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The Genetic Basis of Morphometric and Meristic Traits in Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract

In fishes, body shape, is a complex trait involving several genetic and environmental factors. Understanding the genetic basis of phenotypic variation in body form could lead to breeding strategies aimed at adapting body shape to captive environments.

In the present study, QTLs associated with morphometric and meristic traits in rainbow trout were identified using a genetic linkage map created from a cross of two clonal lines divergent for morphology and life history (wild steelhead trout and domesticated rainbow trout). Genome regions associated with differences in morphological (body depth, mouth orientation, caudal peduncle shape, anal and dorsal fin length) and meristic (number of skeletal elements of median and paired fins and of caudal fin) characters were identified. The identification of genomic locations influencing body morphology, even if only at a gross level, could be of pivotal importance to direct breeding strategies in commercial hatcheries towards the production of more desirable body types.

ABBREVIATIONS

QTL: Quantitative Trait Loci; WR: Whale Rock; AR: Arlee; CDFG: California Department of Fish and Game; RTS: Mt. Shasta Strain; DH: Double Haploids; SL: Standard Length; GLS: Generalized Least-Squares; TPS: Thin-plate Spline; PCA: Principal Component Analysis; CVA: Canonical Variate Analysis; DFA: Discriminant Function Analysis; ANOSIM: Analysis of Similarities; UPCR: Upper Principal Caudal Rays; LPCR: Lower Principal Caudal Rays; MS 222: Tricaine Metasulfonate 222; AFLP: Amplified Fragment Length Polymorphism; PCR: Polymerase Chain Reaction

INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss)*, [1] are one of the most widely-cultured species in Europe and North America due to their adaptability to artificial rearing, and have been cultured for more than a century [2]. Prolonged artificial selection for desired traits (e.g., enhanced growth rate and late sexual maturity) [3] and incidental effects of domestication carried on throughout the last century have led to the development of domesticated strains sharing phenotypic traits, different from wild populations [4].

Besides its importance in aquaculture, rainbow trout are well suited for research because they are amenable to chromosomal manipulations which have been exploited to produce rainbow trout clonal lines with variable phenotypes. The characterization of shape variability [5,1] and of meristic expression [6,7,8] in these lines has facilitated the selection of clonal lines with divergent phenotypes for subsequent QTL analyses aimed at identifying genome regions linked with variation in morphological (including both morphometric and meristic) traits.

Heritable variation between and within populations has been well-documented for traits important in salmonid aquaculture (i.e., body size, feeding behaviour, growth rate, muscle growth, survival, disease resistance and age at sexual maturity) [9]. However, body form has received much less attention.

Some attempts at linking shape-related traits with genetic variation and at identifying the genetic architecture underlying morphometric variation in fishes were conducted on both marine, sea bass *Dicentrarchus labrax* L. [10,11], and sea bream *Sparus aurata* L. [12], and freshwater (zebrafish *Danio rerio* [13], rainbow trout [5,14] and Atlantic salmon *Salmo salar* L. [15]

species. It is important to understand whether the phenotypic variation in body form has a small or large genetic component and whether the genetic component is determined by many genes of small effect or by a few major loci of large effect. This could lead to breeding strategies that are aimed at adapting body shape to the rearing environment.

Fish morphology has been demonstrated to affect organismal performance, [16,17] such as prey-capture success [18], predator avoidance [19], and swim performance [20]. In nature, conflicting selective pressures affect fish body morphology. Generally, morphology represents an adaptation to the prevailing environmental conditions [21], such as water velocity [22] and environmental variability [23], but also to biotic factors, such as the presence of predators [24,25] or prey availability [26-28]. Thus, understanding how morphological traits are modified under artificial propagation is important for both conservation efforts and aquaculture. Over the past few decades, body shape has become a valuable appearance trait in commercial fish. Thus, to satisfy market demand and increase profitability, producers may desire to modify fish body shape [29]. This is not easy, as fish body shape is a complex trait, involving several genetic and environmental factors. Progress in this field will depend in part on dissecting the underlying genetics of these traits for future implementation of modern selection strategies, such as marker-assisted selection, which is particularly relevant when seeking to enhance selection efficiency for traits that are difficult to assess in practice.

The aim of this study was to identify QTLs associated with morphometric and meristic traits in rainbow trout using a genetic linkage map based on progeny obtained from a cross of two clonal lines which are highly divergent in their morphology. Results must be conservatively interpreted, as the sample is limited to a cross of two different haploid genomes. However, the clonal lines used do provide convenient, reproducible experimental material and results can allow hypotheses to be developed which can be broadly tested in future studies.

MATERIALS AND METHODS

Genetic crosses

Genetic analyses of morphology-related characters were conducted using doubled-haploid progeny produced from a cross between the Whale Rock (WR – XX, steelhead and wild) and Arlee (AR – YY, rainbow and domesticated) lines. These two divergent clonal lines and four others were previously characterized for juvenile shape and meristic counts [1,8]. The Whale Rock line was originally a sea-run steelhead population, before the Whale Rock Dam was built on Old Creek (California) in 1961 and the Whale Rock Reservoir was created. The natural population now migrates into the reservoir to feed and grow. The milt used to obtain the clonal line came from wild fish migrating up to the creek from the reservoir [30].

The Arlee line was derived from a fully domesticated strain from the Jocko River State hatchery (Arlee, Montana). As for almost all the important rainbow trout strains used by fish and game agencies in the U.S., it can be originally tracked to the McCloud River of North California [31]. The Arlee line is related to one of the nine strains artificially propagated from the California Department of Fish and Game (CDFG): the Mt. Shasta strain (RTS). This strain was derived in the 1950's from a cross between the Hot Creek strain and a strain from Meader's Trout Farm (Idaho) [32].

