

Habitat-associated morphological divergence in two Neotropical fish species

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We examined intraspecific morphological diversification between river channel and lagoon habitats for two Neotropical fish (*Bryconops caudomaculatus*, Characidae; *Biotodoma wavrini*, Cichlidae). We hypothesized that differences between habitats (e.g. flow regime, foraging opportunities) might create selective pressures resulting in morphological divergence between conspecific populations. We collected fish from four channel-lagoon habitat pairs in the Río Cinaruco, Venezuela, and compared body morphology using geometric morphometrics. There were two aspects of divergence in both species: (1) placement of maximum body depth and (2) orientation of the mouth. For both species, maximum body depth was positioned more anteriorly (i.e. fusiform) in the river channel than in lagoons. Both species exhibited a relatively terminal mouth in lagoons compared to the channel. The mouth of *B. caudomaculatus* was relatively upturned, whereas the mouth of *B. wavrini* was relatively subterminal, in channel habitats. Observed morphological patterns are consistent with functional morphological principles suggesting adaptive divergence. We also show that spatial distance between habitats, presumably reflecting rates of population mixing, appears to have constrained diversification. For both species, morphological divergence increased with distance between habitats. Thus morphological divergence between channel and lagoon habitats apparently reflects a balance between diversification driven by natural selection, and homogenization driven by population mixing. © 2003 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2003, 80, 689–698.

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INTRODUCTION

A foundational concept in evolutionary biology is that divergent selective regimes often generate and maintain phenotypic diversification (e.g. Ehrlich & Raven, 1969; Endler, 1977; Rice & Hostert, 1993; Robinson & Wilson, 1994; Smith & Skúlason, 1996; Schluter, 2000). Divergent selection can lead to phenotypic differences through either genetic differentiation or phenotypic plasticity (Levins, 1968; West-Eberhard, 1989; Robinson & Wilson, 1994; Orr & Smith, 1998;

Schluter, 2000). Either source of divergence can drive microevolutionary change within a species, and can lead to speciation (West-Eberhard, 1989; Rice & Hostert, 1993; Losos *et al.*, 2000; Schluter, 2000; Agrawal, 2001; Kaneko, 2002). However, population mixing (e.g. migration, gene flow) may constrain adaptive diversification between alternative environments (e.g. Haldane, 1948; Felsenstein, 1976; Endler, 1977; Slatkin, 1985; Storfer & Sih, 1998; Hendry, Day & Taylor, 2001; Lenormand, 2002). Thus, evolutionary divergence can largely be considered a balance between selection and the mixing of organisms between alternative environments.

Intraspecific diversification is well documented in fishes (reviewed in Robinson & Wilson, 1994; Smith & Skúlason, 1996; Taylor, 1999; Jonsson & Jonsson,

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2001). Aquatic environments can exhibit great spatial or temporal variability in both abiotic and biotic habitat parameters (e.g. Lowe-McConnell, 1987; Goulding, Carvalho & Ferreira, 1988). A common example is the difference in resource composition and availability between near-shore, littoral zones and off-shore, open-water habitats. A number of species exhibit morphological variants (e.g. benthic and limnetic forms) between these environments (reviewed in Robinson & Wilson, 1994). Several species of threespine stickleback appear to have originated largely as a result of this diversification (reviewed in Bell & Foster, 1994; Schluter, 1996; McKinnon & Rundle, 2002).

Understanding general patterns and causes of diversification requires an examination of divergence in multiple species (e.g. Cody & Mooney, 1978; Endler, 1982; Johnson & Belk, 2001; Jennions & Telford, 2002; Van Buskirk, 2002) and an evaluation of potential constraints on divergence (e.g. Endler, 1977; Slatkin, 1987; Losos, 1996; DeWitt, Sih & Wilson, 1998; Hendry *et al.*, 2001). Greater confidence in ecological causes of phenotypic patterns arises when multiple, phylogenetically distinct species converge on similar ecology–phenotype associations (Cody & Mooney, 1978; Endler, 1982; Winemiller, 1991, 1992; Robinson & Wilson, 1994; Losos *et al.*, 1998; Johnson & Belk, 2001). Furthermore, while there is a large theoretical literature on the constraining role of migration and gene flow, few empirical studies have examined the role of population mixing among divergent habitats in constraining diversification (Riechert, 1993; Smith *et al.*, 1997; Storfer, 1999; Hendry, Taylor & McPhail, 2002).

