Morphological variation in the freshwater blenny *Salaria fluviatilis* from Corsican rivers: adaptive divergence, phenotypic plasticity or both?

P. Magnan*‡, R. Proulx*, P. Berrebi§, J. Blondel‖, P. Perret‖
and B. Roché¶†

Centre de recherche sur les interactions bassins versants – écosystèmes aquatiques (RIVE), Université du Québec à Trois-Rivières, C.P. 500, Trois-Rivières, QC, G9A 5H7 Canada, §Institut des Sciences de l'Évolution, UMR 5554 UM2-CNRS-IRD, Université Montpellier II, CC065, place E. Bataillon, 34095 Montpellier, Cedex 5, France, ‖Centre d'Écologie Fonctionnelle et Évolutive, CNRS Montpellier, 34293 Montpellier, Cedex 5, France and ¶Service de l'eau et des milieux aquatiques, Direction régionale de l'environnement de Corse, 20600 Bastia, France

The first goal of this study was to determine whether morphological variation in the freshwater blenny *Salaria fluviatilis* results in spatially structured populations distributed around Corsica, France, which would suggest genetically differentiated populations through reproductive isolation by distance. The second goal was to determine whether some morphological traits are related to water velocity, one of the most contrasting habitat characteristics in these rivers, which would suggest an adaptation to local conditions. The results showed that the morphology of *S. fluviatilis* differed among the three main geographic areas studied in Corsica and that geographically distant populations of *S. fluviatilis* were less similar morphologically and genetically than close ones. The results also indicated that the morphological differences among populations conformed to functional expectations. Overall, the results suggest that the morphological variation of *S. fluviatilis* from Corsican rivers is an adaptive response to water velocity and that these populations are in a process of reproductive isolation by distance.

© 2013 The Fisheries Society of the British Isles

INTRODUCTION

Reproductive isolation by distance (Pope *et al.*, 2006; Mock *et al.*, 2007) or physical barriers (Poissant *et al.*, 2005; Crispo *et al.*, 2006) is the first step of allopatric speciation. Geographically separated populations may diverge *via* genetic drift (Crispo & Chapman, 2008) and from responses to different selection regimes. Furthermore, even if pre- and post-zygotic isolating mechanisms arise as a by-product of genetic divergence in allopatry, their evolution can be accelerated by divergent selection

‡Author to whom correspondence should be addressed. Tel.: +1 819 376 5011 ext. 3375; email: Pierre.Magnan@uqtr.ca

†Deceased. We dedicate this paper to our late colleague.

(Schluter, 2001; Turelli *et al.*, 2001). Species that are distributed over large geographical areas and experience various environmental conditions must be able to adapt to variations in local conditions in order to maximize their fitness. One example of adaptive phenotypic plasticity of populations within the island of Corsica is that of the blue tit *Cyanistes caeruleus* which exhibits a tremendous variation in morphology and in life-history traits such as breeding time and clutch size (Blondel *et al.*, 2006). Phenotypic plasticity and the resulting reaction norms are important ecological agents for expanding the habitat range of a species (Stearns, 1989; Kawata, 2002; DeWitt & Scheiner, 2004) and for creating opportunities for new selective pressures to act (Stearns, 1989; Pigliucci, 2001; West-Eberhard, 2003; Pfennig *et al.*, 2010). Pfennig *et al.* (2010) provided examples in which phenotypic plasticity can play an important role in driving diversification and speciation. In this context, determining whether variation of species traits is related to genetic differences and functional consequences is crucial for understanding how populations adapt to their local environment.

The freshwater blenny *Salaria fluviatilis* (Asso y del Río 1801) is one of the four freshwater representatives of the large marine family Blenniidae (Kottelat, 2004; Brigg, 2010). Its range extends through rivers and lakes that drain into the Mediterranean Sea as well as some Atlantic rivers in Morocco (Zander, 1972; Changeux & Pont, 1995; Perdices *et al.*, 2000). There is phylogenetic evidence that *S. fluviatilis* and the peacock blenny *Salaria pavo* (Risso 1810) diverged from a common ancestor around the Middle Miocene (Almada *et al.*, 2009). The allozyme variation of some populations from the Iberian peninsula suggests that isolation by distance could be the main factor explaining the genetic distance among river populations following a single colonization and dispersal event (Perdices *et al.*, 2000). The ancestor of *S. fluviatilis* could have migrated into fresh water at the beginning of the Pliocene, some 4 million years ago, evolving into the strictly freshwater *S. fluviatilis* (Zander, 1972; Almada *et al.*, 2009). As a result, salinity could act as a physiological barrier to gene flow among river populations of the species despite its capacity to acclimate to sea water under experimental conditions (Plaut, 1998). During their evolution from a marine to a freshwater form, however, *S. fluviatilis* populations from different rivers might have been in contact through brackish, oligosaline and even freshwater conditions, the last two possibly occurring at every glacial maximum in some areas of the Mediterranean Sea (Bianco *et al.*, 1996; Almada *et al.*, 2009). In this context, geographically close populations would be expected to be genetically more similar than more distant ones. Some traits, however, should still be largely shaped by environmental drivers producing phenotypic plasticity because Mediterranean rivers exhibit large gradients of environmental conditions, both spatially and temporally. The Corsican Island represents a good system for studying the effects of both genetic diversification and phenotypic plasticity in *S. fluviatilis* because populations are distributed in geographically distinct catchments, and yet weakly connected to one another along the coastline.

