



## Morphological variation in hybrids between *Salmo marmoratus* and alien *Salmo* species in the Volarja stream, Soca River basin, Slovenia

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There were significant correlations between colour pattern, *LDH-5\** genotype and certain meristic characters in 59 hybrid trout *Salmo* sp. from the Volarja stream, Soca River basin, Slovenia. It is concluded that panmixia between native *Salmo marmoratus* and introduced *S. trutta* of Atlantic, Danubian and Mediterranean origin had not been reached in this zone, despite the long period of introgression. The result is in agreement with other studies dealing with introgression in *Salmo*, and for management purposes certain morphological characters, especially colour pattern, can be a valuable tool in restoring the marble trout population in the Soca River.

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Key words: introgression; hybridization; allozymes; morphometrics; *Salmo marmoratus*.

### INTRODUCTION

Hybridization and introgression among fish species is well documented (Verspoor & Hammar, 1991; Leary *et al.*, 1995). Among the Eurasian *Salmo* species, hybrids between Atlantic salmon *Salmo salar* L. and brown trout *S. trutta* L. are reported frequently from the entire range of sympatry of the two species (McGowan *et al.*, 1992; Elo *et al.*, 1995).

The taxonomy of Eurasian trout does not benefit from consensus. Earlier morphological studies, mainly concerning trout from geographically restricted areas, divided Eurasian trout into more than 100 species-level taxa. According to the compilation of Kottelat (1997), there could be about 20 valid species of *Salmo* in Europe. Recent molecular data, including both variation in the mitochondrial and nuclear genome, tend to divide Eurasian trout into five geographic groups (Bernatchez *et al.*, 1992; Garcia-Marin *et al.*, 1999) which are not considered generally to be distinct species, with the occasional exception of *Salmo marmoratus* (Cuvier).

Hybridization between *Salmo* species occurs mainly as a consequence of human activities. Stocking of Atlantic *S. trutta* seems to be one of the causes

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(Largiadér & Scholl, 1996; Morán *et al.*, 1996; Poteaux *et al.*, 1998a, 1998b, 1999; Berrebi *et al.*, in press). This form has been introduced in non-Atlantic regions at a large scale, and also in other regions and several continents where it was absent (Baglinière, 1991). In the case of the frequently reported hybridization between *S. salar* and Atlantic basin *S. trutta*, hybrids are highly viable (Galbreath & Thorgaard, 1995) and introgression, even though reported on (Verspoor & Hammar, 1991) and possible to demonstrate under laboratory conditions (Wilkins *et al.*, 1993), seems to be of limited importance. However, in other *Salmo* species, introgression can be a major threat. The marble trout *S. marmoratus* is heavily affected by stocked alien Atlantic *S. trutta* and Danubian and/or Mediterranean *Salmo* species and presently there are only a few populations of *S. marmoratus* that are believed to be unaffected by introgression (Giuffra *et al.*, 1996; Berrebi *et al.*, in press).

*Salmo marmoratus* is distributed natively in rivers emptying into the Adriatic Sea from northern Italy to Albania (Povz *et al.*, 1996). With a single exception, viz. the 'marmorated trout' from River Otra in Norway (Skaala & Solberg, 1997), it is diagnosable from other *Salmo* species by its marbled colour pattern alone. The 'marmorated trout' has allozymes similar to other Scandinavian *S. trutta* populations (Skaala & Solberg, 1997). Osteological characters (Dorofeyeva *et al.*, 1991) and genetic markers, both allozymes (Berrebi *et al.*, in press; Giuffra *et al.*, 1996) and mtDNA (Bernatchez *et al.*, 1992; Giuffra *et al.*, 1994), support the recognition of *S. marmoratus* as a species distinct from *S. trutta*. The most informative allozyme locus is *LDH-5\**. Most of the non-Atlantic trout except *S. marmoratus* are homozygous for the 100 allele, widely distributed among salmonids and therefore often referred to as the ancestral allele (Hamilton *et al.*, 1989). Atlantic *Salmo trutta* populations are polymorphic having an autapomorphic 90 allele in addition to the 100 allele (Hamilton *et al.*, 1989). The 100 allele dominates in several upstream trapped populations (Hamilton *et al.*, 1989), but it is very rare in hatchery strains (Poteaux *et al.*, 1998a; Guyomard, 1989). Unaffected *S. marmoratus* populations are usually fixed for an autapomorphic 120 allele (Giuffra *et al.*, 1996; Berrebi *et al.*, in press). In Slovenia native *S. marmoratus* are found in the Soca River and its tributaries. Within the Soca River basin, seven water courses in the upper reaches of the river seem to hold populations unaffected by stocking of alien *Salmo* species (Povz *et al.*, 1996; Berrebi, unpubl. data). The Soca River has been stocked repeatedly since 1906 with non-native *Salmo* species of both Atlantic and Danubian (or, less likely, Mediterranean) origins (Povz *et al.*, 1996).

