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# Population differentiation in the swordtail characin (*Corynopoma riisei*): a role for sensory drive?

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speciation.

## Abstract

Sensory drive, where the efficacy of a sexual signal depends on the environment in which it is employed, is a potential mechanism behind divergent evolution of secondary sexual traits. Male swordtail characins are equipped with a narrow and transparent extension of the gill cover with a flag-like structure at its tip. This opercular flag mimics a prey item and is employed by males as a 'lure' to attract the attention of females during mating attempts. We conducted a study of genetic and morphological differentiation across swordtail characin populations throughout their native range in Trinidad. The morphology of the opercular flag varied across populations and several aspects of this variation match the predicted hallmarks of sensory drive. First, morphological differentiation of the flag across populations was unrelated to genetic similarity at neutral genetic markers. Second, the shape of the flag covaried with those aspects of body shape that should reflect adaptation to different feeding regimes. Third, and most importantly, the shape of the flag covaried across populations with those environmental characteristics that should most closely reflect differences in local prey abundance. Overall, our results are consistent with a scenario where the evolution of this male sexual signal tracks food-related shifts in female sensory biases across populations, thus providing at least provisional support for a role for sensory drive in population differentiation.

## Introduction

Sexual selection by mate choice is a potent evolutionary generator of reproductive isolation. This is so partly because it acts on traits involved in reproduction (e.g., Coyne & Orr, 2004) but also because it efficiently generates linkage disequilibrium across loci (Kirkpatrick & Ravigné, 2002), and is thus often considered an important engine of incipient speciation (Panhuis *et al.*, 2001). Yet our understanding of if and why sexual selection is divergent in allopatric populations of any given species is very limited. One possibility is that evolutionary trajectories may come to differ during population differentiation as a result of largely random events, because of the arbitrary nature of certain forms of sexual selection (Lande, 1981; Arak & Enquist, 1993, 1995; Schluter & Price, 1993). However, the efficacy of many sexual signals and other types of secondary sexual traits depends to a large extent on the environment in which they are employed. This form of dependency between environmental conditions and the sexual selection regime leads to an adaptive process known as sensory drive (West-Eberhard, 1983; Endler, 1992). Sensory drive is predicted to generate covariation across populations or higher order taxa between abiotic and biotic ecological conditions on the one hand and aspects of sexual signalling traits on the other. Because conditions invariably differ across populations, sensory drive is thus potentially a common driver of evolutionary divergence in reproductive traits (Boughman, 2002). There are, however, surprisingly few clear examples of sensory drive. In addition to the handful of cases discussed by Boughman (2002), sensory drive has recently been implicated in sexual signal divergence in haplochromine cichlids (Seehausen *et al.*, 2008), dwarf chameleons

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(Stuart-Fox *et al.*, 2007) and treehoppers (McNett & Cocroft, 2008). The relative paucity of examples of sensory drive is likely a reflection of the large empirical effort required to demonstrate sensory drive (Boughman, 2002; Fuller *et al.*, 2005).

Although little is known about the evolutionary origin of sexual signals (Arnqvist, 2006), there is no doubt that male signals commonly evolve to exploit pre-existing sensory biases in females through a process known as sensory exploitation (West-Eberhard, 1979, 1984; Ryan, 1990; Ryan & Rand, 1993; Endler & Basolo, 1998). Sensory biases in females are often the results of strong natural selection (i.e., adaptive sensory biases; *sensu* Arnqvist, 2006), reflecting adaptations to, for example, foraging and predator avoidance (Christy, 1995; Rodd *et al.*, 2002). There are at least two reasons to believe that sensory drive may be a particularly important generator of population divergence for sexual signals that have originated by, or is maintained in part by, sensory exploitation. First, selection on female responses to male signals will be dominated by direct natural selection, which is generally a stronger force than indirect sexual selection on female mate preferences (e.g. Kirkpatrick & Barton, 1997). Second, because natural selection regimes often differ across populations as a direct effect of spatially varying environmental conditions (Schluter, 2000), natural selection will often generate divergence between populations in those female mate preferences that result from sensory biases (Rodd *et al.*, 2002). Yet we are unaware of any example of population divergence through sensory drive involving sexual signals that result from sensory exploitation.