The first goal of this study was to determine whether morphological variation of *S. fluviatilis* is spatially structured in seven rivers from different regions of Corsica. If true, this would suggest a divergence through reproductive isolation by distance. To test this hypothesis, genetic analyses (intron polymorphisms) were carried out on a sub-set of four populations (two on the east coast and two on the west coast). The second goal was to determine whether some morphological traits were related

to water velocity (among and within rivers). Water velocity is one of the most contrasting habitat characteristics in these rivers and such a relationship would suggest an adaptation to local conditions. It has been shown that morphological traits exhibit responses to water velocity in many species of fishes and that these responses allow individuals to cope with varying conditions of their environments (Peres-Neto & Magnan, 2004; Fischer-Rousseau *et al.*, 2010). *Salaria fluviatilis* prefer zones of high water velocity with a substratum dominated by boulder, stone and gravel (Freeman *et al.*, 1990; Roché, 2001), and it is recognized that water velocity is a driving factor in the ecology of the species (Freeman *et al.*, 1990). *Salaria fluviatilis* provides a good model for investigating these questions because it is found in many rivers flowing into the Mediterranean Sea (Zander, 1972; Roché, 2001) and under a large gradient of environmental conditions, especially water velocity.

MATERIALS AND METHODS

STUDY AREA, FISH SAMPLING AND WATER VELOCITY MEASUREMENTS

The study was carried out in seven rivers on the island of Corsica. Three of these rivers were located on the east coast (Fium'Orbo, Golo and Tavignano) and the other four on the west coast (Fango, Liamone, Rizzanese and Taravo) (Fig. 1). Rivers Fango, Golo (site Leccia) and Liamone were sampled from 9 to 16 June 1999, Fium'Orbo, Golo (sites Casamozza and Querrio) and Tavignano from 5 to 7 June 2000 and Rizzanese and Taravo on 19 June 2001. Between 29 and 71 fish were sampled at each site using a Dream Electronique electrofishing device (model Héron; 200–1000 V; 1–4 A; www.dream-electronique.com). The fish were kept alive until morphological measurements were taken in the laboratory, within 6–36 h. Water velocity was measured with a Mini Pygmy Current Meter (www.rickly.com) and estimated at each site from 15 to 35 readings. One continental population from the European mainland (Var River, south of France; 43° 39′ N; 7° 11′ E) was included in the molecular analysis as a reference for the populations inhabiting most of the estuaries of the north Mediterranean coast. Continental populations in rivers opening to the sea are considered at the core of the species range while lake and island populations (including Corsica) are considered to be at the periphery of the species range.

MORPHOLOGICAL MEASUREMENTS

Fish were sacrificed with an overdose of Alka-Seltzer (Summerfelt & Smith, 1990) prior to morphological measurements. Eleven morphological characteristics were measured on each fish: body depth, body width, head depth, head width, lower jaw length, pectoral-fin length, dorsal-fin base length, pelvic-fin length, anal-fin length, caudal-fin length and peduncle depth (Fig. 2). Total length (L_T, ±0·01 mm), mass (±0·1 g) and sex of each fish were also determined. Morphological measurements were taken using a Mytutoyo digital calliper connected to a data transmitter and a computer database.

MOLECULAR ANALYSES

Intron polymorphism analyses were carried out on four Corsican populations (Golo and Tavignano Rivers from the east coast, and Rizzanese and Taravo Rivers from the west coast) and on one continental population from the European mainland (Var River) for a polymorphism level comparison. Corsican populations are considered to be in a peripheral zone, isolated from the core of the *S. fluviatilis* populations. A classical effect of peripheral populations is their low polymorphism (Eckert *et al.*, 2008). The continental population was
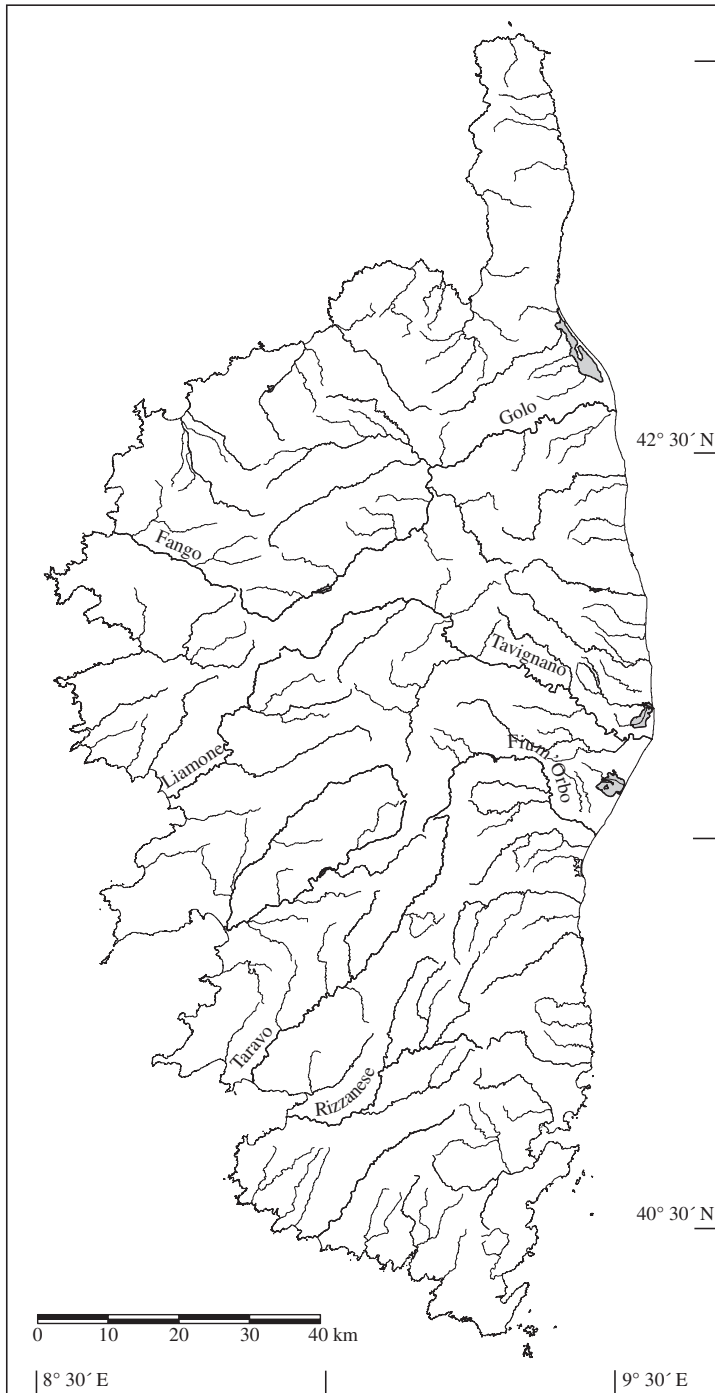
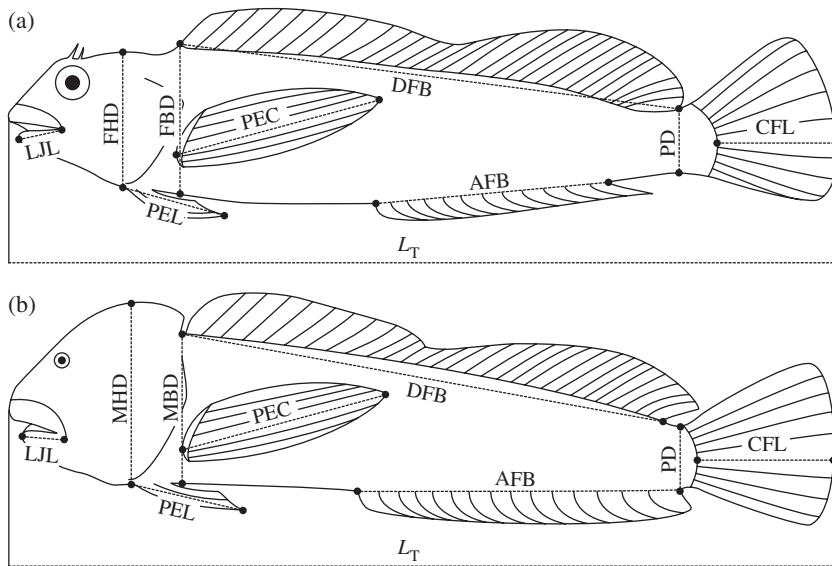FIG. 1. Location of the study sites in Corsica, France.