In general, most studies on hybrids among salmonids in the wild use genetic markers only and morphology (e.g. colour pattern) is used most often only as a sorting tool (Largiadér & Scholl, 1996), or as an indication of possible hybrids. However, Hammar *et al.* (1991) demonstrated intermediate morphology in electrophoretically determined hybrids of *Salvelinus alpinus* (L.) and *Salvelinus fontinalis* (Mitchill). Hedenskog *et al.* (1997) found hybrids between *Salmo salar* and *S. trutta* to be intermediate in their morphology, whereas Wilkins *et al.* (1994), also studying hybrids between *S. salar* and *S. trutta*, found hybrids less often intermediate in phenotype, resembling one or other parent species. Ielli & Duchi (1990) used the presence of red spots as an indication of hybridization in *S. marmoratus* from Italy. According to Povz *et al.* (1996) and Berrebi *et al.*

(in press), certain genetically pure marble trout populations, have red spots on the side of the body.

The main goal of this study was to investigate hybrid trout from the Volarja stream—a tributary of the Soca River—and search for correlations between several hypothetically independent data sets, viz. colour pattern, morphometry, meristics, and *LDH-5\** genotype in a sample of trout from a population with a relatively long history of introgression. Trout from the Volarja stream have been sampled and investigated genetically repeatedly, and have contributed to a long-time study of *S. marmoratus* in the Soca River basin (Povz *et al.*, 1996). Allozyme electrophoresis on the trout from the Volarja stream showed that a majority of the investigated specimens were probably Fn-hybrids between *S. marmoratus* and introduced *Salmo* species (Berrebi, unpubl. data). The genetic constitution of this large population is approximately a third of each origin: autochthonous *S. marmoratus*, introduced commercial Atlantic *S. trutta* and translocated neighbour Danubian (or Mediterranean) trout (*S. trutta* and other species). The presence of alien trout and hybrids in the Volarja stream and in the Soca River basin in general is confirmed also by extensive variation in colour pattern (Povz *et al.*, 1996).

## MATERIALS AND METHODS

### METHODS

#### *Sampling*

A total of 60 specimens was collected by electrofishing in the Volarja stream. Each specimen was tagged and one or both eyes were removed and transferred to liquid nitrogen for later electrophoresis. Then the specimens were fixed in 10% formalin and later transferred to 75% ethanol. Due to the loss of one specimen, 59 specimens were included in comparative analyses. The sample is herein referred to as the Volarja trout. The Volarja station is located about 1 km from its confluence with the Soca River, and because no obstacle limits its exchange with the main river, this sample is considered representative of the Soca River. The entire material, both Volarja trout and comparative material (see below) is deposited in the collection of the Swedish Museum of Natural History (NRM). The specimens have catalogue numbers NRM 41534-41538 (<http://www.nrm.se/>).

#### *Electrophoresis*

The long-term survey of the Slovenian *S. marmoratus* populations in terms of hybridization assessment is processed currently using only *LDH-5\** analysis. Electrophoresis is carried out on horizontal starch gels, using TP 7.4 buffer system and staining solution given in Pasteur *et al.* (1987).