Clonal lines have been propagated at Washington State University for a number of generations. Fish that are homozygous for all loci remain genetically uniform and are no longer affected by selection or domestication. This means that their original genetic status is maintained while propagated.

Clonal line production is described elsewhere [1]. Briefly, eggs from outbred females obtained from a commercial source of O. *mykiss* (Troutlodge) were irradiated with gamma radiation to destroy maternal nuclear DNA. Sperm from clonal males (Arlee) were used to fertilize irradiated eggs and the first embryonic cleavage was blocked by a heat shock to restore diploidy with all-paternal (androgenetic) nuclear DNA inheritance, and embryonic development proceeded as in normal diploid embryos. Eggs from homozygous clonal females (Whale Rock) were activated to develop using UV-irradiated sperm [33] and the second polar body of the egg was retained by heat shock to produce homozygous gynogenetic diploids. Embryos were maintained in recirculating stack egg incubators at a constant 11°C until complete yolk absorption, then fish were transferred to nine-liter tanks maintained in a recirculating system (Aquaneering, Inc.; www.aquaneer.com) at the same densities, temperature (13°C) and feeding regime (*ad libitum* two or three times a day with Biodiet trout starter flakes). Three months after hatching, all the survivors were euthanized with a lethal dose of MS 222, measured (standard length, LS), weighed (W) and immediately fixed in formalin (10%) for further analyses (Whale Rock: n = 40, SL = 2.54 ± 0.25 cm, W = 0.29 ± 0.10 g; Arlee: n = 19, SL = 3.08 ± 0.29 cm, W = 0.70 ± 0.18 g)

Eggs from a WR female were fertilized with milt from an AR male to produce a family of F1 progeny. Milt from a single F1 male hybrid clone was used to produce doubled haploid progeny by androgenesis [13,34,35]. Eggs from three females were used during androgenesis, but do not contribute genetically to the nuclear inheritance in the offspring. Doubled haploids (DH) were reared for three months at the Washington State University research hatchery [36]. DH rearing conditions were the same as previously described for clonal lines. The DH were split into three tanks (T1, T2 and T3) on the same recirculating system at similar densities to reduce tank effects, but several traits were significantly different between tanks (two-tailed Welch's t-test). With genotypes randomly assigned to tanks, these differences should effect the QTL analysis very little (i.e., if at locus A there are genotypes AA and AB, the AA and AB genotypes in tank 1 would have the same conditions even if the conditions between tank 1 and tank 2 are different).

All surviving fish (n = 118; SL: 2.84 ± 0.28 cm; W: 0.40 ± 0.13 g) were analyzed. Fish were euthanized with a lethal dose of neutralized MS-222 (pH 7), measured, weighed and immediately fixed in buffered formalin (10% in phosphate buffer 0.1M, pH 7.2) for further analyses (Washington State University Institutional Animal Care and Use Committee approved protocol #02991-005 and #02991-06).

Phenotyping

The power of geometric morphometrics lies in the possibility to visualize shape differences, to decompose such differences into a uniform component (which describes stretching, compression or shearing of the entire landmark configuration) and a non-uniform, localised component. Co-variation among regions of the body are taken into account by the model; the numerical output can be analysed with traditional multivariate statistics.

Each doubled-haploid fish was photographed in the left lateral aspect with a digital camera mounted on a copy stand. On each specimen, 15 landmarks (Figure 1) were digitized using TpsDig 2.0 software [37]. The landmark coordinates for each specimen were aligned and superimposed by the generalized least-squares Procrustes superimposition method (GLS) [38,39]. The Procrustes method eliminates size, location and orientation of specimens in three steps:

1. Scale each configuration to a unit centroid size, defined as the square root of the sum of squared distances between each landmark of a configuration and its centroid [40].

2. Superimpose the centroids of all configurations by subtracting the mean x and y coordinates of each configuration from the coordinates of all its landmarks;

3. Rotate the configurations around the centroids to an optimal fit that minimizes the sum of squared distances of the landmark of each specimen to the corresponding landmarks of the overall mean configuration.

Residuals from the superimposition were analysed with the thin-plate spline (TPS) interpolating function [40]. Shape data on parental lines were from a previous study on rainbow trout clonal lines [1].

As an ordination method in order to display the major features of shape variation, a principal component analysis [41], a dimension-reduction tool aimed at reducing a large set of variables to a small set that still contains much of the information in the large set, was performed on the covariance matrix (as the variables were homogeneous with respect to their variances).

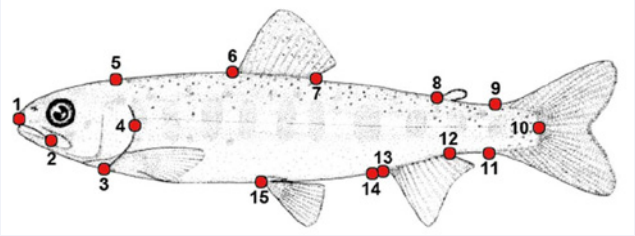

Figure 1 Landmarks (red circles) tagged on specimens of O. mykiss. (1) Anterior tip of premaxilla; (2) most posterior point of maxilla; (3) insertion of the operculum on the ventral profile; (4) most posterior point of the operculum; (5) projection of the operculum on the dorsal profile; (6) anterior and (7) posterior of base of the dorsal fin; (8) anterior of base of adipose fin; (9) anterior attachment of dorsal membrane from caudal fin; (10) base of middle caudal rays; (11) anterior attachment of ventral membrane from caudal fin; (12) anterior and (13) posterior of base of anal fin; (14) anus; (15) origin of the left pelvic fin.