We compared body morphology of two distantly related fish species (*Bryconops caudomaculatus* Günther, Characidae; *Biotodoma wavrini* Gosse, Cichlidae) between habitats within a Venezuelan floodplain river. Two habitats, river channel and lagoon sand banks, are common environments in tropical floodplain rivers and differ in several major environmental variables (e.g. water velocity). For each species, we addressed three questions: (1) does body morphology differ between habitats? (2) what is the nature of morphological divergence?, and (3) does spatial distance between habitats affect morphological divergence? While morphological differences between channel and lagoon habitats would not necessarily indicate divergent selection, documenting patterns of differentiation is an important step in the investigation of divergent natural selection.

MATERIAL AND METHODS

STUDY SYSTEM

We conducted the study in the Río Cinaruco, an oligotrophic, blackwater, floodplain river in south-western

Venezuela (6°32'N, 67°24'W). For a detailed description of this system, see Jepsen, Winemiller & Taphorn (1997) and Arrington (2002). The physical structure of the system comprises a meandering river channel with numerous connected lagoons. Lagoons vary – both spatially and temporally – in size and degree of connection. However, all lagoons differ physically from the main river channel in that they have no water flow, lower water transparency, higher water temperature, and greater leaf litter accumulation on the substratum (C.A. Layman, K.O. Winemiller & J.V. Montoya, unpubl. data). Tropical floodplain lagoons typically have considerably higher densities of zooplankton than channel habitats (Saunders & Lewis, 1988, 1989; Hamilton *et al.*, 1990); a pattern that is apparent in the Río Cinaruco (J.V. Montoya, unpubl. data). Thus, a suite of selection pressures may differ between these environments, analogous to selection differences between lakes and rivers/streams (e.g. Swain & Holtby, 1989; Lavin & McPhail, 1993; Taylor *et al.*, 1997; Hendry *et al.*, 2000; Brinsmead & Fox, 2002). We hypothesized that these differences between habitats would result in morphological divergence in fishes that inhabit them.

DATA COLLECTION

Two fish species (*B. caudomaculatus*, *B. wavrini*) are common in both channel and lagoon littoral habitats (Arrington & Winemiller, 2003; C.A. Layman & K.O. Winemiller, unpubl. data). A total of 110 individuals of *B. caudomaculatus* (13.75 ± 0.96 individuals per sample) and 111 *B. wavrini* (13.88 ± 0.99 individuals per sample) were collected from channel and lagoon littoral habitats. All lagoons from which we collected fish maintain a deep (>2 m) connection to the river channel throughout the low-water period (January–March). Samples were taken during the dry season (February 2002) using a 12.8 m × 1.8 m beach seine (4 mm mesh) and 244 cm cast net (10 mm mesh). We collected individuals from channel and lagoon habitats in a pairwise fashion. At four locations on the river, we made two collections: (1) a sample taken along the shoreline near the back of a lagoon, and (2) a sample taken in the main river channel on a sand bank within 75 m of the lagoon mouth. Each pair of channel–lagoon samples was separated by at least 2 km. Using this collecting scheme, we compared morphology between channel and lagoon environments across naturally replicated occurrences of channel–lagoon habitat pairs.

To obtain morphological information, fish were placed on a laminated grid mounted on a wooden plank. We captured a lateral image of the left side of each individual using a digital camera (Sony DSC-S30) mounted on a tripod, level with the plank. Image

characteristics of this camera-lens system were verified a priori not to produce visual distortions of shape (e.g. fish-eye effects or excessive parallax). Images were captured at a resolution of 0.2 mm/pixel. Fish were then placed in 10% formalin and later preserved in 70% alcohol. Specimens are catalogued at Texas A & M University.