#### *Sorting in four categories*

Specimens in ethanol were sorted into four groups according to colour pattern, viz. pure marble, marble-like, brown-like, and pure brown. This was done without *a priori* knowledge of the allozyme genotype. The marble trout is characterized by a marbled colour pattern, absence of black spots with white rings, and red spots if present, only along the lateral line in larger specimens. Atlantic *S. trutta* and other *Salmo* species usually lack any marbled patterning, whereas black and red spots (often enclosed by a white ring) are observed commonly. Thus specimens denoted as pure correspond to these

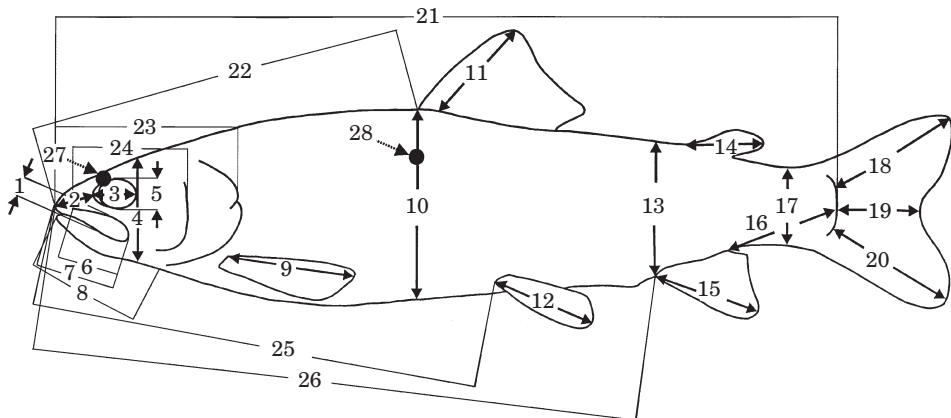


FIG. 1. Measurements taken on *Salmo* specimens: 1, upper jaw depth, as largest depth of the maxilla and supramaxilla; 2, snout length, from symphysis of premaxilla to osseous orbit margin; 3, orbital horizontal diameter, between osseous orbital margin; 4, head depth, just posterior to orbit; 5, orbital vertical diameter, between osseous orbital margin; 6, length of maxillae, from premaxillad end to posterior end of maxilla; 7, upper jaw length, from symphysis of premaxilla to posterior end of maxillae; 8, lower jaw length, from symphysis of dentary to retroarticular; 9, pectoral fin length, from base of first ray to tip of longest ray; 10, body depth, at level of origin of dorsal fin; 11, dorsal fin length, from base to tip of longest ray; 12, pelvic fin length, from base of first ray to tip of longest ray; 13, body depth, at level of origin of anal fin; 14, adipose fin length, from origin to tip; 15, anal fin length, from base of first ray to tip of longest ray; 16, caudal peduncle length, from end of anal fin to middle base of caudal fin; 17, least depth of caudal peduncle; 18, length of upper caudal fin lobe, from base to tip of longest ray; 19, length of middle caudal fin ray, from base to tip of shortest ray; 20, length of lower caudal fin lobe, from base to tip of longest ray; 21 standard length ( $L_s$ ), from upper jaw symphysis to middle base of caudal fin; 22, predorsal length from upper jaw symphysis to origin of dorsal fin; 23, head length, from upper jaw symphysis to posterior tip of operculum; 24, premaxilla to preoperculum length, from premaxillad end of maxillae to posterior margin of preoperculum; 25, prepelvic length, from upper jaw symphysis to origin of pelvic fin; 26, preanal length, from upper jaw symphysis to origin of anal fin; 27, interorbital width, transverse at narrowest part of skull, 28, body width, transverse at widest part of body at level of dorsal fin origin, above abdominal cavity.

two descriptions respectively. Assignment to either of the categories, marble-like or brown-like, includes an element of arbitrariness, but ranges from black-spotted specimens looking like brown trout except for some traces of marble pattern on the back (i.e. brown-like) to marbled specimens having too many red spots and/or traces of black spots (i.e. marble-like).

### Measurements and counts

Measurements were taken with digital callipers on the left side of the specimens and rounded to the nearest 0.1 mm (Fig. 1). Numbers of pored scales along the lateral line to the end of the caudal peduncle (left side), of scales in an oblique row from the base of the adipose fin backwards down to the lateral line including lateral line scales (left side), of gill rakers on the lower and upper limbs of the first gill arch separately (right side), and of branchiostegal rays on both sides, were counted under a binocular dissection microscope. Numbers of abdominal vertebrae, caudal vertebrae, unbranched and branched rays in the dorsal and anal fins, of pterygiophores supporting the anal and dorsal fins, of caudal fin upper and lower procurrent rays, of interneurals, of expanded neural spines in the caudal skeleton, and of vertebrae having expanded neural spines were recorded from radiographs. The position of the dorsal and anal fins was estimated in relation to vertebral count.