Canonical variate analysis [42], was used to separate known groups in the data, providing an ordination that maximized the separation of the group means relative to the variation within groups. CVA is used to determine which variables discriminate between two or more a priori determined groups. It is broken into a two-step process: (1) testing the significance of a set of discriminant functions (a step identical to a multivariate analysis of variance), and (2) classification. CVA automatically determines some optimal combination of variables so that the first function provides the most overall discrimination between groups, the second provides the second most, and so on. Subjects are classified in the groups in which they have the highest classification scores. Discriminant Function Analysis (DFA) was also performed to obtain pairwise comparisons. DFA procedure carries out a leave-one-out cross-validation to assess the reliability of classification [20]. Statistical analyses were performed using the software MorphoJ [43].

The same specimens, once photographed, were in to double stained using red alizarin for bones and alcian blue for cartilage [44], and the following skeletal elements were counted: 1) number of total vertebrae, 2) number of epurals and hypurals, 3) number of upper (UPCR) and lower (LPCR) principal caudal rays, 3) number of left and right pectoral fin rays, 4) number of left and right pelvic fin rays, 5) number of dorsal fin pterygiophores and rays, and 6) number of anal fin pterygiophores and rays. Observations were performed on both sides of the fish under a dissecting microscope (Wild, LEITZ). Meristic counts were performed twice by the same individual, at a time-length equal to the time employed to complete one round. Since the repeated measurements were exactly the same, measurement error did not influence meristic counts. Meristic data on parental lines were from a previous study on rainbow trout clonal lines [45].

A modified version of the analysis of similarities (ANOSIM) procedure [46], was applied to compare the pattern of meristic expression among the lines (clonal lines and DH progeny). ANOSIM is based on the comparison between the mean rank distances between- and within-groups. A metric was used to convert the raw data in a square matrix of dissimilarity among individuals. The distance between two individuals, for a given meristic character, was computed as the absolute distance between the values for the meristic character considered. Then, the overall distance between two individuals for all the meristic characters inspected was computed as the cumulated sum of distances obtained for the single characters. The cumulated distances were computed between all the available individuals, whatever their origin, and the obtained distances were then converted to ranks. The use of rank distances is justified by the discrete and non-normal distribution of data, which prevents the usability of standard approaches such as ANOVA. The ANOSIM statistic R is based on the difference of mean ranks between groups (rB) and within groups (rW):

$$R = (r_B - r_w)/(N(N-1)/4)$$

where N is the total number of individuals. A bootstrap procedure was applied to assess its significance by comparing the observed value of R with the significance interval defined by the 95% ($\alpha = 0.005$) threshold of the distribution obtained from the bootstrap. This approach sought to obtain a realistic assessment

of variation between meristic patterns. It represented the simplest distances between two points in an n-dimensional space in which each dimension was characterized by discrete values.

In order to test whether the medians of meristic counts were different between the parental lines and the DH progeny, non-parametric Kruskal-Wallis (followed by two-tailed Mann-Whitney pairwise tests) were performed on those characters showing variation among medians (all except epurals, hypurals and caudal rays).

ANOSIM and Kruskal-Wallis tests were performed using PAST 3.04 [47].

Genotyping and QTL analysis

Doubled haploid fry were anesthetized with MS-222 and fin clips were rinsed before DNA extraction. Phenol-chloroform DNA extractions [48], were performed before an AFLP protocol was used to amplify polymorphic markers [49]. Dominant AFLP markers are unambiguous in the doubled-haploid progeny, as all individuals are homozygous. AFLP products were run on polyacrylamide gels and visualized on a Typhoon 9400 variable mode imager (Amersham Biosciences). Multiple restriction enzyme combinations were used to cut genomic DNA. After ligation of adapters, various primer combinations were used to amplify a subset of these genomic fragments. After visualization, the fragments were considered markers if they followed Mendelian inheritance [35]. Microsatellites from Rexroad et al., [50], were genotyped by PCR amplification, polyacrylamide gel electrophoresis, and visualized on a Typhoon 9400 imager.

A genetic map was created using R/qtl [3], from the polymorphic markers and microsatellites [33], were used to assign linkage groups to their respective chromosomes [36]. The genetic map size was 1,471 cM, with a mean distance of 7.58 cM between 230 markers. Quantitative trait loci (QTL) analysis using interval mapping was performed using R/qtl for all traits. A permutation test using 1,000 permutations was used to set the significance threshold of a QTL (p-value ≤ 0.05, two marginal p-values of 0.055 and 0.054 were included as well).

RESULTS AND DISCUSSION

Body shape

Parental clonal lines were clearly separated along PC1 (accounting for 31% of the explained variance) (Figure 2A): WR was confined in the positive and AR in the negative portion of the axis. Doubled haploid progeny (DH) positioned between the parental lines, showing intermediate shapes.

Groups were significantly ($T^2 = 576.98$, $p < 0.001$) separated along the first axis (69.4% of the explained variance) in the CVA plot (Figure 2B). Mahalanobis distances among groups were all significant ($p < 0.05$) (10,000 permutations). All the pair wise comparisons performed by DFA were highly significant and the relative classification very reliable: a negligible misclassification error (0.85%) occurred in the comparison between DH and AR (one individual misclassified) (Table 1).