We used a medial axis technique (Bookstein, 1985) to capture overall body shape information. Using MorphoSys (V. 1.29) software (Meacham, 1993), we digitized three landmarks directly (snout, dorsal insertion of caudal fin, ventral insertion of caudal fin) and computed eight points geometrically from the medial axis (line connecting snout and midpoint of caudal peduncle) for each individual. These eight landmarks were calculated as pairs of points on the body outline perpendicular to the medial axis at 20%, 40%, 60% and 80% distance from the snout. Thus, a total of 11 landmarks were digitized on the image of each fish. As neither species is sexually dimorphic in body shape (Chernoff & Machado-Allison, 1999; Weidner, 2000), we pooled sexes for analyses.

We used geometric morphometric techniques to analyse body shape differences among fish from the two habitats (separately for each species). Geometric morphometrics is a new tool for analysing shape variation that retains information on spatial covariation among landmarks (Rohlf & Marcus, 1993). This sets it apart from traditional approaches that simply use one-dimensional distances without consideration of where a given measurement is taken relative to others. We used tpsRegr software (Rohlf, 2000a) to obtain superimposed landmark coordinates for analyses. TpsRegr rotates, translates, and scales landmark coordinates into alignment via generalized least squares superimposition, and describes the variation in relative landmark positions using thin-plate-splines (Bookstein, 1991). For each species, superimposed landmark configurations were used to calculate partial warps using tpsRegr. Partial warps are bending energies describing morphological variation, and serve as our shape variables in morphometric analyses. This technique also provides visualization of shape differences by producing thin-plate-spline transformations of landmark positions. For a more detailed description of geometric morphometrics, see Bookstein (1991), Rohlf & Marcus (1993) and Marcus *et al.* (1996).

As lagoons varied in size, the distance between channel and lagoon samples varied among the four locations of the river. However, all lagoons were highly similar in environmental characteristics (e.g. no water movement, high leaf litter, lower water transparency, higher water temperature). Thus, we did not expect selection pressures to covary with spatial distance between habitats. This provided an opportunity to test

for effects of spatial distance between habitats on morphological divergence. Spatial distance between samples was calculated as the shortest possible aquatic route using GPS coordinates. Our measure of spatial distance is meant to serve as a surrogate for population mixing between habitats. In this paper, we use 'population mixing' to denote the movement of organisms, gametes, or propagules between populations (*sensu* Hendry *et al.*, 2001), and 'gene flow' to refer to the exchange of genes typically resulting from population mixing.

STATISTICAL METHODS

With geometric morphometrics, centroid size is typically used as an estimate of overall body size (Bookstein, 1991). Centroid size is the square root of the summed, squared distance of all landmarks from their centroid. Centroid size was highly correlated with standard length ($r = 0.99$, $P < 0.0001$ for both species) and was used as our measure of body size in all analyses.

All statistical analyses were conducted using JMP software (Version 4.04, SAS Institute Inc.). Our analyses consisted of both univariate and multivariate analyses of variance. Each statistical model was tested for effects on a response variable(s) attributable to habitat (channel or lagoon), location (four channel-lagoon pairs), and the interaction between habitat and location. We statistically treated each factor as a fixed effect as we wished to test the following null hypotheses: (1) there is no difference in means among habitats (2) there is no difference in means among locations (3) the effect of habitat on the dependent variable(s) does not depend on the particular location (Hocking, 1985; Bennington & Thayne, 1994; Sokal & Rohlf, 1995; Newman, Bergelson & Grafen, 1997). For all analyses, data were analysed separately for each species.

We conducted univariate analyses (ANOVA) to examine body size differences between habitats. We found a trend towards larger body size in channel habitats for both species (*B. caudomaculatus*: $F_{1,102} = 18.99$, $P < 0.0001$, *B. wavrini*: $F_{1,103} = 2.90$, $P = 0.09$). However, as we were interested in body shape differences, and as size distributions were highly overlapping between habitats, we focused our analyses on size-independent body morphology.

Does body morphology differ between habitats?

We performed multivariate analyses to examine body shape differences. For all multivariate analyses, partial warps ($N = 18$) served as dependent variables describing body morphology. We first performed a discriminant function analysis (DFA) to test for effects of habitat on body morphology. This procedure predicts

the habitat of origin for each individual, with the null hypothesis that 50% are correctly classified by chance. Second, we conducted a multivariate analysis of covariance (MANCOVA) for each species. MANCOVA used centroid size as a covariate to control for shape variation due to body size (i.e. multivariate allometry). This statistical model examined effects on body morphology attributable to centroid size (covariate), habitat, location, and the interaction between habitat and location. Heterogeneity of slopes was tested and never found for models using a covariate.