TABLE I. Colour pattern, *LDH-5\** genotype, and  $L_S$  (mm) in 59 specimens of *Salmo* from Volarja Stream, Soca River basin, Slovenia. The ranks used in Spearman rank order correlation are given in bold

<i>LDH-5*</i>	Colour pattern	Pure brown <b>1</b>	Brown-like <b>2</b>	Marble-like <b>3</b>	Pure marble <b>4</b>
9090	<b>1</b>	1	3		
90100	<b>1</b>	2	2	1	
100100	<b>1</b>		2	5	
90120	<b>2</b>	2	8	6	
100120	<b>2</b>		2	6	3
120120	<b>3</b>		2	8	6
$L_S$ (mm)		125–207	98–252	104–240	122–359

## COMPARATIVE MATERIAL

### *Salmo marmoratus*

A total of 41 specimens (104–280 mm  $L_S$ ) was selected from samples having catalogue numbers NRM 41516, 41519, 41522, 41523, 41528 and 41529. The specimens had been collected in three tributaries to the Soca River, viz. the upper Idrijca, Trebuscica, and Zadlascica. The populations from Trebuscica and Zadlascica are regarded as being genetically pure (Povz *et al.*, 1996) based on four informative allozyme loci (Berrebi *et al.*, in press). Purity of the Idrijca sample is based on *LDH-5\** only (Berrebi *et al.*, in press). However, both the Zadlascica and Trebuscica populations have some alleles, usually not be found in *S. marmoratus* and the former is almost fixed for the 100 allele in the *LDH-5\** locus (Berrebi *et al.*, in press). Nevertheless, the Zadlascica River is considered as inhabited by a pure *S. marmoratus* population. The high proportion of the 100 allele is explained by a putative ancient introgression (Berrebi *et al.*, in press).

### *Salmo trutta*

A total of 121 specimens (105–305 mm  $L_S$ ) was selected from samples having catalogue numbers NRM 20125, 20126, 23404, 23406, 23661, 24343–24345, 24357, 24361, 24881, 24847, 24849, 24866, 24885, 24894, 24895, 24903, 36995, 41373, 41781, 41783, 41785, 41789–41791, 41793, 41794, 42539–42543, 42548, 42549, 42551, 42558 and 42559. The specimens came from several different Atlantic drainage localities in Sweden, Norway, Russia and Scotland. A rather large number of specimens was chosen to compensate for the lack of knowledge of the exact origin of the Atlantic alleles in the Soca basin. Further, such a heterogeneous sample also provides information about variation among *Salmo* populations usually referred to a single species, viz. *S. trutta*.

## ANALYSIS

Statistical analyses were performed using the SYSTAT 5.0 (Wilkinson *et al.*, 1992) or the Statistica (StatSoft, 1995) software packages. The correlation between colour pattern and allozyme genotype in the Volarja trout was tested using the Spearman rank order correlation. Colour patterns were coded from 1 to 4 (pure brown–pure marble). Allozyme genotypes were coded as: 1=alien (9090, 90100, 100100), 2=heterozygote (90120, 100120), 3=homozygote (120120) (Table I). As there was no *a priori* reason to treat either of the two alien alleles as closer to *S. marmoratus*, both were treated as alien.

Meristic characters were subjected to a discriminant analysis, using the comparative material as a training set (Wilkinson *et al.*, 1992) and *a priori* probabilities were adjusted from the default setting (0.5 for two groups of equal sample size) in accordance with the proportions of *S. marmoratus* (0.25) and *S. trutta* (0.75). The Volarja trout was weighted as zero (having no impact) and the analysis predicted them to be either *S. trutta* or

TABLE II. Classification of Volarja trout with the aid of discriminant analysis using comparative material meristics as a training set. Specimens grouped by colour pattern and by *LDH-5\** genotype

Colour pattern	Pure brown	Brown-like	Marble-like	Pure marble		
<i>S. trutta</i>	4	10	1	1		
<i>S. marmoratus</i>	1	9	23	7		
<i>LDH-5*</i>	9090	90100	100100	90120	100120	120120
<i>S. trutta</i>	3	2	1	8	2	
<i>S. marmoratus</i>	1	3	5	8	8	15