Shape differences, shown by the splines in Figure (2C), were mostly relative to body height, caudal peduncle morphology, mouth orientation and dorsal fin length.

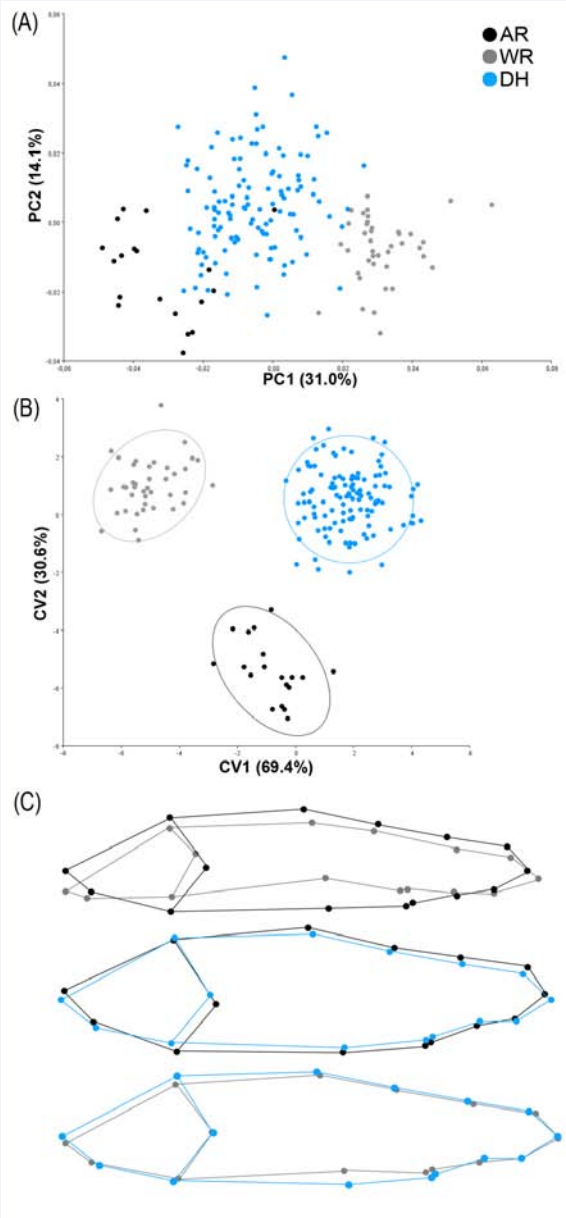

Figure 2 (A) Principal Component Analysis (PCA) ordination plot performed parental clonal lines and doubled haploid progeny. (B) Canonical Variate Analysis (CVA) ordination plot performed on the three groups, 95% confidence ellipses are drawn. (C) Splines showing shape differences between clonal lines and doubled haploid progeny. (AR: Arlee; WR: Whale Rock; DH: doubled haploid progeny).

All PCA axes (percentages of variance explained shown in Figure (3) were used for QTL analysis.

Meristic counts

Parental clonal lines were clearly separated along PC1 (accounting for 31% of the explained variance) (Figure 2A): WR was confined in the positive and AR in the negative portion of the axis. Doubled haploid progeny (DH) positioned between the parental lines, showing intermediate shapes.

Groups were significantly ($T^2 = 576.98$, $p < 0.001$) separated

Table 1: Discriminant Analysis results: differences between means (Mahalanobis Distance, T2, p value) and classification tables after cross-validation (10,000 permutations).

AR vs WR				
	Mahalanobis D	**T^2**	***p***	
	13.624	2390.866	< 0.0001	
True	**Allocated to**	**Total**	**Error %**	
	AR	**WR**		
AR	19	0	19	0
WR	0	40	40	0
AR vs DH				
	Mahalanobis D	**T^2**	***p***	
	6.701	735.466	< 0.0001	
True	**Allocated to**	**Total**	**Error %**	
	AR	**DH**		
AR	19	0	19	0
DH	1	117	118	0.85
WR vs DH				
	Mahalanobis D	**T^2**	***p***	
	6.956	1445.429	< 0.0001	
True	**Allocated to**	**Total**	**Error %**	
	WR	**DH**		
WR	40	0	40	0
DH	0	118	118	0

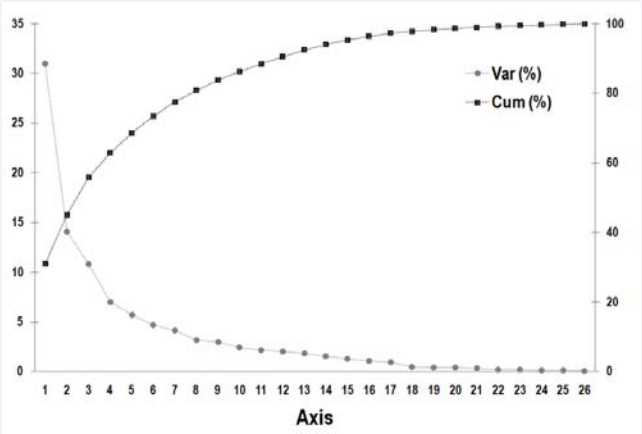

Figure 3 Percentage of variance (Var) explained by each PC axis. Cum: Cumulative Variance.

along the first axis (69.4% of the explained variance) in the CVA plot (Figure 2B). Mahalanobis distances among groups were all significant ($p < 0.05$) (10,000 permutations). All the pair wise comparisons performed by DFA were highly significant and the relative classification very reliable: a negligible misclassification error (0.85%) occurred in the comparison between DH and AR (one individual misclassified) (Table 1).