The swordtail characin (*Corynopoma riisei*) is a freshwater Glandulocaudinae tetra, endemic to lowland river systems in Trinidad and northern Venezuela (Nelson, 1964; Kenny, 1995; Weitzman & Menezes, 1998). Although the diet of *C. riisei* varies across natural populations, it consists almost exclusively of various invertebrate prey (N. Kolm & G. Arnqvist, unpubl.). This species exhibits an exceptional form of sexual dimorphism. Not only do adult males grow longer fin rays and develop a sword-like extension of the tail fin but also sexually mature males are equipped with a striking and unique extension on each of both gill covers (Kutaygil, 1959; Nelson, 1964), which is entirely absent in females. The stem of these extensions is narrow and transparent but the tip is flattened and forms a flag-like structure containing a dark bean-shaped patch (see Fig. 1). These opercular flags are not in any way engaged in swimming: they are held flattened against the body and are then very inconspicuous. They are exclusively engaged during courtship, when males use these signals to attract females in the following manner. Males initiate matings by approaching females and extending one of their opercular flags conspicuously in front of the female. Females typically show obvious interest in the tip of the flag, and react by approaching the male, nipping and biting at the flag (Kutaygil, 1959; Nelson, 1964; Amcoff *et al.*, 2009) and even other males that are nearby sometimes bite at the extended opercular flags of courting males (Nelson, 1964). Wickler (1968) originally recognized that these extraordinary male signals in some sense mimic invertebrate prey items and subsequent work on the courtship behaviour of these fish imply that sensory exploitation has been involved in the evolution of these traits (see Arnqvist & Rowe, 2005). For example, females trained on coloured food items prefer to approach and bite at artificial flags artificially given the same colour as their food (M. Amcoff & N. Kolm, unpubl.). Displaying their 'lures' allow males to position the female appropriately for successful sperm transfer, as insemination in this internally fertilizing species (Burns *et al.*, 1995) is very swift and only takes place after the female bites at the flag (Nelson, 1964). The exact positioning of the female is important in Glandulocaudinae (Kutaygil, 1959; Nelson, 1964), in part because males lack a gonopodium, and male mating attempts only occur if the female is appropriately positioned relative to the male (Nelson, 1964). Remarkably, four types of analogous but not

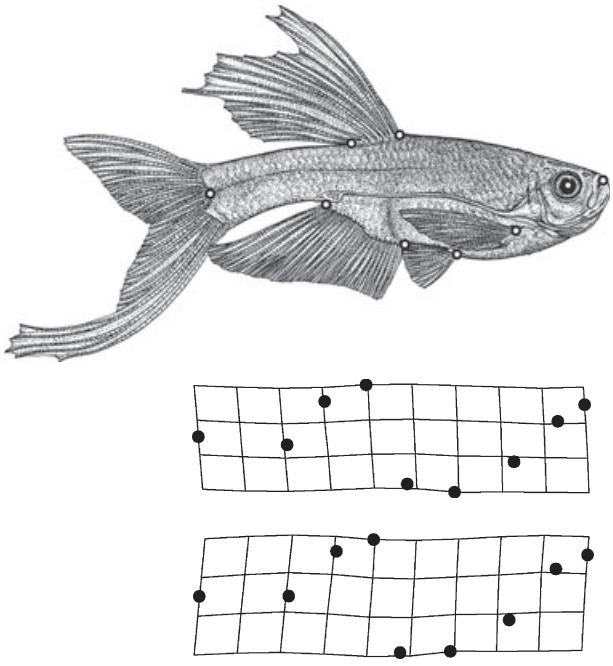

**Fig. 1** Male *Corynopoma riisei*. Note the extension on the gill cover, carrying a pigmented and flattened flag-like tip. Black circles represent the location of the nine landmarks used to study variation in body shape. Bottom of figure shows thin-plate spline deformation grids, illustrating mean body shape associated with positive and negative scores along the third discriminant function of habitat variation. Fish in populations inhabiting wide streams with much shading vegetation (top grid) tended to have more elongated and shallow bodies with a more upwards pointed mouth compared to fish in narrow streams with little shading vegetation (bottom grid). Drawing by Tamara Clark (from Burns & Weitzman, 2005).