What is the nature of morphological divergence?

We produced thin-plate-spline transformations of landmark positions using canonical axes derived from MANCOVA. We used tpsSuper (Rohlf, 2000b) to generate photographic representations of these transformations on the body shape of a specimen. This procedure warped one of our original images for each species (individual with intermediate canonical score) using canonical values from the habitat canonical axis. Thus, images were produced depicting morphological differences between channel and lagoon habitats, while statistically controlling for size, location, and habitat \times location effects on shape.

Does spatial distance between habitats affect morphological divergence?

Theory predicts that increasing distance between populations – reflecting decreasing levels of population mixing – should result in increasing phenotypic divergence (Haldane, 1948; Felsenstein, 1976; Slatkin, 1985; Hendry *et al.*, 2001). We plotted mean canonical values for each sample along an axis of spatial separation of habitats. If geographical distance between environments constrains divergence, we should observe the least amount of divergence when habitats are closest in proximity. We also calculated, for each species, the relative magnitude of divergence in each location. Magnitude of divergence was calculated as the difference between habitat canonical centroids (i.e. Euclidean distance) within each location. We then cal-

culated relative magnitude of divergence, which placed each species on a comparable scale, by dividing each Euclidean distance by the maximum distance for each species (range of 0–1, $N = 8$). We used linear regression to test for a positive relationship between the relative magnitude of divergence and spatial distance between habitats.

RESULTS

Does body morphology differ between habitats?

Discriminant function analyses indicated significant morphological differences in both species between channel and lagoon habitats (*B. caudomaculatus*: $F_{18,91} = 2.35$, $P = 0.004$; *B. wavrini*: $F_{18,92} = 1.88$, $P = 0.027$). Based on body shape, as measured by partial warps, DFA correctly classified 74.5% of *B. caudomaculatus* and 68.5% of *B. wavrini* into the correct habitat. MANCOVA revealed significant morphological divergence, independent of size, between habitats for each species (Table 1). The habitat canonical axis (axis describing morphological differences between habitats) explained 39.5% of the variance for *B. caudomaculatus* and 28.6% of morphological variance for *B. wavrini*. The covariate (centroid size) was significant ($P < 0.0001$) in all cases, indicating multivariate allometry.

What is the nature of morphological divergence?

Morphological differences between channel and lagoon samples were visualized through thin-plate-spline transformation grids and photographic representations using canonical axis values from MANCOVA (Fig. 1). Inspection of visualizations suggested two major shifts in body shape among environments for both species: (1) placement of maximum body depth and (2) orientation of the mouth. For both species, maximum body depth was anteriorly positioned in the river channel relative to lagoons. This shift produced a more fusiform body shape in the channel habitat by deepening the anterior portion of the body, and exhibiting a more shallow caudal

Table 1. MANCOVA tests for effects of habitat, location, and their interaction on body morphology (i.e. 18 partial warps) for *Bryconops caudomaculatus* and *Biotodoma wavrini*. Wilks' lambda values were used to approximate F -values for location and the interaction term

Species	Effect	F	d.f.	P
<i>B. caudomaculatus</i>	Habitat (H)	3.04	18, 84	0.0003
	Location (L)	2.47	54, 254.1	<0.0001
	H \times L	2.17	54, 254.1	<0.0001
<i>B. wavrini</i>	Habitat (H)	1.90	18, 85	0.0269
	Location (L)	2.19	54, 251.1	<0.0001
	H \times L	1.35	54, 251.1	0.0667