Shape differences, shown by the splines in Figure (2C), were mostly relative to body height, caudal peduncle morphology, mouth orientation and dorsal fin length.

All PCA axes (percentages of variance explained shown in Figure (3) were used for QTL analysis.

QTL

Body morphology: Six QTLs for principal component axes were identified (Figure 4,5, & Table 4). one of them non significant. For PC2, two QTLs were identified on two chromosomes, 17 and 27, explaining 9.44 and 9.78% of the phenotypic variation in PC2 (which explained 13.5% of PCA variation). Looking at splines relative to the extreme values of PC2 (Figure 6), variability appears to be mainly focused on body depth (dorso-ventral height, landmarks 6-7 and 14-15), mouth length and orientation (landmarks 1 and 2), and caudal peduncle length and height (landmarks 9-11).

Morphological differences displayed along PC5, PC6, and PC13 were less pronounced and related, above all, to the head region and to the trunk depth (Figure 6). For each one of these morphological characters, a single QTL was identified: one QTL for PC5 (explaining 7.1% of PCA variation), positioned on chromosome 13, and explaining 5.7% of phenotypic variation; one for PC6 (6.2% of PCA variation; 11.4% of phenotypic variation), co-localizing on chromosome 26 with the non significant QTL ($p = 0.06$) for PC24 (0.2% of PCA variation; 11.27% of phenotypic variation); one QTL for PC13 (1.9% of PCA variation; 11.12% of phenotypic variation).

Shape variation along PC5 was mostly related to head length, with the negative extreme characterized by a shorter mouth length and a slightly asymmetric caudal peduncle. PC6 captured variation on head length, and shape configuration at the negative extreme was more fusiform, with a longer cephalic region. Along PC13, body shape mostly varied in the trunk region, i.e. at the negative extreme a projecting abdomen contrasted with a thin caudal peduncle. Shape variation along PC24 was limited to mouth and dorsal fin length (Figure 6).

Meristics

Ten QTLs for meristic counts were identified (Figure 4,5 & Table 4). Two QTLs for dorsal fin ray numbers were detected:

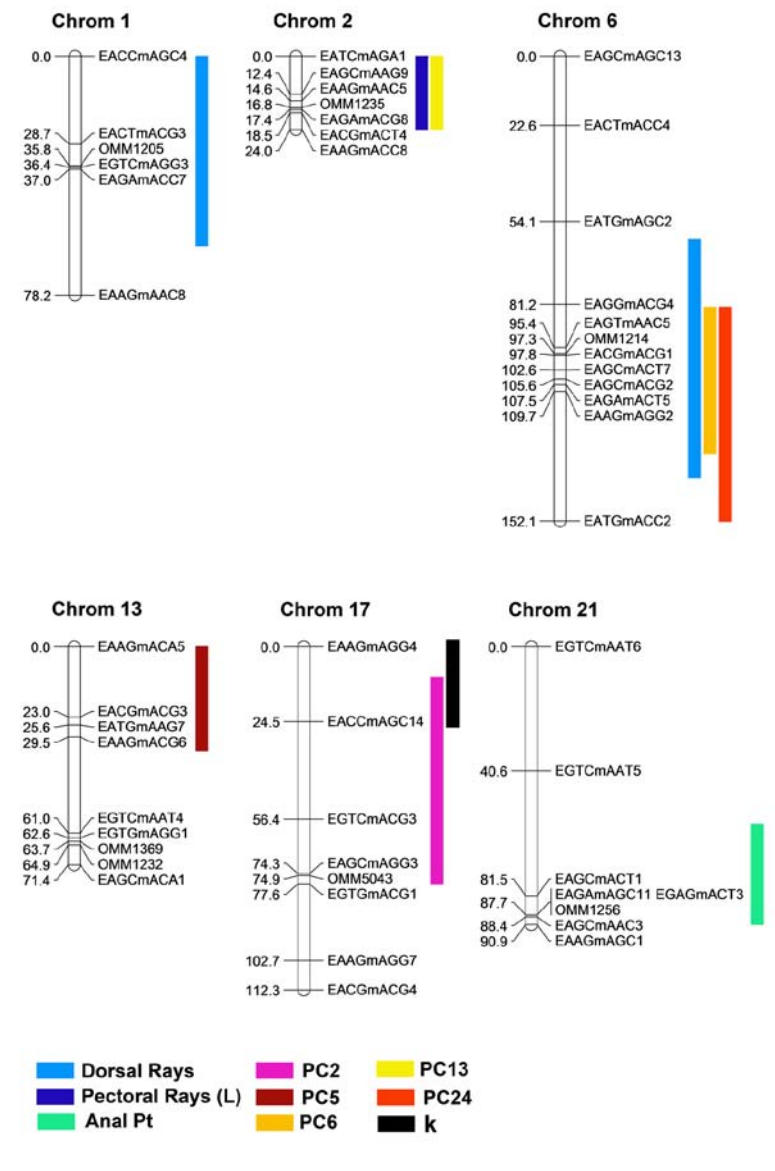

Figure 4 QTL Results WR x AR Panel. The distance of a QTL was determined by a drop in the LOD score of 1.0. QTL for condition factor (k) is from Christensen et al., [8].

Table 2: Medians and ranges for each set of meristic counts in doubled haploid (DH) progeny and parental clonal lines, Clonal line data are from Pulcini et al., [39].