FIG. 2. Positions of the 13 morphological characteristics for (a) females and (b) males associated with swimming used in this study. AFB, anal-fin base length; CFL, caudal-fin length; DFB, dorsal-fin base length; FBD, female body depth; FHD, female head depth; LJL, lower jaw length; L_T, total length; MBD, male body depth; MHD, male head depth; PD, peduncle depth; PEC, pectoral-fin length; PEL, pelvic-fin length. The head width and body width were taken dorsally at the position of head depth and body depth measurements.

included to test for this effect. DNA was extracted from the fin tissues of 29–31 individuals per river using the Chelex–proteinase K method (Estoup *et al.*, 1996). Polymerase chain reactions (PCRs) were carried out in a total volume of 10 μl, which contained 1 μl of 10× buffer (Promega; www.promega.com), 2·5 mM MgCl$_2$, 0·2 mM of each dNTP (Invitrogen; www.invitrogen.com), 0·5 mM of each primer (MWG-Biotech AG, labelled with CY5 or fluorescein; www.mwg.biotech.com), 0·3 U of Taq polymerase (Sigma Aldrich; www.sigmaaldrich.com) and 1 μl of DNA template (at *c*. 50–150 mg ml^{-1}). Thermocycling conditions in an Eppendorf Mastercycler (www.eppendorf.com) consisted of an initial denaturation at 94° C for 3 min followed by 35 cycles of denaturation at 94° C for 1 min, annealing at 53 and 56° C for (loci *CK7* and *GPD2*) for 1 min, extension at 72° C for 1 min 20 s and a final extension at 72° C for 10 min.

Only length polymorphisms of intron amplification were analysed. All necessary details concerning the informative intron systems are given in the study of Berrebi *et al.* (2006). Among the 26 tested intron systems, three were successfully amplified (to give three polymorphic loci), but only two gave interpretable patterns (loci *CK7* and *GPD2*), presumably due to the poor preservation of some samples. The term system is used to designate all the production of a given PCR, which can correspond to several loci due to sequence duplications in the *S. fluviatilis* genome (*e.g.* tandem duplications and pseudogenes; Hassan *et al.*, 2002; Atarhouch *et al.*, 2003). To ensure that only target loci were amplified, each system was first tested by a thermal gradient PCR in an Eppendorf Mastercycler gradient to determine the maximum annealing temperature that allowed amplification. When several loci were amplified, the fixation sequences could be confidently assumed to be the same or similar to the universal primers' complements. One microlitre of PCR products from each individual was loaded onto an 8% denaturing polyacrylamide gel (Bio-Rad; www.bio-rad.com). The PCR products were visualized with a FMBIO fluorescent imaging system (Hitachi; www.hitachi.com). Allele sizes were determined using a fluorescently labelled ladder of known size (Promega) with the FMBIO ANALYSIS 8.0 image analyser programme (Hitachi).

STATISTICAL ANALYSES

All morphological characteristics were significantly related to L_T and sex ($P < 0.05$). The variance associated with these two variables was controlled by computing the residuals of the allometric relationship between each morphological variable and L_T for each sex separately (Fleming *et al.*, 1994; Proulx & Magnan, 2004). Data for each morphological descriptor were first $\log_{10}$ transformed and standardized (mean of 0 and S.D. of 1). Each descriptor was then expressed as the deviation of individuals (regression residuals) from the pooled within-group regression line describing the relationship between the descriptor and L_T. These residuals are considered to be independent of fish size and should reflect the variation resulting from measurement error and the biological deviation of individuals from the predicted characteristic and length relationship (Kuhry & Marcus, 1977).