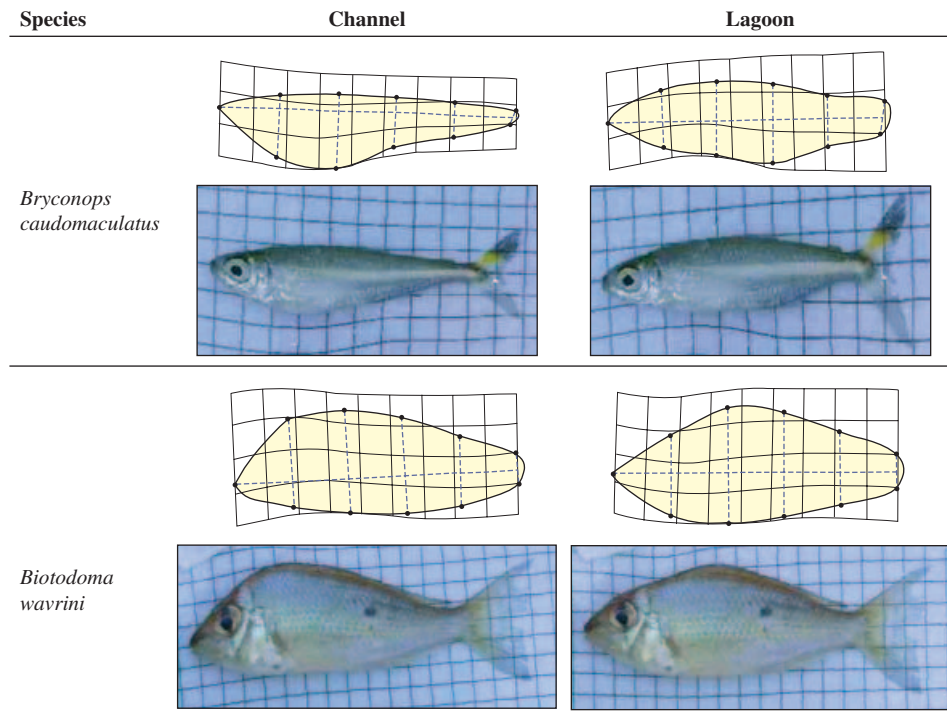


Figure 1. Thin-plate-spline transformations depicting morphological differences in *Bryconops caudomaculatus* and *Biotodoma wavrini* between channel and lagoon habitats (magnified $3\times$). Visualizations describe two major shifts in body shape: (1) placement of maximum body depth (i.e. more anterior in channel) and (2) orientation of the mouth (i.e. relatively terminal in lagoons). Note that tail shape was extrapolated in photos above and apparent differences are not significant (see text for details).

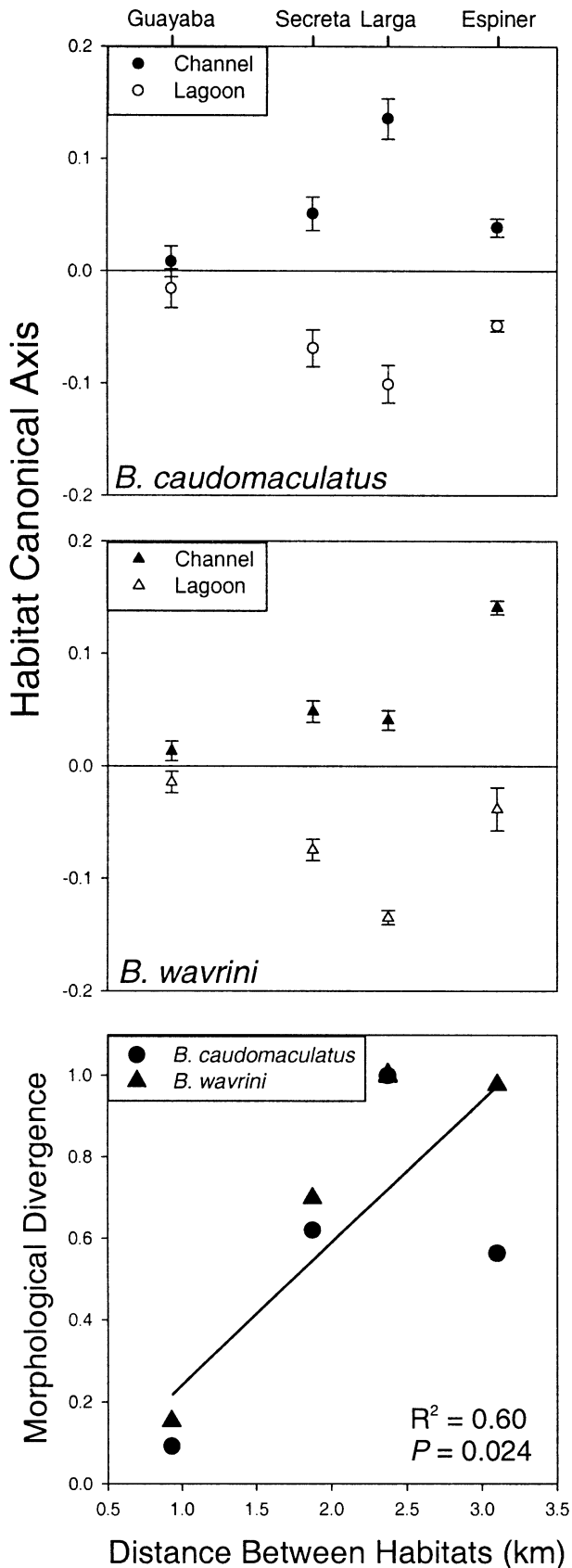
peduncle. While both species exhibited an anterior deepening in channel habitats, this was achieved in different ways for the two species: a ventral deepening in *B. caudomaculatus*, and heightening of the nape region in *B. wavrini*. Both species had an overall elliptical body shape in lagoon environments, with maximum body depth relatively posteriorly positioned. In the channel, the mouth position of *B. caudomaculatus* was relatively upturned, while the mouth of *B. wavrini* was relatively subterminal. Both species had a relatively terminal mouth in lagoons.