			Vt	Ep	Hyp	UPCR	LPCR	Pectoral	Pelvic	Dorsal	Anal				
								L	R	L	R	Pt	R	Pt	R
DH	**Median**		61	3	6	10	9	13	13	10	10	14	17	12	15
	Range	min	56	2	5	10	9	11	9	8	9	12	14	10	13
		max	64	3	6	11	10	15	15	11	11	17	18	14	18
AR	**Median**		60	3	6	10	9	14	14	9	9	14	18	12	16
	Range	min	57	2	5	10	9	13	10	9	9	14	17	11	15
		max	62	3	6	11	10	15	14	10	10	15	18	13	17
WR	**Median**		60	3	6	10	9	13	13	9	9	13	16	11	15
	Range	min	56	2	6	9	9	11	12	8	8	12	15	11	14
		max	62	3	6	10	9	14	14	10	10	14	17	12	16

Abbreviations: DH: Double Haploids; AR: Arlee; WR: Whale Rock; Vt: Vertebrae; Ep: Epurals; Hyp: Hypurals; UPCR: Upper Principal Caudal Rays; LPCR: Lower Principal Caudal Rays; Pectoral L: left Pectoral fin rays; Pectoral R: Right pectoral fin rays; Pelvic L: Left pelvic fin rays; Pelvic R: Right pelvic fin rays; Dorsal Pt: Dorsal fin pterygophores; Dorsal R: Dorsal fin Rays; Anal Pt: Anal fin pterygophores; Anal R: Anal fin rays.

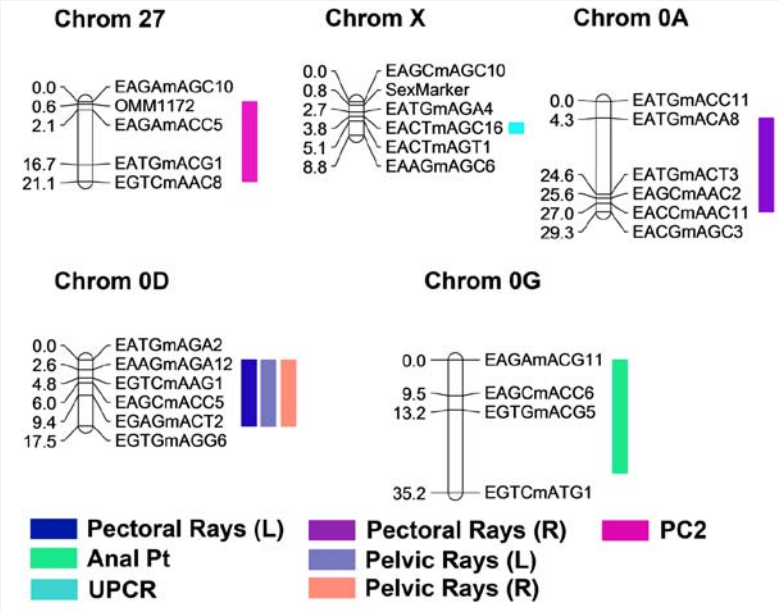

Figure 5 Continued from Figure 4.

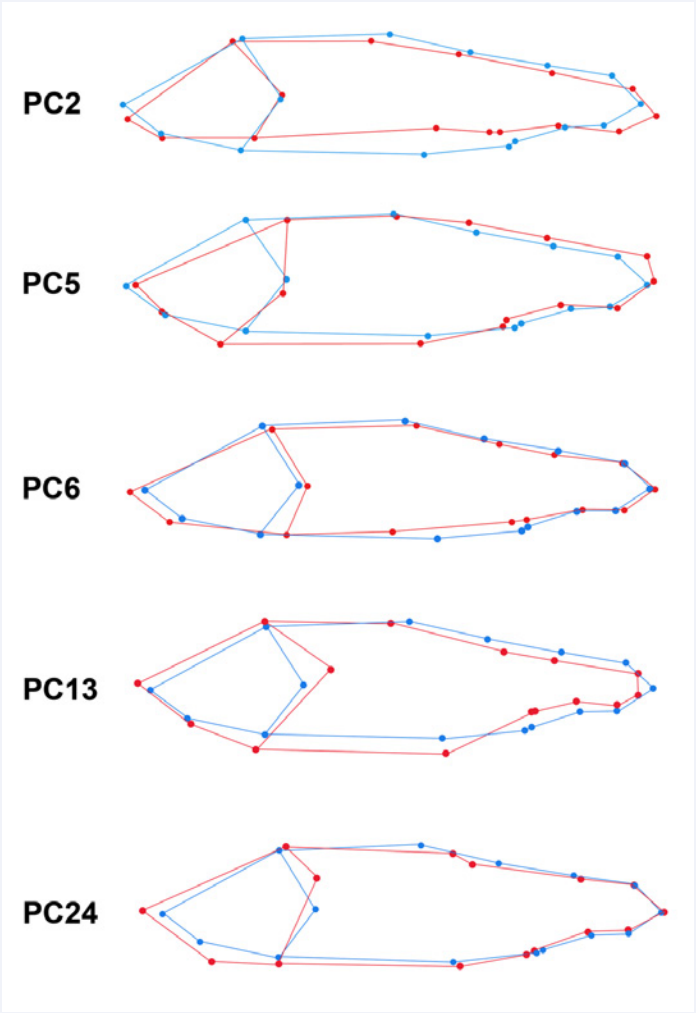

Figure 6 Splines representing shape variation along the PC axes for which QTLs were detected. Blue: positive extreme; red: negative extreme.

Table 3: Kruskal-Wallis test for equal medians and Bonferroni corrected p values of Mann-Whitney pairwise post-hoc tests for meristic counts among lines (clones and doubled haploid progeny).