*S. marmoratus*. Each meristic character was studied also with the aid of Spearman rank order correlation for the Volarja trout only, with the same coding as described above. Measurements were omitted from discriminant analysis for two reasons: (i) the impossibility to adjust them for size in a theoretically acceptable way (Bookstein, 1985); and (ii) the absence of significant differences in morphometry between Atlantic basin *S. trutta* and *S. marmoratus*. Instead, measurements and counts were subjected to principal component analysis (PCA). The PCA was performed in two steps using a covariance matrix on log-transformed measurements and a correlation matrix on square-rooted counts (Bookstein, 1985). Principal component I (PC I) for morphometric data mainly represents overall size, whereas PC II and further components are less correlated to size (Bookstein, 1985). For the meristic data set, several uninformative characters (based on the comparative material) were omitted. The comparative material was included in this analysis. Then the morphological variation was studied by plotting scores of the most informative principal components in each data set, labelling the Volarja trout with their colour pattern or genotype. Contrary to discriminant analysis, PCA does not require *a priori* grouping of the material and the distribution of the comparative material and the Volarja trout in the biplots can be viewed as an assumption-free ordination of data.

## RESULTS

Classification based upon colour pattern and *LDH-5\** genotype is summarized in Table I. A Spearman rank order correlation ( $r=0.49$  and  $P=0.000076$ ) confirmed the obvious trend for the 120 allele to be more common in the marbled specimens. If the two alien alleles were compared there was also a tendency for 100100 homozygotes to be more marbled compared with the 9090 homozygotes. The tendency was similar for the heterozygotes.

The discriminant analysis on meristic characters classified the comparative material with 100% accuracy (not shown). Both colour pattern and *LDH-5\** genotype tended to be correlated with the meristic characters (Table II), e.g. out of 32 specimens denoted as pure marble or marble-like, 30 were classified as *S. marmoratus* and 31 out of 41 specimens having the 120 allele were classified also as *S. marmoratus* according to the meristics. As for the colour pattern, there was also a tendency for specimens having the 100 allele to be more marble-like in their meristics, compared with specimens having the 90 allele.



The most important characters in the discriminant analysis were those related to vertebral counts, number of expanded neural spines and number of procurent caudal fin rays (Table III). *Salmo marmoratus* has a higher number of vertebrae (mode 61) compared with *S. trutta* (mode 59). *Salmo marmoratus* also has a higher number of expanded neural spines (usually 5 or 6) compared with *S. trutta* (usually 4 or 5) but fewer upper and lower procurent caudal fin rays (mode 12 and 12, except for the Zadlascica population, mode 14 and 12) compared with mode 15 and 13 in *S. trutta*. For the Volarja trout, Spearman rank order correlations (Table III) were carried out as independent tests, whereas actually several characters were correlated highly with each other, e.g. number of abdominal vertebrae and anal fin position. When significant, the signs (+ or -) of correlation coefficients ( $r$ ) were in agreement with prior knowledge from the comparative material, except for unbranched dorsal fin rays and dorsal fin pterygiophores. One of the most important characters, the number of abdominal vertebrae, is summarized for the entire material in Table IV.

Morphometric characters were less informative. According to the PCA (Table V), body width and fin measurements had the highest loading on PC II, i.e., explain most of the variation not related to size. *Salmo marmoratus* had a wider, more cylindrical body, and shorter more rounded fins compared with *S. trutta*. Morphometric PC II was uncorrelated to size (log  $L_s$ ) ( $r=0.005$ ,  $P=0.94$ ).

Plotting meristic PC I on morphometric PC II placed the comparative material in two distinct clusters [Fig. 2(a), (b)]. The contribution from morphometry was small but not null. The Volarja trout overlapped with both *S. marmoratus* and *S. trutta* and there was a tendency for displacement along PC I towards the *S. marmoratus* cluster. When the Volarja trout specimens were labelled with their colour pattern [Fig. 2(a)], a majority of the specimens classified as pure marble or marble-like was found within the *S. marmoratus* cluster. If labelled by genotype instead [Fig. 2(b)], 14 out of 15 120120 homozygotes were found above or within the *S. marmoratus* cluster.

## DISCUSSION

There was a rather strong correlation between morphology and *LDH-5\** genotype, despite the continuous stocking of non-native trout since the turn of the 20th century. Colour pattern and meristic characters taken from radiographs were the most informative whereas morphometry held very little information. Regarding colour pattern (Table I) one cannot exclude the possibility of a strong linkage between the 120 allele and a marbled pattern. However, the Zadlascica population, almost fixed for the ancestral 100 allele (Berrebi *et al.*, in press), had a prominent marbled colour pattern and was thought previously to be the only pure *S. marmoratus* population in the Soca River.