A principal component analysis (PCA) was conducted on the correlation matrix among size-adjusted morphological characteristics to evaluate their correlation structure across rivers. PCA summarizes all characteristics into a set of statistically independent variables, the principal components (PCs) (Tabachnick & Fidell, 2001). PCA has the advantage of making no assumption about the existence of groups and thus allows for their independent identification (Humphries *et al.*, 1981). The loading coefficients represent correlations of the characteristics with the PCs. Informative PC axes were retained following a visual inspection of the screen plot and a quantitative assessment based on the Kaiser–Guttman criterion (Legendre & Legendre, 1998). To determine if there was a relationship between water velocity and the morphological characteristics (mean PC scores) of *S. fluviatilis*, each PC axis was independently regressed against water velocity.

With the genotype matrix, factorial correspondence analyses (FCAs) were first performed, which is a multidimensional technique well suited to binary genotypic data (She *et al.*, 1987). This technique allowed interpretation of the intron polymorphisms of the four populations studied and provided a first estimation of the overall genetic structure. These analyses were performed with the GENETIX software (Belkhir *et al.*, 1998). Because this kind of analysis can demonstrate the existence of unexpected taxa, it is a prerequisite before other calculations are made. Individuals were first coded according to the presence of the different alleles with values 0 (allele absent), 1 (heterozygotic for the allele) or 2 (homozygotic for the allele). The computation then aims at finding composite axes that are a combination of the variables (the alleles) that optimize differences between individuals. These multidimensional analyses require a matrix with no missing data. For this reason, FCAs were performed with 96 individuals instead of the 127 initially involved. Two FCAs are presented here, one including the Var River (mainland) together with Corsican samples, and the other with data from the Corsican rivers only.

The heterozygosity parameters (H_{nb}; Nei, 1978; calculated with GENETIX) were based on the two polymorphic loci (*CK7* and *GPD2*) and thus must be interpreted with caution as majority of monomorphic loci (>30) were not involved in the calculation. Results do not represent the estimated polymorphism of the species but rather the relative polymorphism among samples. In the same manner, observed heterozygosity (H_{obs}) and the mean number of alleles per locus were computed based on the two loci.

The F_{st} values were estimated between populations to illustrate their level of differentiation. The estimator θ of Weir & Cockerham (1984) was calculated using GENETIX and its significance was based on 5000 permutations. For a given pair of samples, the permutations produced 5000 artificial matrices where individuals were permuted randomly between samples, giving unstructured matrices (null hypothesis). The percentage of artificial matrices where θ is equal to or higher than the true value gives the empirical level of significance of θ.

RESULTS

MORPHOLOGICAL ANALYSES

The first three PCs summarized 32, 13 and 11% of the total variation in the morphology of individuals sampled in the study rivers (Table I). PC1 mostly accounted for inter-individual variation in caudal fin, lower jaw, head length and

TABLE I. Canonical loading of the first three principal components (PCs) derived from the size-adjusted morphological characteristics of *Salaria fluviatilis* from Corsica

Morphological trait	PC1 (32%)	PC2 (13%)	PC3 (11%)
Pectoral fin length	0·26	−0·67	−0·30
Anal fin length	−0·17	−0·61	0·26
Dorsal fin length	−0·28	0·02	−0·81
Pelvic fin length	0·58	−0·53	0·07
Caudal fin length	0·66	−0·30	−0·26
Lower jaw	0·69	0·08	0·26
Head depth	0·58	0·12	0·07
Head length	0·81	0·15	0·06
Peduncle depth	0·51	0·21	−0·38
Body width	0·71	0·32	−0·06

Coefficients represent the correlation of each characteristic with component scores.

body width; PC2 for pectoral-, anal-, and pelvic-fin lengths and PC3 accounted for dorsal-fin length and peduncle depth. The PCA considering all morphological characteristics of *S. fluviatilis* provided an overall description of differences in body shape, at least for characteristics that correlated across rivers (Fig. 3). PC1 clearly separated the northern populations of Corsica from the southern ones while PC2 separated the three Golo sites from the other two eastern populations (Fig. 3). PC3 separated the north-east and north-west populations (Fig. 3), with the exception of one site in the Golo River.

No significant relationship was found between water velocity and the mean PC1 and PC2 scores ($P > 0.05$). In contrast, the water velocity explained 55% of the variation in the mean PC3 scores ($P < 0.05$; Fig. 4). The loading of canonical coefficients on PC3 indicated that the dorsal-fin length and peduncle depth were the best variables describing the relationships between morphological traits and water velocity (Table I), and their coefficients indicated that they were inversely related to water
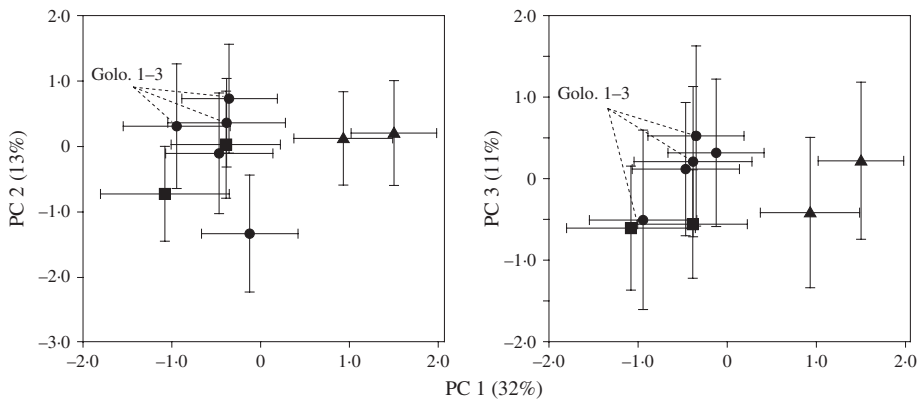