	Kruskal-Wallis	Mann-Whitney pairwise				
	H	**p**		**DH**	**AR**	**WR**
Vt	32.05	< 0.0001	**DH**	-	< 0.001	< 0.0001
			AR	< 0.001	-	0.883
			WR	< 0.0001	0.883	-
	H	**p**		**DH**	**AR**	**WR**
Pectoral L	33.04	< 0.0001	**DH**	-	< 0.0001	< 0.05
			AR	< 0.0001	-	< 0.0001
			WR	< 0.05	< 0.0001	-
	H	**p**		**DH**	**AR**	**WR**
Pectoral R	26.45	< 0.0001	**DH**	-	< 0.001	< 0.001
			AR	< 0.001	-	< 0.0001
			WR	< 0.001	< 0.0001	-
	H	**p**		**DH**	**AR**	**WR**
Pelvic L	19.58	< 0.0001	**DH**	-	0.243	< 0.0001
			AR	0.243	-	< 0.05
			WR	< 0.0001	< 0.05	-
	H	**p**		**DH**	**AR**	**WR**
Pelvic R	20.30	< 0.0001	**DH**	-	1.000	< 0.0001
			AR	1.000	-	< 0.05
			WR	< 0.0001	< 0.05	-
	H	**p**		**DH**	**AR**	**WR**
Dorsal Pt	52.38	< 0.0001	**DH**	-	< 0.0001	< 0.0001
			AR	< 0.0001	-	< 0.0001
			WR	< 0.0001	< 0.0001	-
	H	**p**		**DH**	**AR**	**WR**
Dorsal R	63.33	< 0.0001	**DH**	-	< 0.0001	< 0.0001
			AR	< 0.0001	-	< 0.0001
			WR	< 0.0001	< 0.0001	-
	H	**p**		**DH**	**AR**	**WR**
Anal Pt	17	< 0.001	**DH**	-	0.129	< 0.05
			AR	0.129	-	< 0.001
			WR	< 0.05	< 0.001	-
	H	**p**		**DH**	**AR**	**WR**
Anal R	16.62	< 0.001	**DH**	-	< 0.05	< 0.05
			AR	< 0.05	-	< 0.001
			WR	< 0.05	< 0.001	-

Abbreviations: Vt: vertebrae; Pectoral L: Left pectoral fin rays; Pectoral R: Right pectoral fin rays; Pelvic L: left Pelvic fin rays; Pelvic R: right Pelvic fin rays; Dorsal Pt: Dorsal fin Pterygophores; Dorsal R: Dorsal fin Rays; Anal Pt: Anal fin Pterygophores; Anal R: Anal fin Rays; DH: Doubled Haploid progeny; AR: Arlee; WR: Whale Rock; DH: Doubled Haploid progeny.

Table 4: Quantitative trait loci for morphometric and meristic traits.

QTL	Position	Closest marker	d.f.	*n*	LOD	E.H.	*p* value	*Chr*
UPCR	8.76	EAAGmAGC6	2	118	3.97	0.151	~0	X
Anal Pt	81.5	EAGCmACT1	1	118	3.09	0.119	0.02	21
Anal Pt	0.0	EAGAmACG11	1	118	3.24	0.125	0.02	"0G"

Pectoral L	0.0	EATCmAGA1	1	118	2.9	0.112	0.05	2
Pectoral L	2.6	EAAGmAGA12	1	118	2.8	0.109	0.055	"0D"
Pectoral R	27	EACCmAAC11	1	118	3.51	0.134	~0	"0A"
Pelvic L	0	EATGmAGA2	1	118	3.2	0.123	0.02	"0D"
Pelvic R	9.37	EGAGmACT2	1	118	4.79	0.179	~0	"0D"
Dorsal R	28.7	EACTmACG3	1	118	3.19	0.123	0.02	1
Dorsal R	95.4	EAGTmAAC5	1	118	2.89	0.112	0.03	6
PC2	52	EGTGmACG1	1	118	3.01	0.116	0.03	17
PC2	14	EGTCmAAC8	1	118	3.39	0.130	0.01	27
PC5	23	EACGmACG3	1	118	3.8	0.145	~0	13
PC6	103	EAGCmACT7	1	118	3.08	0.119	0.02	6
PC13	17.4	EAGAmACG8	1	118	2.87	0.111	0.04	2
PC24	122	EATGmACC2	1	118	2.73	0.106	0.06	6

Abbreviations: QTL: Quantitative Trait Loci; LOD: Logarithm of the Odds; E.H: Estimated Heritability; Chr: Chromosome; UPCR: Upper Principal Caudal Rays; Pt: Pterygiophores; L: Left; R: Right; R: Rays; PC: Principal Component.

one (explaining 11.49% of phenotypic variance) located on chromosome 1, and another (9.91%) on chromosome 6. Two QTLs were identified for left pectoral fin rays: one explaining 4.11 % of phenotypic variance, on chromosome 2; the second (4.08%) on an unidentified chromosome "0D". In the same chromosome interval, one QTL for left pelvic fin rays (11.76%) and one for right pelvic fin rays (17.03%) were identified.

The two QTLs identified for anal pterygiophores were positioned on chromosome 21, (11.54 %), and on an unidentified chromosome "0G", (11.88%). Finally, one QTL for right pectoral fin rays, explaining 13.03% of variance, was identified on the unknown chromosome "0A".

In farmed fish, body shape is a prominent trait that influences suitability for marketing: a fish with an improved appearance has greater consumer acceptance and a higher sale value [52-54]. There has been some progress in this area with commercial fish, mainly through selective breeding or classical genetic analysis [54]. Information gained by identifying QTLs or genes underlying body shape could be used to improve selective breeding based on molecular markers.