The meristic characters showed strong correlations also, both when the Volarja trout were grouped according to colour pattern or *LDH-5\** genotype. Of course, also there could have been some linkage between colour pattern or *LDH-5\** genotype and meristic characters, but at least one specimen classified as pure marble had typical Atlantic *S. trutta* meristics [Fig. 2(a),  $\Delta$  within the *Salmo trutta* cluster]. Further, the Zadlascica population (15 out of the 41

TABLE III. Canonical loadings from discriminant analysis and character loadings from principal component analysis on meristic characters taken on 59 specimens of *Salmo* species from Volarja, 41 specimens of *S. marmoratus* and 119 specimens of *S. trutta*. Characters are sorted, starting with the one having the highest loading in discriminant analysis. The Volarja trout had no impact on canonical loadings. Results from Spearman rank order correlations are for the Volarja trout only. Ranks are given in text and Table I

	Canonical loadings	Meristic PC I	Spearman rank order correlations		
			Colour pattern <i>r</i>	LDH-5* genotype <i>r</i>	<i>p</i>
Abdominal vertbrae	0.572	0.889	0.42	0.00085	0.27
Dorsal fin position	0.494	0.831	0.35	0.0071	0.27
Anal fin position	0.412	0.756	0.39	0.0021	0.30
Expanded neural spines	0.358	0.729	0.65	<0.00001	
Vertebrae having expanded neural spine	0.316	0.678	0.31	0.017	0.36
Caudal fin upper procurent rays	- 0.293	- 0.591	- 0.27	0.041	0.006
Scales from adipose fin to lateral line	- 0.222	- 0.404			
Interneurals	0.207	0.623	0.39	0.0024	0.38
Caudal fin lower procurent rays	- 0.189	- 0.383			0.0031
Gill rakers on upper arch	- 0.177	- 0.407			
Caudal vertebrae	- 0.175				
Branched dorsal fin rays	0.120				
Anal fin pterygiophores	- 0.089				
Gill rakers on lower arch	- 0.087				
Branched anal fin rays	- 0.073				
Lateral line scales	0.068				
Unbranched dorsal fin rays	- 0.064				
Right side branchiostegal rays	0.059				0.26
Dorsal fin pterygiophores	0.025		0.42	0.0013	0.28
Unbranched anal fin rays	- 0.025				
Left side branchiostegal rays	- 0.021				
Variance explained (PCA)		42.6%			



TABLE IV. Number of abdominal vertebrae in comparative material of *S. marmoratus* and *S. trutta*, and in the Volarja trout sorted by colour pattern or *LDH-5\** genotype

	Number of abdominal vertebrae						
	33	34	35	36	37	38	39
<i>S. marmoratus</i> reference sample					12	20	9
<i>S. trutta</i> reference sample	5	31	40	26	10	2	
Volarja trout sorted by colour pattern							
Pure marble		1			6	2	
Marble-like			1	2	11	12	
Brown-like			2	8	7	2	
Pure brown			1	3	1		
Volarja trout sorted by <i>LDH-5*</i>							
120120					8	8	
100120		1	1	1	6	2	
90120			2	8	3	3	
100100					4	3	
90100				2	3		
9090			1	2		1	
Σ Volarja trout		1	4	13	25	16	

specimens of *S. marmoratus*) had the distinguishing meristic characters similar to the Idrijca and Trebuscica populations, with the notable exception of the number of upper procurent caudal fin rays.

Comparing the two alien alleles both when homozygous or heterozygous with the 120 allele we found that the presence of the 100 allele seemed to have less impact on morphology of *S. marmoratus*. Apparently the 100 allele came from a *Salmo* species more similar to *S. marmoratus* in morphology than to Atlantic *S. trutta*. Thus, the tendency for the entire Volarja sample to look more like *S. marmoratus* than *S. trutta* in the characters studied (Fig. 2) might be explained by the origin of the 100 allele.