FIG. 3. Mean ± S.D. canonical scores derived from the size-adjusted morphological characteristics of *Salaria fluviatilis* sampled in the seven study rivers from north-east (●), north-west (■) and south-west (▲) Corsica (computed from the correlation matrix of the principal component analysis).
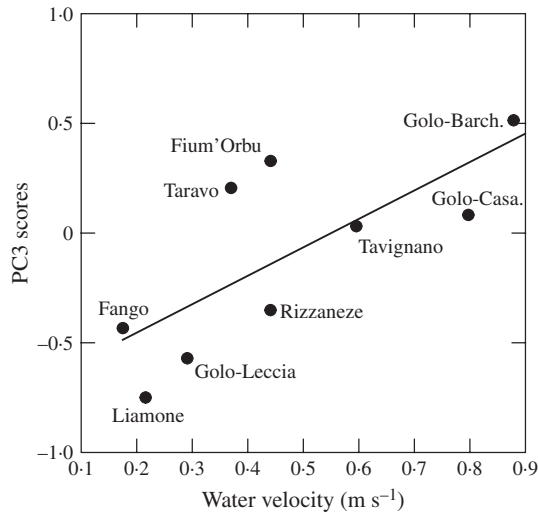
FIG. 4. Relationship between water velocity and mean PC3 scores. The curve was fitted by $y = 1.36x - 0.77$ ($r^2 = 0.55$, $P < 0.05$).

velocity. The relationship between water velocity and the mean PC3 scores was also observed for the three stations of the Golo River (Fig. 4), but this relationship was not significant ($P > 0.05$), probably due to the low power of the analysis ($n = 3$ sites).

MOLECULAR ANALYSES

Population heterozygosity ranged between 0·40 and 0·74 (H_{nb}; Table II). The present results show a clear difference in heterozygosity between the mainland Var (0·74) and Corsican populations (0·40–0·50). Furthermore, heterozygosity of the Corsican populations was nearly the same (*c*. 0·53) except for the Golo population ($H_{nb} = 0.40$). These observations were supported by the higher mean number of alleles in the Var (9) compared to the Corsican (2·5–4·5) populations (Table II). The difference between the unbiased expected heterozygosity (H_{nb}) and the observed heterozygosity (H_o) indicated a panmictic disequilibrium (Table II).

The first FCA clearly discriminates the Var and the Corsican populations [Fig. 5(a)]; this reflects the higher polymorphism of the Var population (Table II). The second FCA does not show a clear genetic structure among Corsican populations [Fig. 5(b)]. In contrast, F_{st} estimates showed that only two of the six comparisons were not significantly different: Golo and Tavignano from the east coast and Rizzanese and Taravo from the west coast (Table III).

DISCUSSION

The results of this study show that the morphology of *S. fluviatilis* significantly differs among the three main geographical areas studied in Corsica (north-east, north-west and south-west). Furthermore, intron analyses suggest that a part of these differences could have a genetic basis, at least between the north-east and south-west

TABLE II. Allele frequencies of the two polymorphic intron loci (*GPD2* and *CK7*), sample size (*n*), unbiased expected (H_{nb}; Nei 1978) and observed (H_{obs}) heterozygosity, and mean allele number per locus (*A*) for *Salaria fluviatilis* from the Rizzanese, Taravo, Golo and Tavignano Rivers (Corsica) and Var River (continental France)

	Rizzanese	Taravo	Golo	Tavignano	Var
GPD2 (*n*)	30	16	25	26	30
250	–	–	–	–	–
258	0·6167	0·5625	1	0·8077	0·6
260	0·35	0·4375	–	0·1731	0·2333
262	0·0167	–	–	–	0·0667
264	–	–	–	–	0·0667
266	0·0167	–	–	–	0·0333
268	–	–	–	0·0192	–
CK7 (*n*)	28	22	9	16	29
206	–	–	–	–	–
234	–	–	–	–	–
290	–	–	–	0·0313	–
292	0·5893	0·6591	0·1667	0·3125	0·1897
294	–	–	–	0·0313	0·1207
296	–	–	–	–	0·1207
298	–	–	–	–	0·0172
300	0·0179	0·0455	–	0·125	–
302	–	–	–	–	0·0345
304	–	–	–	–	–
306	0·3393	0·2405	0·2222	0·2813	0·1034
308	0·0536	0·0682	0·3889	0·2188	0·2414
310	–	–	0·2222	–	0·0345
312	–	0·0227	–	–	0·0172
314	–	–	–	–	0·0172
316	–	–	–	–	0·069
318	–	–	–	–	0·0172
322	–	–	–	–	0·0172
H_{nb}	0·5273	0·5304	0·3958	0·5272	0·735
H_{obs}	0·4815	0·3571	0·1875	0·3125	0·3966
A	4	3·5	2·5	4·5	9

populations. Finally, two points suggest that the morphological differences among populations are an adaptive response to local habitats, namely: (1) the significant relationship between water velocity and morphology (PC3 scores) of the Corsican populations and (2) the fact that morphological differences among populations conform to functional expectations (adaptation to water velocity). This morphological variation may result not only from adaptive phenotypic plasticity (reaction norms) but it may also result from developmental fixed traits that vary among sites because of local, genetically determined adaptation (microevolutionary) processes.