Previous studies confirm the existence of significant genetic variation for body morphology and meristic counts in rainbow trout [1,8], indicating potential for genetic improvement. In particular, the parental clonal lines selected for this study were clearly distinguishable on the basis of their external morphology: the domesticated resident Arlee line had a higher profile along the entire body and a higher condition factor (0.23 ± 0.02) than the wild migratory Whale Rock line (0.17 ± 0.02). The caudal peduncle was also more robust and shorter, the head was longer and the mouth more upward oriented in the Arlee line. Dorsal fins were also noticeably longer. Such differences ensured a 100% of correct classification in a discriminant function analysis between the two lines. Several meristic traits significantly differed between the two lines: the number of rays of paired fins (pectoral and pelvic) and the skeletal elements (pterygiophores and rays) of dorsal and anal fins. The fact that the observed morphometric and meristic differences were maintained in a common-garden experiment suggests that they resulted from genetic differences among clonal lines and, by extension, possible differences among the source populations from which the lines were derived. However, caution must be exercised, as clonal lines represent very limited samples of the source populations.

In this study, 10 QTLs for meristic counts and six for morphometric characters were identified. The detection of two QTLs for dorsal fin rays and two for anal pterygiophores on different chromosomes suggests that at least two large-effect genes or closely linked genes affect variation in this trait, while for paired fin rays (pectoral and pelvic) a pleiotropic effect of the gene/gene complex on chromosome "0D" is conceivable.

Despite significant differences between parental clonal lines for numbers of dorsal fin pterygiophores and anal fin rays, this study failed to detect any QTL for these meristic traits that differed between the parental lines segregating in the DH progeny. One explanation for this result could be that a very high number of loci are affecting these traits and thus QTLs for these traits are not being detected in our cross.

While the number of genes responsible for the development of meristic characters has been presumed to be large [55], this study identified one or two QTL for each. Two explanations are possible: 1) only a few major genes were segregating for differences in meristic trait values in this family, or 2) if many genes play a small role in the development of skeletal meristic elements, the sample size of this study could have failed to detect these loci (as the power of QTL analysis is affected by both the number of progeny segregating for QTL and the magnitude of the differences observed between parental lines) [56]. Evidence herein suggests that meristic trait QTL are generally not pleiotropic for more than one trait, except for the paired fins. Different meristic characters are determined at different times during development, and from different embryonic tissues, and

that is likely why, as previously observed by Nichols et al. [6], they do not co-localize to the same linkage group. In their study on different rainbow trout families, Nichols et al. [6] detected one QTL explaining 23% of variation of vertebrae number on linkage group OA-VI, two QTLs for count of scales above the lateral line on linkage groups OA-VIII and OA-XIV, accounting for 27.5 % and 15.9 % of variation, respectively, and one for anal fin ray counts on linkage group OA-XXII, explaining 13.4% of variation in this trait. Nichols et al., [6] also rejected the hypothesis of a negative correlation between embryonic developmental rate and meristic values in rainbow trout.

Concerning morphometric traits, the detection of two QTLs on separate chromosomes for PC2 suggests that the variation in the body depth among the doubled haploid progeny is greatly affected by at least two large-effect genes or gene complexes. The co-localization of one of the two QTL for dorsal ray with the QTLs for PC6 and PC24 on chromosome 6 suggests that the genomic region may have a pleiotropic effect or that multiple genes affecting morphology and meristic traits co-localize.

On the same specimens, Christensen et al., [36] calculated Fulton's condition factor (k), a synthetic index of fish body shape, and found a QTL on chromosome 17, partially co-localizing with the large effect QTL PC2, substantially capturing variation on body depth (Figure 4).

In salmonids, progress has been made in this field through QTL research, mainly for growth-related traits (fork length, body weight, condition factor) and meristic traits [57]. Results on Atlantic salmon suggest that a significant portion of quantitative variation in body weight and condition factor is under the control of a few QTLs with relatively large effects [58].

Because of differences in linkage maps between studies and moderate precision of QTL positions, as well as differences among experimental designs, comparison with other studies is quite hard. In rainbow trout, ten QTLs for morphometric traits were identified from Nichols et al., [5] in their study about smoltification-related traits in a cross between a resident line (Oregon State University rainbow trout) and a migratory (Clearwater steelhead) line. QTLs were identified for principal component axes capturing dorso-ventral variability in body depth (on linkage groups OC13, OC31, OC8 and OC20) and dorsal fin length (OC9 and OC20). None of these QTLs were on the same chromosomes as those identified in this study.

Quillet et al., [59] mapped QTLs for body size (15, both for body length and body weight) and conformation (12) in rainbow trout, in order to find possible links between stress response and production traits. Conformation QTLs were found at similar locations to the body size, suggesting pleiotropic effects of the QTL and underpinning the picture of a genetic link between size and shape. Thus, an undesired correlation between large body mass and rotund shape [52], could be more efficiently controlled.

CONCLUSION

This study revealed genomic loci associated with morphological and meristic traits in a cross between a landlocked steelhead trout line of wild origin and a domesticated rainbow trout line. Both lines were derived from California. Different genomic regions were associated with multiple morphometric and meristic traits, suggesting multiple important genes involved in body shape characteristics. The identification of genomic locations influencing body morphology, even if only at a gross level, could be important both for conservation efforts or to direct breeding strategies in commercial hatcheries towards the production of more desirable body types [52].

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