However, other studies on salmonid hybrids, i.e. hybrids between *S. salar* and *S. trutta* (Wilkins *et al.*, 1994; Hedenskog *et al.*, 1997) and hybrids between *Salvelinus alpinus* and *S. fontinalis* (Hammar *et al.*, 1991), show that hybrids are not necessarily symmetrically distributed between the parental species. *Salmo trutta* × *salar* (F1) looks more like and overlaps with *S. trutta* (fig. 2 in Hedenskog *et al.* 1997). Also there is a tendency for *S. alpinus* × *fontinalis* to look more like and overlap slightly with *S. alpinus* (fig. 3 in Hammar *et al.*, 1991) even though at least one hybrid was a supposed back-cross with *S. fontinalis*.

Preferably, our study should have included also comparative material of non-Atlantic *Salmo*, representing the alien 100 allele. Most probably the 100 allele originates from the Danube (Berrebi *et al.*, in press). However, as the 100 allele is found in all *Salmo* species in the neighbourhood, except for *S. marmoratus* and introduced hatchery strains of Atlantic *S. trutta*, and as those species taken together are thought to possess more morphological variation in

TABLE V. Character loadings on principal components I-III for 28 measurements taken on 221 specimens, both Volarja trout and comparative material. Five highest loadings indicated in bold

	PC I	PC II	PC III
Standard length	0.288	0.009	0.015
Preanal length	0.292	0.008	0.014
Prepelvic length	0.300	0.013	0.006
Predorsal length	0.295	0.022	-0.001
Head length	0.292	0.016	-0.018
Premaxilla to preoperculum length	0.309	0.008	-0.025
Caudal peduncle length	0.261	0.012	0.028
Caudal peduncle depth	0.273	0.013	0.024
Length of upper caudal fin lobe	0.285	- <b>0.049</b>	0.011
Length of lower caudal fin lobe	0.283	- <b>0.046</b>	0.006
Length of middle caudal fin ray	0.303	-0.020	-0.006
Dorsal fin length	0.279	-0.035	0.014
Pectoral fin length	0.268	- <b>0.050</b>	0.012
Pelvic fin length	0.284	- <b>0.051</b>	0.018
Adipose fin length	0.271	0.038	0.023
Anal fin length	0.299	-0.027	0.009
Body width	0.232	<b>0.067</b>	0.012
Body depth at dorsal fin	0.287	0.011	<b>0.052</b>
Body depth at anal fin	0.291	0.011	<b>0.044</b>
Head depth	0.287	0.000	-0.004
Horizontal orbit diameter	0.225	-0.027	- <b>0.037</b>
Vertical orbit diameter	0.220	-0.038	- <b>0.046</b>
Interorbital width	0.324	0.005	0.008
Snout length	0.316	0.028	-0.020
Upper jaw length	0.323	0.015	-0.031
Length of maxilla	0.329	0.011	- <b>0.036</b>
Height of maxilla	0.273	0.034	- <b>0.036</b>
Lower jaw length	0.327	0.012	-0.032
Variance explained (%)	96.0	1.01	0.73

studied characters compared with Atlantic *S. trutta*, any inclusion of Danubian/ Mediterranean material without knowledge of the origin of the 100 allele would obscure the analysis.

If the Soca hybrid population (to which the Volarja trout belongs), has interbred freely for a long time, genetic and morphological markers would be perfectly mixed and no correlation between any kind of marker would be possible. The fact that strong correlations between each kind of marker have been demonstrated here, indicates that panmixia has not been reached in this zone. The most obvious explanation of the observed pattern is that the Volarja trout has not behaved as a random mating population, e.g., that *S. marmoratus* and the alien *Salmo* species were spatially, behaviourally, and/or temporarily segregated during spawning. It is also possible that hybridization was selected against either because the stocked alien *Salmo* species were less competitive compared with *S. marmoratus* or because hybrid genotypes have a lower fitness (Leary *et al.*, 1995). If this is the case, high proportions of *S. trutta* phenotype