Although we did not include morphological characteristics of the caudal fin in our primary analyses, the photographic transformation suggested a difference in the size and shape of the caudal fin for *B. caudomaculatus* (Fig. 1). This resulted because thin-plate-spline transformation extrapolated changes in body landmark positions to the tail (tail landmarks were not included in the analysis). As this extrapolation might reflect underlying phenotypic integration among the body and caudal fin (i.e. correlative changes among traits due to shared developmental processes), we examined caudal fin morphology in *B. caudomaculatus* by digitizing three additional

landmarks: most distal dorsal tip of caudal fin, most distal ventral tip of caudal fin, and tail fork. We found no significant difference in size ($F_{1,101} = 0.02, P = 0.88$) or shape ($F_{6,96} = 1.32, P = 0.25$) of the caudal fin among habitats, although trends were in the directions suggested in Figure 1.

Does spatial distance between habitats affect morphological divergence?

Plotting the average canonical value for each sample along an axis of spatial separation of habitats revealed that the magnitude of divergence depended on the distance between habitats (Fig. 2). For both species, magnitude of divergence was smallest when habitats within a pair were closest (i.e. Guayaba location). The canonical values indicated that the magnitude of divergence varied among locations, but the nature of divergence did not (i.e. all channel sites positive, all lagoon sites negative). Furthermore, there was a significant, positive relationship between the relative magnitude of divergence and spatial separation of habitats ($R^2 = 0.60, P = 0.024$; Fig. 2). Thus, we observed greater morphological divergence in channel-lagoon pairs where mixing of individuals between habitats was less likely.



As we found that spatial distance between habitats seemed to limit morphological divergence, we repeated our analyses of body morphology (i.e. DFA and MANCOVA) excluding fish collected from the Guayaba location, where habitats were considerably closer in proximity. This procedure evaluated the relative contribution of the Guayaba location in constraining divergence. When the Guayaba location was removed from DFA, morphological divergence increased in significance (*B. caudomaculatus*: $F_{18,67} = 3.62$, $P < 0.0001$; *B. wavrini*: $F_{18,62} = 2.40$, $P = 0.0058$) and predictive ability improved (*B. caudomaculatus*: 83.7% correct; *B. wavrini*: 79.0% correct). When Guayaba was removed from MANCOVA, significance of the habitat effect increased (*B. caudomaculatus*: $F_{18,62} = 4.34$, $P < 0.0001$; *B. wavrini*: $F_{18,57} = 2.55$, $P = 0.0038$) and significance of the interaction term was removed (*B. caudomaculatus*: $F_{36,124} = 1.40$, $P = 0.092$; *B. wavrini*: $F_{36,114} = 0.83$, $P = 0.74$). A loss of significance of the interaction term suggests that the Guayaba region, by exhibiting less morphological divergence, was responsible for the significant interaction term when included in analyses. The interaction term thus reflected variation in the magnitude of morphological differences among locations, not in the nature of those differences. Also, variance explained by the habitat canonical axis increased when fish from the Guayaba location were removed from the analyses (55.7% of shape variance in *B. caudomaculatus*, 44.6% in *B. wavrini*). Results of analyses with Guayaba excluded suggest that the relatively small spatial distance between habitats in this channel–lagoon pair served to constrain the magnitude of divergence.