All known populations of *S. fluviatilis* are strictly restricted to fresh water (Perdices *et al.*, 2000). In an experimental study, Plaut (1998) showed that this species is able to osmoregulate both in fresh and sea water after a 3 month acclimation to 0, 40 and 100% sea water (salinity of 0·0, 14·4 and 36·0). In an extension of this study,
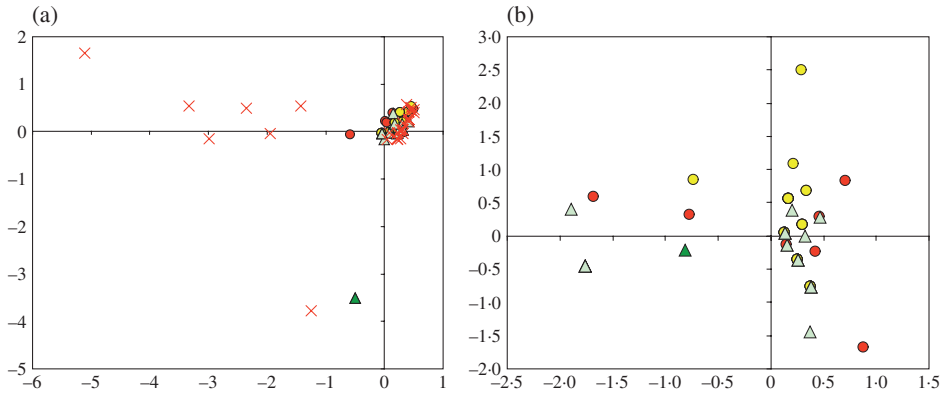
FIG. 5. Factorial correspondence analysis based on intron polymorphism for *Salaria fluviatilis* from the west (●, Rizzanese; ●, Taravo) and east (▲, Golo; △, Tavignano) coasts of Corsica and the Var Rivers (×): (a): all populations and (b) Corsican populations.

however, Plaut (1999) showed that standard metabolic rates of *S. fluviatilis* were significantly higher in fresh water than in iso-osmotic conditions (375 mOsm l^{-1}) and in sea water (1000 mOsm l^{-1}); the author suggested that the reduced metabolic activity associated with salinity could be a reaction to chronically suboptimal environmental conditions. In this context, salinity could have acted as a limitation to gene flow among Corsican rivers. Therefore, isolation by distance is likely to be the main factor explaining the morphological and genetic differences between north-east and south-west populations. It will, however, be necessary to run experimental acclimations of larvae to sea water to determine whether they can drift or swim from one river to another. Frequent floods occurring in some rivers after heavy rains (Beaudou *et al.*, 1995; P. Magnan, pers. obs.) are probably more susceptible to flush small individuals into the river estuaries. Furthermore, more detailed genetic analyses with numerous polymorphic loci (*e.g.* with microsatellite markers) could reveal gene flow between rivers.

To broaden the understanding of morphological variation beyond this limited area (Corsican rivers), genetic relationships were analysed among some other populations

TABLE III. F_{st} and statistical significance of the differences between populations (based on 5000 permutations of individuals randomly reassigned in the two populations). The F_{st} between each pair of populations was considered significantly different from zero ($P <$ 0·05; in bold) when the observed value was outside the central 95% values of the generated distribution from the permutations

	Taravo	Golo	Tavignano	Var
F_{st}				
Rizzanese	−0·00871	**0·23988**	**0·05776**	**0·09077**
Taravo		**0·30630**	**0·09341**	**0·09569**
Golo			0·03768	**0·08607**
Tavignano				**0·02484**

of *S. fluviatilis*. First, an analysis was carried out to see whether the insular populations were similar to continental ones, considering that the series of estuarine populations along the French Mediterranean Sea coast should be similar. The results from this study suggest that continental estuarine populations of *S. fluviatilis* are nearly 50% more diverse than the insular populations (0·50 v. 0·75), at least for neutral nuclear markers. Such a low polymorphism in Corsica suggests the recent establishment of these insular populations from one or a few continental mother populations. Second, a genetic organization of the Corsican populations is supported by the F_{st} estimations. Of the six among-river comparisons, four differed significantly or highly significantly. Only two F_{st} values were not statistically different from zero: the Golo and Tavignano Rivers ($F_{st} = 0·038$, $P > 0·05$), which are two eastern rivers, and the Taravo and Rizzanese Rivers ($F_{st} = 0$, $P > 0·05$), which are both located on the west coast of the island and flow into the same marine bay. These results suggest that populations inhabiting the same side of the island are more similar than populations located on opposite sides. The rivers were geographically close on each side of the island, but further analyses will be necessary to determine whether there is a relationship between the genetic diversity of the populations of *S. fluviatilis* and the geographical distance of the estuaries of their home rivers. The molecular allozyme data provided by Perdices *et al.* (2000) suggest isolation by distance in Iberian populations.

The intron loci tested (26) were not useful because of a low rate of polymorphism. Another category of neutral nuclear markers must be determined in order to investigate the polymorphism of this species more completely. Introns are generally good markers to describe phylogeographic structures (Berrebi *et al.*, 2005, 2006). A general heterozygote deficiency was observed in this study, but it is not possible at this stage to discriminate which mechanism is involved: genotyping errors, selection, inbreeding or the Walhund effect (*e.g*. migration and isolated age cohorts).

There is a large body of literature on the effect of water velocity on fish morphology. Most experimental evidence indicates that changes in morphology (often over relatively short periods of time) are phenotypic responses to water velocity (Peres-Neto & Magnan, 2004; Fischer-Rousseau *et al.*, 2010). It is not possible from the results of this study, however, to determine if the relationship between morphology and water velocity is a genetic adaptation or an expression of phenotypic plasticity within a reaction norm (or an interaction between these). Only an experimental study on forced swimming at different water velocities could confirm this hypothesis (Peres-Neto & Magnan, 2004).

