. .)
Single_GWAS_N.PR.Tot	1	20	31985710	coding	methionine synthase reductase-like	*MTRR*	FMN binding, oxidoreductase activity	oxidation reduction process	Jones et al. (2018)	Folate-related genes (UV radiation sensitive) associated to skin pigmentation
MultGWAS_7	1	29	34327580	coding	netrin-G1-like	*NTNG1*			Kang et al. (2013) da Silva Romero et al. (2018)	Coat color variation
MultGWAS_2	4	20	17551935	Downstream 2871 bp	P2Y purinoceptor 2-like	*P2RY2*	signal transducer activity, G-protein coupled receptor activity	signal transduction, G-protein coupled receptor signaling pathway	Ganesan et al. (2008) Jin et al. (2014)	Impact melanogenesis
MultGWAS_7	6	12	15186741	Downstream 1234 bp	transcription factor SOX-10	*SOX10*	DNA binding	Upstream 4864 pb de UPF0193 protein EVG1	Nüsslein-Vollard and Singh (2017) Mahalwar et al. (2014) Zhu et al. (2016)	Essential to pigmentation patterning in fish
Single_GWAS_N.PN.Tot	9	1	17060287	Upstream 24905 bp	shroom in melanosome 1 protein-like	*SLC45A2*			Belleno & Omacea (2014) Zhu et al. (2016) Sevane et al. (2019)	Ion transport in melanosomes
-	9	1	17132468	coding	-	*SLC45A2*			-	-
RDA	9	1	29826721	Upstream 12812 bp	Bone morphogenetic protein 4	*BMP4*	growth factor activity	regulation of signaling receptor activity	Singh et al. (2012) Gramann et al. (2019)	Melanin synthesis, melanocyte development (zebrafish)
RDA and Single_GWAS_N.PN.Tot	9	1	30355164	Upstream 21549 bp	Gap junction delta-2 protein	*GJD2*		action potential, synaptic transmission	Djurdjevic et al. (2019)	Differentially expressed in trout skin
RDA	10	13	98943518	Upstream 14366 bp	semaphorin-4D	*SEMA4D*			Soong et al. (2012)	Melanocyte survival and growth
RDA	11	5	35618944	coding	zinc finger protein ZIC 4	*ZIC4*	nucleic acid binding, DNA binding, metal ion binding	regulation of transcription by RNA polymerase II	Ohtsuka et al. (2004)	Dorso-ventral patterning in medaka
RDA	11	5	41442470	coding	X-linked interleukin-1 receptor accessory protein-like 2	*IL1RAPL1*		signal transduction	Jin et al. (2016)	Role in propagation of vitiligo and hypopigmentation
RDA	16	1	114514891	Upstream 2269 bp	vesicle-associated membrane protein 8-like	*VAMP8*		vesicle-mediated transport	Wade et al. (2001)	Regulate fusion events within the late endosomal pathway, critical role in melanogenesis
MultGWAS_2	17	15	78637963	coding	E3 ubiquitin-protein ligase Itchy-like	*ITCH*	ubiquitin-protein transferase activity, transferase activity, ligase activity, ubiquitin protein ligase activity	ubiquitin-dependent protein catabolic process, protein ubiquitination	Raposo & Marks (2007) Nazari-Ghadikolaei et al. (2019)	Melanocyte-based coat colour variation
Single_GWAS_N.PR.Tot	17	15	87874821	Upstream 11038 bp	melanocyte protein PMEL-like	*PMEL*			Schouthaler et al. (2005) Raposo & Marks (2007) Helbiriian et al. (2011) Luo et al. (2019)	Color variation (silvering, dilution), including zebrafish and koi carp
RDA	22	4	34326580	coding	pro-neuregulin-2 membrane-bound isoform-like	*NRG2*			Kido et al. (2009) Gallagher et al. (2013)	Regulated by *MITF1* (a master pigmentation gene) and role in the migration of melanosome