DISCUSSION

Many fish species exhibit morphological differences between habitats (reviewed in Robinson & Wilson, 1994; Smith & Skúlason, 1996; Taylor, 1999; Jonsson & Jonsson, 2001). For both species examined in this study, we found significant morphological differences between channel and lagoon habitats. We observed two primary morphological shifts: (1) placement of maximum body depth and (2) orientation of the

Figure 2. Relationships of morphological divergence between channel–lagoon habitat pairs and spatial distance between habitats. The top two panels depict mean canonical variate values (± 1 SE) of each sample (top: *Bryconops caudomaculatus*, middle: *Biotodoma wavrini*). The lower panel shows the relationship between relative magnitude of morphological divergence (i.e. relative Euclidian distance between habitat centroids for each location) and spatial distance between habitats.

mouth. Both species exhibited an anterior shift in maximum body depth (i.e. fusiform shape) in channel sites relative to lagoons. In channel habitats, the mouth position of *B. caudomaculatus* was relatively upturned, while *B. wavrini* exhibited a relatively subterminal mouth. Both species were characterized by a more posterior position of maximum depth and relatively terminal mouths in lagoons.

Intraspecific polymorphism is typically believed to arise from divergent selection pressures between alternative environments (reviewed in Robinson & Wilson, 1994; Smith & Skúlason, 1996; Schluter, 2000). Phenotypic diversification can result from either genetic differentiation or phenotypic plasticity; both sources of diversification can represent adaptive responses to divergent selection (Via & Lande, 1985; West-Eberhard, 1989; Robinson & Wilson, 1994; Orr & Smith, 1998; Schluter, 2000). In this study, habitats were chosen a priori because they differ in several major abiotic and biotic variables. Body shape differences observed between channel and lagoon habitats are consistent with those known in other fishes – differences that often reflect variations in the swimming and feeding of fishes. Divergent selection for fishes inhabiting channel and lagoon habitats might arise from two major differences between these environments: (1) water velocity and (2) resource availability/composition.

Many aquatic environments contain habitats with varying water velocities, and several fish species are known to exhibit distinct morphologies between lotic and lentic habitats (Robinson & Wilson, 1994; Taylor *et al.*, 1997; Hendry *et al.*, 2000; Pakkasmaa & Piironen, 2000; Brinsmead & Fox, 2002). Hydrodynamic theory posits that a more fusiform body shape reduces drag, and hence reduces the energetic expenditure necessary to maintain position in flowing water (reviewed in Keast & Webb, 1966; Blake, 1983; Webb, 1984; Videler, 1993; Vogel, 1994). In this study, we found that two distantly related fish species – distinguished at the level of superorder – exhibited a more fusiform body shape in channel habitats, where water velocity is relatively high compared to the still-water habitats of lagoons. It seems that, for many fish species, a fusiform body shape provides a general solution to the problem of coping with high water velocity. However, the two species in our study achieved this solution in different ways (Fig. 1). The extent to which these shifts represent alternative routes to functionally equivalent outcomes needs further study.

Intraspecific trophic diversification is also well known in fishes (Robinson & Wilson, 1994; Wimberger, 1994; Robinson & Wilson, 1995, 1996; Smith & Skúlason, 1996; Ruzzante *et al.*, 1998; Mittelbach, Osenburg & Wainwright, 1999; Holtmeier, 2001). The observed differences in mouth position among habitats could