It is generally accepted that among- and within-species differences in morphology are adaptive because they conform to functional expectations (Webb, 1982, 1984; Walker, 1997; Robinson & Parsons, 2002), even if these relationships have little experimental support (Rouleau *et al.*, 2009). The significant relationship between water velocity and the mean PC3 scores of the Corsican populations reveals that the length of the dorsal fin, and to some extent those of the pectoral and caudal fins, as well as peduncle depth are inversely related to water velocity. These relationships are consistent with the usual predictions of functional morphology: fin length and width and caudal-peduncle depth are inversely related to water velocity because they reduce drag at higher swimming speeds and require less energy for sustained cruising (Webb, 1982, 1984). The reduction in fin length and peduncle depth with increasing water velocity is presumably adaptive.

In conclusion, this study shows that the morphology of *S. fluviatilis* differs among the three main geographical areas studied in Corsica and that geographically distant populations are less similar morphologically and genetically than close ones. The results presented here also suggest that the morphological variation among populations studied conforms to functional expectations and, thus, should be adaptive. Further experiments will be needed to determine the relative contribution of genetic adaptation and phenotypic plasticity to the relationship between morphology and water velocity in *S. fluviatilis*.

References

Almada, V. C., Robalo, J. I., Levy, A., Freyhof, J., Bernardi, G. & Doadrio, I. (2009). Phylogenetic analysis of Peri-Mediterranean blennies of the genus *Salaria*: molecular insights on the colonization of freshwaters. *Molecular Phylogenetics and Evolution* **42,** 424–431.

Atarhouch, T., Rami, M., Cattaneo-Berrebi, G., Ibanez, C., Augros, S., Boissin, E., Dakkak, A. & Berrebi, P. (2003). New primers for EPIC amplification of intron sequences for fish and other vertebrate population genetic studies. *BioTechniques* **35,** 676–682.

Beaudou, D., Baril, D., Roché, B., Le Baron, M., Cattaneo-Berrebi, G. & Berrebi, P. (1995). Recolonisation d'un cours d'eau corse dévasté: contribution respective des truites sauvages et domestiques. *Bulletin Francais de la Pêche et de la Pisciculture* **337,** 259–266.

Berrebi, P., Boissin, E., Fang, F. & Cattaneo-Berrebi, G. (2005). Intron polymorphism (EPIC-PCR) reveals phylogeographic structure of *Zacco platypus* in China - a possible target for aquaculture development. *Heredity* **94,** 589–598.

Berrebi, P., Retif, X., Fang, F. & Zhang, C.-G. (2006). Population structure and systematics of *Opsariichthys bidens* (Osteichthyes: Cyprinidae) in south-east China using a new nuclear marker: the introns (EPIC-PCR). *Biological Journal of the Linnean Society* **87,** 155–166.

Bianco, P. G., Ahnelt, H. & Economidis, P. S. (1996). The freshwater fishes from eastern, and large Mediterranean islands with comments on their safety status. *Acta Universitatis Carolinae-Biologica* **40,** 45–60.

Blondel, J., Thomas, D. W., Charmantier, A., Perret, P., Bourgault, P. & Lambrechts, M. M. (2006). A thirty-year study of phenotypic and genetic variation of blue tits in Mediterranean habitat mosaics. *BioScience* **56,** 661–673.

Brigg, J. C. (2010). A plethora of blennies. *Environmental Biology of Fishes* **87,** 89–92.

Changeux, T. & Pont, D. (1995). Current status of the riverine fishes of the French Mediterranean basin. *Biological Conservation* **72,** 137–158.

Crispo, E. & Chapman, L. J. (2008). Population genetic structure across dissolved oxygen regimes in an African cichlid fish. *Molecular Ecology* **17,** 2134–2148.

Crispo, E., Bentzen, P., Reznick, D. N., Kinnison, M. T. & Hendry, A. P. (2006). The relative influence of natural selection and geography on gene flow in guppies. *Molecular Ecology* **15,** 49–62.

DeWitt, T. J. & Scheiner, S. M. (2004). *Phenotypic Plasticity: Functional and Conceptual Approaches*. New York, NY: Oxford University Press.

Eckert, C. G., Samis, K. E. & Lougheed, S. C. (2008). Genetic variation across species' geographical ranges: the central–marginal hypothesis and beyond. *Molecular Ecology* **17,** 1170–1188.

Estoup, A., Largiader, C. R., Perrot, E. & Chourrout, D. (1996). Rapid one-tube DNA extraction for reliable PCR detection of fish polymorphic markers and transgenes. *Molecular Marine Biology and Biotechnology* **5,** 295–298.

Fischer-Rousseau, L., Chu, K. P. & Cloutier, R. (2010). Developmental plasticity in fish exposed to a water velocity gradient a complex response. *Journal of Experimental Zoology* **314B,** 67–85.

Fleming, I. A., Jonsson, B. & Gross, M. R. (1994). Phenotypic divergence of sea-ranched farmed, and wild salmon. *Canadian Journal of Fisheries and Aquatic Sciences* **51,** 2808–2821.

Freeman, M. C., Viñolas, D., Grossman, G. D. & De Sostoa, A. (1990). Microhabitat use by *Blennius fluviatilis* in the Rio Matarraña, Spain. *Freshwater Biology* **24,** 335–346.

Hassan, M., Lemaire, C., Fauvelot, C. & Bonhomme, F. (2002). Seventeen new exon-primed intron-crossing polymerase chain reaction amplifiable introns in fish. *Molecular Ecology Notes* **2,** 334–340.

Humphries, J. M., Bookstein, F. L., Chernoff, B., Smith, G. R., Elder, R. L. & Poss, S. G. (1981). Multivariate discrimination by shape in relation to size. *Systematic Zoology* **30,** 291–308.