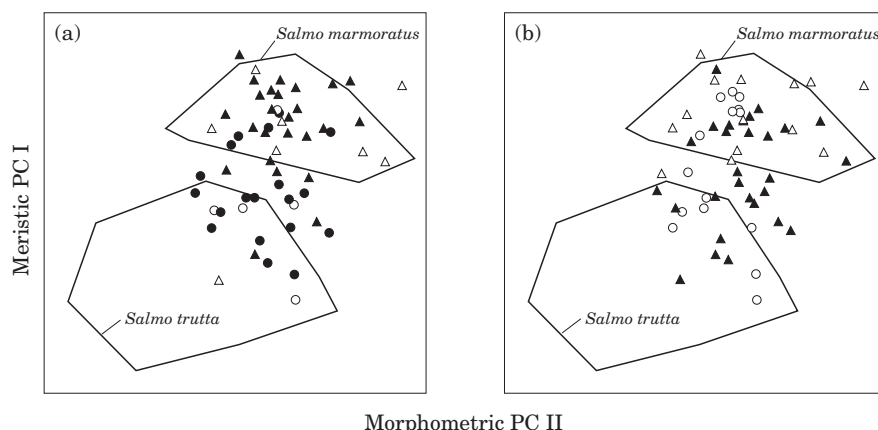


FIG. 2. Plots of scores of first meristic principal component on second morphometric principal component. Corresponding loadings are given in Tables III and V. For the comparative material, *S. marmoratus* and *S. trutta*, only the outlines of the clusters are given. (a) Volarja trout labelled by colour pattern:  $\Delta$ , pure marble;  $\blacktriangle$ , marble-like;  $\circ$ , pure brown;  $\bullet$ , brown-like. (b) Volarja trout labelled by *LDH-5\** genotype:  $\Delta$ , 120120;  $\blacktriangle$ , 90120, 100120;  $\circ$ , 9090, 90100, 100100.

and hybrids depend largely on the continuous stocking of alien trout. In 1996 the Slovenian Government banned stocking alien *Salmo* species.

It is also probable that the Volarja Stream receives pure *S. marmoratus* continuously from unaffected localities upstream. If so, this natural introduction of pure genotype prevents panmixia being reached even if the above suggested selection against hybrids and alien *Salmo* species is weak.

Studies on introgression between native and introduced trout in the Doubs River, Rhône River basin, support limited hybridization between different *Salmo* species. Based on phenotype (colour pattern) and genotype, Largiadèr & Scholl (1996) showed that the two forms do not hybridize freely in nature. The native Doubs trout is characterized by four broad black vertical bars and thereby is distinguishable easily from the alien *Salmo* species, which Largiadèr & Scholl (1996) concluded to be of Atlantic origin. Largiadèr & Scholl (1996) also found differences in introgression pattern between different localities. The same kind of analysis involving southern french Mediterranean trout populations subject to stocking have also shown important departure from panmixia and linkage disequilibria using allozymes, mtDNA (Poteaux *et al.*, 1998b), and microsatellite markers (Poteaux *et al.*, 1999). On the other hand, natural introgression between Atlantic forms (modern and ancestral *sensu* Hamilton *et al.*, 1989) also show departure from panmixia (Aurelle & Berrebi, 1998; Aurelle, 1999). Thus, limited hybridization is observed often between *Salmo* species, even if formal departure from Mendelian transmission in experimental conditions is difficult to demonstrate (Poteaux *et al.*, 2000). Behavioural explanations that suggest assortative mating are often forwarded (Aurelle, 1999). Other studies, e.g. Morán *et al.* (1991) and Beaudou *et al.* (1994), dealing with the impact of stocking in Spanish and French Mediterranean rivers, respectively show also that intense stocking of alien *Salmo* species, in these cases distinguishable by the 90 allele in the *LDH-5\** locus, seems sometimes to have little or no effect at all on the native population.

Whatever the explanations are, c. 10% of the trout in the Volarja stream both look like *S. marmoratus* and are homozygous for the 120 allele in the *LDH-5\** locus. Consequently, based on present day knowledge these specimens should be regarded as *S. marmoratus* and measures suggested in the Action Plan for *S. marmoratus* (Povz *et al.*, 1996), such as allowing larger bag limits and smaller minimum size for specimens having an *S. trutta* or hybrid like phenotype, can reduce the amount of alien *Salmo* species and their genes in the Soca River and favour the re-establishment of *S. marmoratus* in the Soca River. However, a better description of the colour pattern and its variation among populations of *S. marmoratus* is needed.

In the Soca River it is not possible to discriminate hybrids from pure *S. marmoratus* based on morphological characters only, but colour pattern alone or in combination with molecular data can be a powerful tool in management of *S. marmoratus* and possibly in other phenotypically distinct *Salmo* populations subject to introgression.

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