reflect differences in feeding, such as foraging mode or orientation, or diet composition. *Bryconops caudomaculatus* is a midwater-surface foraging characin and *B. wavrini* is a benthic cichlid (Géry, 1977; Taphorn, 1992; Weidner, 2000). Yet despite typical foraging niches, many fishes consume abundant prey when available, even outside their speciality (McKaye & Marsh, 1983; Lowe-McConnell, 1987; Winemiller, 1989, 1990; Robinson & Wilson, 1994). Because lagoons typically contain more zooplankton than channel habitats (Saunders & Lewis, 1988, 1989; Hamilton *et al.*, 1990; J.V. Montoya, unpubl. data), we might expect *B. caudomaculatus* and *B. wavrini* to forage more frequently on these midwater prey items in lagoons. In channel habitats, these fish may rely more on their foraging specialities. Based on functional morphology of fish feeding, differences in mouth position observed between habitats are consistent with these hypotheses. Midwater foragers typically exhibit terminal mouths, benthic feeders exhibit subterminal mouths, and surface feeders have upturned mouths (Keast & Webb, 1966; Winemiller, 1992; Moyle & Cech, 2000).

We also found that, for both species, the magnitude of morphological divergence tended to increase with increasing spatial distance between habitats (Fig. 2). Empirical studies examining the relationship between intraspecific divergence and population mixing are just emerging, and these studies emphasize the relationship between gene flow and divergence. However, a negative correlation between gene flow and adaptive diversification can arise for two reasons: (1) gene flow may constrain divergence (Riechert, 1993; Storfer, 1999; Hendry *et al.*, 2001; Hendry *et al.*, 2002), or (2) divergence may constrain gene flow (i.e. ecological speciation, Smith *et al.*, 1997; Lu & Bernatchez, 1999; Schluter, 2001; Ogden & Thorpe, 2002). In the first scenario, adaptive diversification is constrained by homogenizing gene flow. In the second scenario, divergent selection, promoting reproductive isolation, constrains gene flow among divergent populations. Empirical tests typically can not distinguish between these two causes. Ideally, one would correlate adaptive diversification with a measurement of population mixing *per se* in order to evaluate the relationship between divergence and mixing. In practice such measures are difficult to obtain.

Geographic distance between divergent populations will often provide a good surrogate for the rate of population mixing. As long as the strength of divergent selection does not increase with spatial distance between habitats, the correlation between distance and divergence should explicitly test whether mixing constrains diversification. Thus, in the present study, it appears that population mixing between habitats served to constrain diversification. In this system,

there is no reason to expect the strength of divergent selection to covary with spatial distance between habitats. No environmental variable that we have measured (water velocity, leaf litter, transparency, water temperature) bears any relationship with habitat separation; these parameters vary little among locations, but considerably between channel and lagoon habitats (C.A. Layman, K.O. Winemiller & J.V. Montoya, unpubl. data).

Whether the observed morphological patterns were produced through genetic differences or phenotypic plasticity is unknown. Populations could diverge via alternative, genetically based morphologies, or through environmentally induced phenotypes. However, gene flow should only constrain the magnitude of genetic differences, not plastic diversification (Via & Lande, 1985; Scheiner, 1993). In fact, theoretical studies show that gene flow between environments can promote, rather than constrain, the evolution of plasticity (Scheiner, 1998; Sultan & Spencer, 2002). Population mixing *per se*, on the other hand, may serve to constrain the magnitude of plastic, as well as genetic diversification. For instance, if habitat-specific morphologies are environmentally induced, greater movement of individuals between relatively close habitats could result in lesser degrees of induced phenotypes. Thus, population mixing might effectively reduce the magnitude of phenotypic diversification, regardless of the source of divergence (i.e. genetic or plasticity). Furthermore, it may often be the case that both sources of diversification simultaneously play a role in intraspecific diversification (Day, Pritchard & Schluter, 1994; Robinson & Wilson, 1996; Chapman, Galis & Shinn, 2000). Exploring the genetic and environmental bases of observed morphological patterns, along with performance experiments in alternative habitats, represent important next steps in the investigation of the divergence observed in these two species.

In this study, ecological factors appeared important in promoting intraspecific divergence. There are several reasons why the morphological patterns may reflect adaptive responses to alternative habitats: (1) functional morphology of fish swimming and feeding suggests adaptive roles for morphological differences, (2) similar forms of divergence were found in distantly related species, and (3) evidence of the same divergence was observed at multiple locations. We also demonstrated that spatial distance between divergent environments might serve as an important constraint on adaptive diversification. Our empirical results are consistent with the evolutionary hypothesis that divergent habitats drive intraspecific diversification, but the degree of divergence is constrained by the mixing of individuals between alternative environments.

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