Kawata, M. (2002). Invasion of vacant and subsequent sympatric speciation. *Proceedings of the Royal Society B* **269,** 55–63.

Kottelat, M. (2004). *Salaria economidisi* a new species of freshwater fish from Lake Trichonis, Greece, with comments on variation in *S. fluviatilis* (Teleostei: Blenniidae). *Revue Suisse de Zoologie* **111,** 121–137.

Kuhry, B. & Marcus, L. F. (1977). Bivariate linear models in biometry. *Systematic Zoology* **26,** 201–209.

Legendre, P. & Legendre, L. (1998). *Numerical Ecology*, 2nd edn. Amsterdam: Elsevier.

Mock, K. E., Bentz, B. J., O'Neill, E. M., Chong, J. P., Orwin, J. & Pfrender, M. E. (2007). Landscape-scale genetic variation in a forest outbreak species, the mountain pine beetle (*Dendroctonus ponderosae*). *Molecular Ecology* **16,** 553–568.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89,** 583–590.

Perdices, A., Doadrio, I., Côté, I. M., Machordom, A., Economidis, P. & Reynolds, J. D. (2000). Genetic divergence and origin of Mediterranean populations of the river blenny *Salaria fluviatilis* (Teleostei: Blenniidae). *Copeia* **2000,** 723–731.

Peres-Neto, P. & Magnan, P. (2004). The influence of swimming demand on phenotypic plasticity and morphological integration a comparison of two polymorphic charr species. *Oecologia* **140,** 36–45.

Pfennig, D. W., Wund, M. A., Snell-Rood, E. C., Cruickshank, T., Schlichting, C. D. & Moczek, A. P. (2010). Phenotypic plasticity's impacts on diversification and speciation. *Trends in Ecology and Evolution* **25,** 459–467.

Pigliucci, M. (2001). *Phenotypic Plasticity: Beyond Nature and Nurture*. Baltimore, MD: The Johns Hopkins University Press.

Plaut, I. (1998). Comparison of salinity tolerance and osmoregulation in two closely related species of blennies from different habitats. *Fish Physiology and Biochemistry* **19,** 181–188.

Plaut, I. (1999). Effects of salinity acclimation on oxygen consumption in the freshwater blenny, *Salaria fluviatilis*, and the marine peacock blenny, *S. pavo*. *Marine and Freshwater Resources* **50,** 655–659.

Poissant, J., Knight, T. W. & Ferguson, M. M. (2005). Nonequilibrium conditions following landscape rearrangement: the relative contribution of past and current hydrological

landscape on the genetic structure of a stream-dwelling fish. *Molecular Ecology* **14,** 1321–1331.

Pope, L. C., Domingo-Roura, X., Erven, K. & Burke, T. (2006). Isolation by distance and gene flow in the Eurasian badger (*Meles meles*) at both a local and broad scale. *Molecular Ecology* **15,** 371–386.

Proulx, R. & Magnan, P. (2004). Contribution of phenotypic plasticity and heredity to the trophic polymorphism of lacustrine brook charr (*Salvelinus fontinalis* M.). *Evolutionary Ecology Research* **6,** 503–522.

Robinson, B. W. & Parsons, K. J. (2002). Changing times, spaces, and faces: tests and implications of adaptive morphological plasticity in the fishes of northern postglacial lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **59,** 1819–1833.

Roché, B. (2001). *Atlas des poisons d'eau douce de Corse*. Bastia: Direction Régionale de l'environnement Corse.

Rouleau, S., Glemet, H. & Magnan, P. (2009). Effects of morphology on swimming performance in wild and laboratory crosses of brook trout ecotypes. *Functional Ecology* **24,** 310–321.

She, J. X., Autem, M., Kotoulas, G., Pasteur, N. & Bonhomme, F. (1987). Multivariate analysis of genetic exchanges between *Solea aegyptiaca* and *Solea senegalensis* (Teleosts, Soleidae). *Biological Journal of the Linnean Society* **32,** 357–371.

Schluter, D. (2001). Ecology and the origin of species. *Trends in Ecology and Evolution* **16,** 372–380.

Stearns, S. C. (1989). The evolutionary significance of phenotypic plasticity. *BioScience* **39,** 436–445.

Summerfelt, R. C. & Smith, L. S. (1990). Anesthesia and surgery. In *Methods for Fish Biology* (Schreck, C. B. & Moyle, P. B., eds), pp. 213–272. Bethesda, MD: American Fisheries Society.

Tabachnick, B. G. & Fidell, L. S. (2001). *Using Multivariate Statistics*, 4th edn. Boston, MA: Allyn and Bacon.

Turelli, M., Barton, N. H. & Coyne, J. A. (2001). Theory and speciation. *Trends in Ecology and Evolution* **16,** 330–343.

Walker, J. A. (1997). Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. *Biological Journal of the Linnean Society* **61,** 3–50.

Webb, P. W. (1982). Locomotor patterns in the evolution of the actinopterygian fishes. *American Zoologist* **22,** 329–342.

Webb, P. W. (1984). Body form, locomotion and foraging in aquatic invertebrates. *American Zoologist* **24,** 107–120.

Weir, B. S. & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution* **38,** 1358–1370.

West-Eberhard, M. J. (2003). *Developmental Plasticity and Evolution*. Oxford: Oxford University Press.

Zander, C.-D. (1972). Evolution of Blennioidei in the Mediterranean. *Revue des travaux de l'Institut des pêches maritimes* **37,** 215–221.

Electronic Reference

Belkhir, K., Borsa, P., Goudet, J., Chikhi, L. & Bonhomme, F. (1998). *GENETIX, logiciel sous Windows^{TM} pour la génétique des populations*. Montpellier: Laboratoire Génome et Populations, Université Montpellier II. Available at http://www.univ-montp2.fr/Bgenep-tix/genetix.htm/