RDA	24	21	53809138	Downstream 11151 bp	integrin alpha-V	*ITGAV*		cell adhesion, integrin-mediated signaling pathway	Beauvais et al. (1995) Wang et al. (2015) and Stresik (2014) Lee	Cell migratory behavior from neural crest cells from which melanophore originate, also dermal thickness and epithelial cell polarity
MultGWAS_7	24	21	53857363	coding	-	*ITGAV*		-	-	-
RDA	26	22	48336761	Downstream 4503 bp	plexin-B1-like	*PLXNB1*	semaphorin receptor activity	signal transduction, semaphorin-plexin signaling pathway	Soong et al. (2017) Soong and Scott (2013)	Survival, growth of melanocytes
RDA	31	9	53901926	Upstream 7837 bp	rap guanine nucleotide exchange factor 2-like	*RAPGEF2*	guanyl-nucleotide exchange factor activity, GTPase activator activity, Rap guanyl-nucleotide exchange factor activity	signal transduction, small GTPase mediated signal transduction, regulation of cell proliferation, positive regulation of GTPase activity	Annen et al. (2006)	Regulation of melanogenesis
RDA	31	9	53902142	Upstream 7621 bp	-	*RAPGEF2*	-	-	-	-
RDA	31	9	57854567	coding	gamma-aminobutyric acid receptor subunit alpha-2-like	*GABRA2*	transmembrane signaling receptor activity, GABA-A receptor activity, ion channel activity, extracellular ligand-gated ion channel activity	ion transport, chloride transport, gamma-aminobutyric acid signaling pathway, ion transmembrane transport	Bertolesi et al. (2016)	Implied in light-regulation of skin color (*Limnpsr*)
RDA	31	9	57854882	coding	-	*GABRA2*	-	-	-	-
RDA	31	9	58319392	Downstream 0546 bp	polycomb group RING finger protein 1-like	*PCGF1*	metal ion binding	transcription DNA-templated, regulation of transcription DNA-templated, aging, histone H2A-K119 monoubiquitination, developmental growth	Huang & Hornyak (2015)	Post-translational modification in melanocyte development and melanogenesis
RDA	31	9	61830541	Upstream 1170 bp	protocadherin-10	*PCDH10*	calcium ion binding	cell adhesion, homophilic cell adhesion via plasma membrane adhesion molecules	Williams et al. (2018)	Melanocyte migration from neural crest cells (zebrafish)
RDA	31	9	61851844	Downstream 0983 bp	-	*PCDH10*	-	-	-	-
RDA	31	9	61851990	Downstream 10129 bp	protoca - therin-10	*PCDH10*	-	-	-	-
RDA	31	9	62603472	Downstream 1358 bp	cystine/glutamate transporter-like	*SLC7A11*	transmembrane transporter activity	transmembrane transport	Chintala et al. (2005) Xu et al. (2014) Wang et al. (2019)	Melanogenesis regulation and pheomelanin production, found in carp and tilapia
RDA	31	9	66591417	coding	protocadherin alpha-C2-like	*PCDHAC2*	calcium ion binding	cell adhesion, homophilic cell adhesion via plasma membrane adhesion molecules	Djurdjevic et al. (2019)	Differentially expressed in trout skin
RDA	31	9	74389966	coding	fibroblast growth factor receptor-like 1	*FGFR1*	nucleic acid binding	DNA integration	Hirobe (2011) et al. (2013) Amin et al. (2018) Cichorek Czyz (2019)	Participate to keratinocyte-melanocyte cooperation in melanogenesis (may result in melanoma in humans). FGF signalling is essential to scale patterning.

Figure 1:

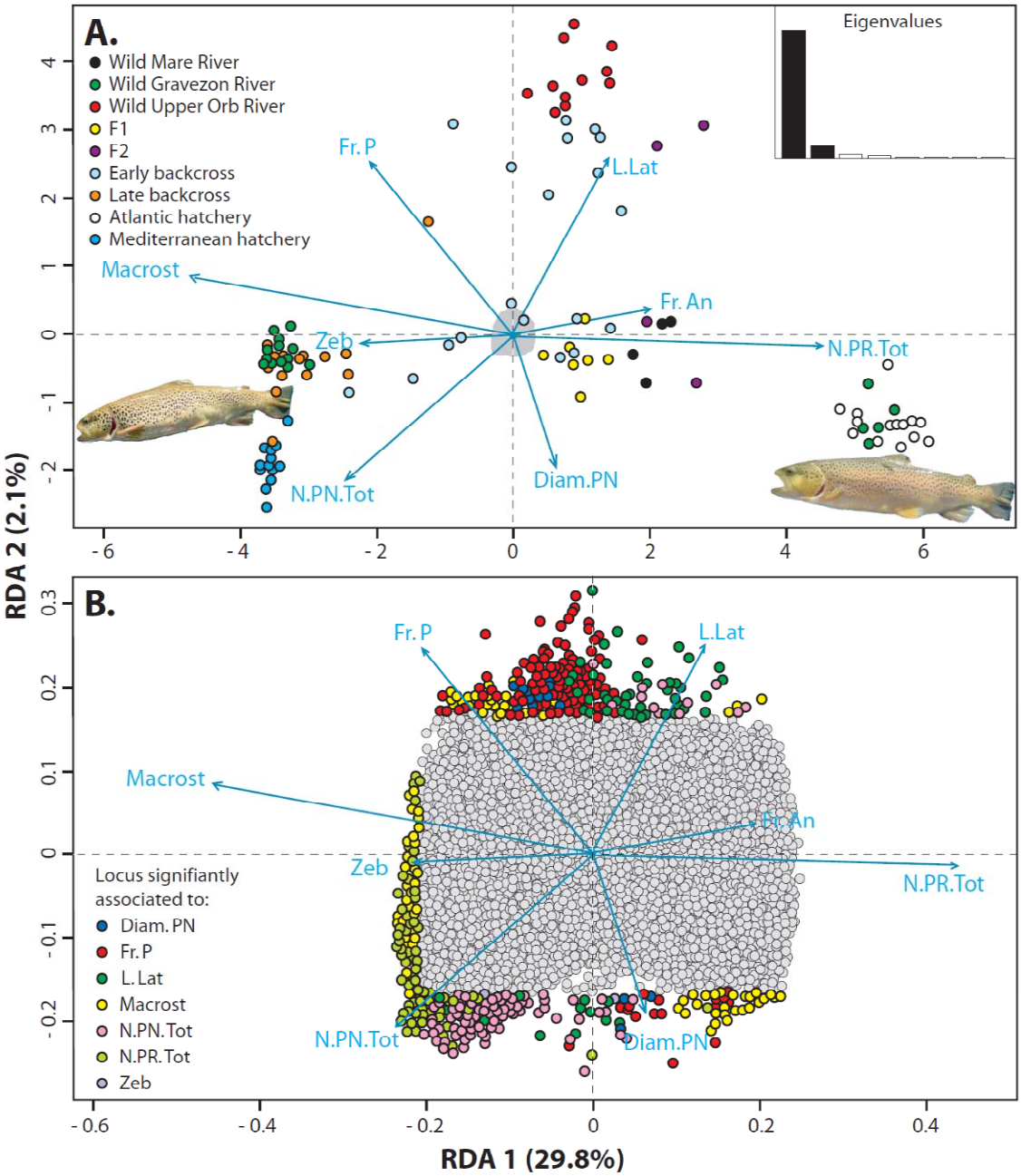

A.
Wild Mare River
Wild Gravezon River
Wild Upper Orb River
F1
F2
Early backcross
Late backcross
Atlantic hatchery
Mediterranean hatchery
Eigenvalues
Fr. P
L.Lat
Macrost
Fr. An
Zeb
N.PR.Tot
N.PN.Tot
Diam.PN
RDA 2 (2.1%)
B.
Fr. P
L.Lat
Macrost
Fr. An
Zeb
N.PR.Tot
N.PN.Tot
Diam.PN
Locus signifiantly associated to:
Diam. PN
Fr. P
L. Lat
Macrost
N.PN.Tot
N.PR.Tot
Zeb
RDA 1 (29.8%)

Figure 2:

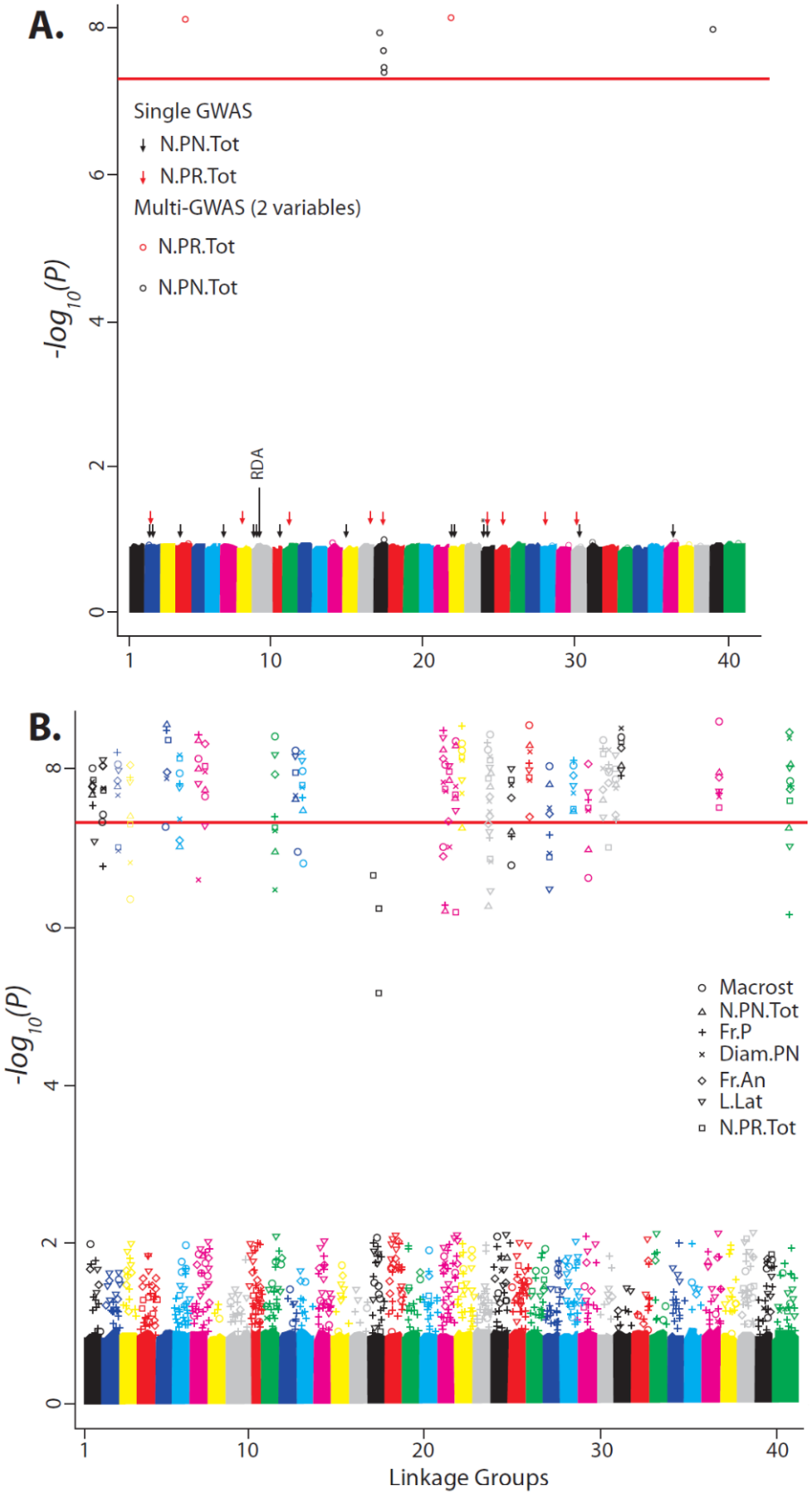

A.
Single GWAS
N.PN.Tot
N.PR.Tot
Multi-GWAS (2 variables)
N.PR.Tot
N.PN.Tot
RDA
-log10(P)
B.
Macrost
N.PN.Tot
Fr.P
Diam.PN
Fr.An
L.Lat
N.PR.Tot
-log10(P)
Linkage Groups

Figure 3:

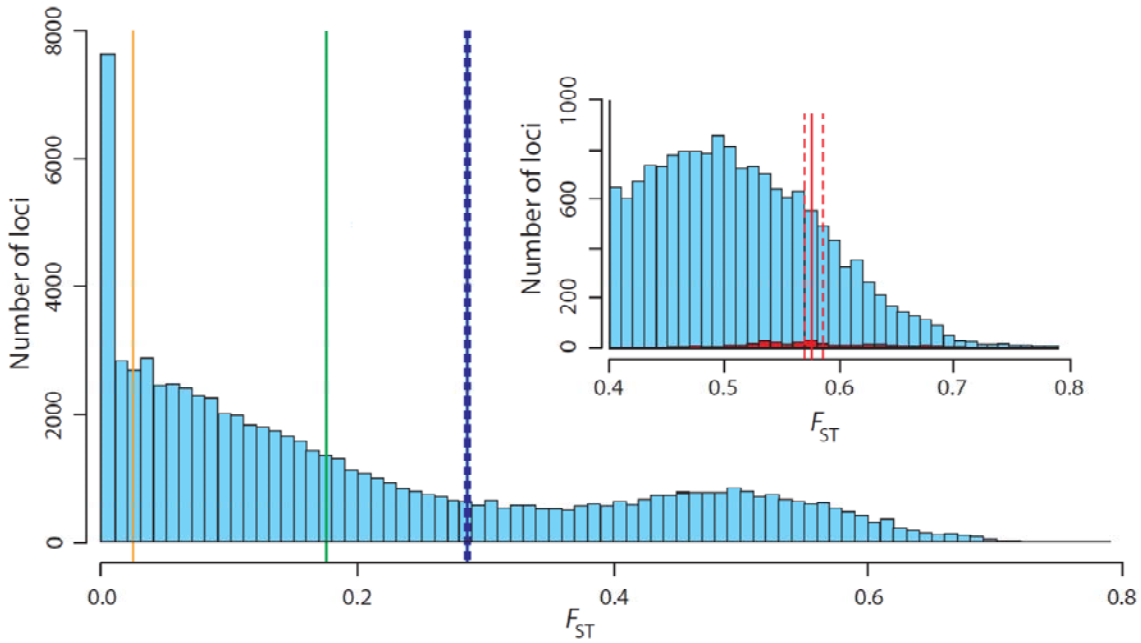
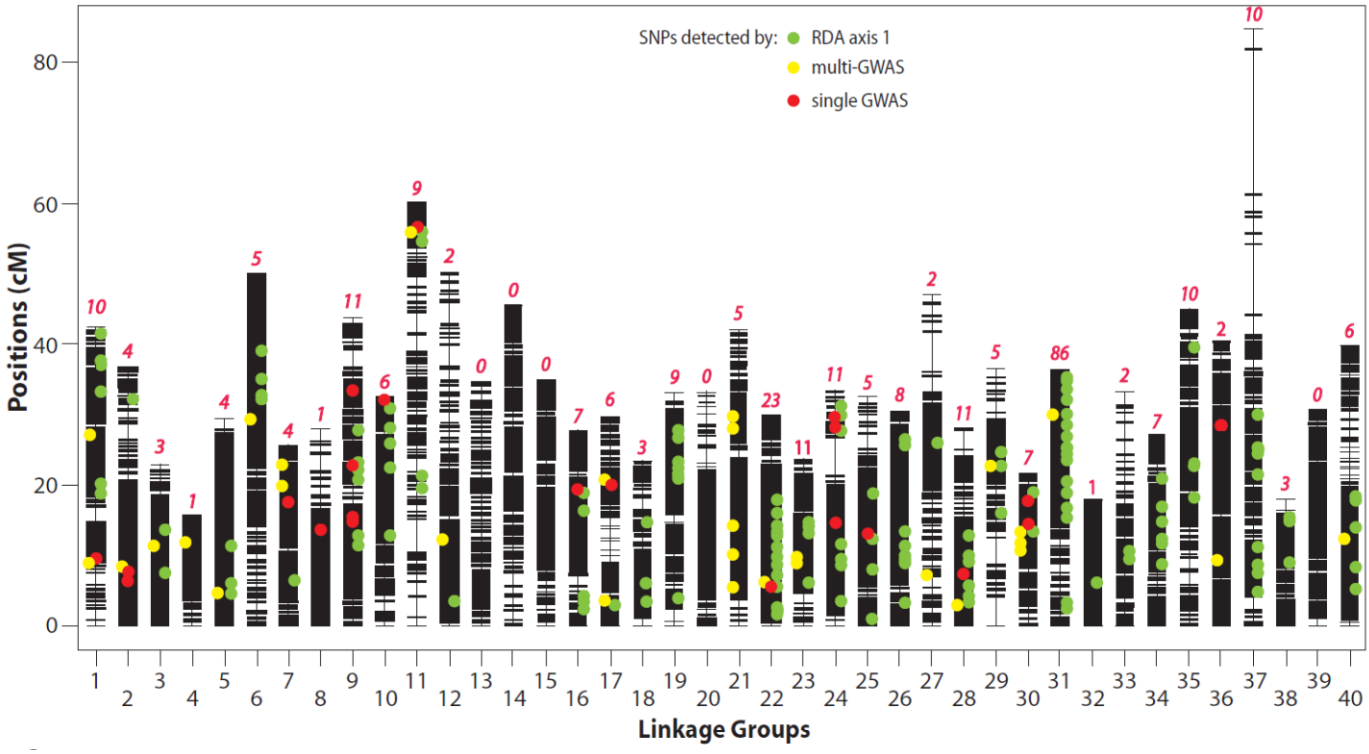

Number of loci
0
2000
4000
6000
8000
0.0
0.2
0.4
0.6
0.8
F_{ST}
Number of loci
0
200
600
1000
0.4
0.5
0.6
0.7
0.8
F_{ST}

Figure 4:


SNPs detected by:
RDA axis 1
multi-GWAS
single GWAS
Positions (cM)
Linkage Groups