homologous male 'lures' have evolved within the Glandulocaudinae tetras (see Arnqvist & Rowe, 2005).

In this study, we first ask whether *C. riisei* populations are genetically subdivided, such that gene flow will not prevent local adaptation (Ogden & Thorpe, 2002). We then assess whether populations have diverged in the morphology of general traits and in sexual signals. Most importantly, we test three explicit predictions. First, we assess whether population divergence in the shape of the sexual signal employed by males covary with environmental conditions across populations. Under a sensory drive scenario, we predict that opercular flag shape should covary with environmental factors that reflect the local abundance of prey items as females should become adapted or habituated to react most strongly to male signals that most closely mimic prey items common in any given population. Second, because body shape is a key trophic adaptation in fish (Webb, 1984; Webb & Weihs, 1986) we predict that body shape, through its covariation with prey utilization across populations, should covary with the shape of the opercular flag. Third, because sensory drive will result in local adaptation, we predict that morphological differentiation across populations should reflect local environmental conditions rather than genetic similarity at neutral genetic markers.

## Methods

### Sampling

We collected samples of *C. riisei* from 18 different populations located in eight distinct drainages covering the entire distribution of this species in Trinidad (Kenny, 1995) (see Fig. 2, Table 1), during May 2005, using two-person push seines. Although all sites are located in the lowland or foothills (*C. riisei* does not occur in mountain streams; Kenny, 1995), the sites were chosen to represent a range of different habitat types. At each site, the aim was to collect at least 10 mature females and 10 mature males. Whole fish were immediately preserved in 95% ethanol for subsequent analyses. At each site, we also recorded a series of environmental variables. To reduce the number of environmental variables and thereby the number of inferential statistical tests, we subsequently performed a principal components analysis of these original variables based on the correlation matrix. The two first principal components, which were the only factors with eigenvalues larger than one (Jackson, 1993), were retained for subsequent analyses. These two variables collectively accounted for 64% of total variation in the recorded environmental variables across sites. The original variables were, with loading on PC1 and PC2 given within brackets, mean stream width (0.62, 0.67), mean water depth (−0.03, 0.92), current velocity (0.81, −0.15), the amount of shading vegetation (0.72, −0.21) and water turbidity (−0.46, 0.22).

**Table 1** The name and location of the 18 populations of *Corynopoma riisei* sampled.

| Site | Location |
|---|---|
| Turure River | 10°39′N 61°10′W |
| Tributary of the Oropuche River | 10°37′N 61°06′W |
| Caigual River | 10°34′N 61°03′W |
| Tributary of the Cunapo River | 10°10′N 61°32′W |
| Coora River | 10°09′N 61°28′W |
| Curamata River | 10°08′N 61°25′W |
| Tributary of the Innis River | 10°08′N 61°21′W |
| Guaracara River | 10°18′N 61°23′W |
| Tributary of the Guaracara River | 10°20′N 61°22′W |
| Savonetta River | 10°24′N 61°23′W |
| Poole River | 10°17′N 61°10′W |
| Navet River | 10°21′N 61°11′W |
| Tributary of the Bois Neuf River | 10°23′N 61°10′W |
| Canque of Nariva River | 10°26′N 61°08′W |
| Sangre Grande River | 10°32′N 61°07′W |
| Aripo River | 10°39′N 61°13′W |
| El Mano River | 10°38′N 61°13′W |
| Guanapo River | 10°38′N 61°15′W |

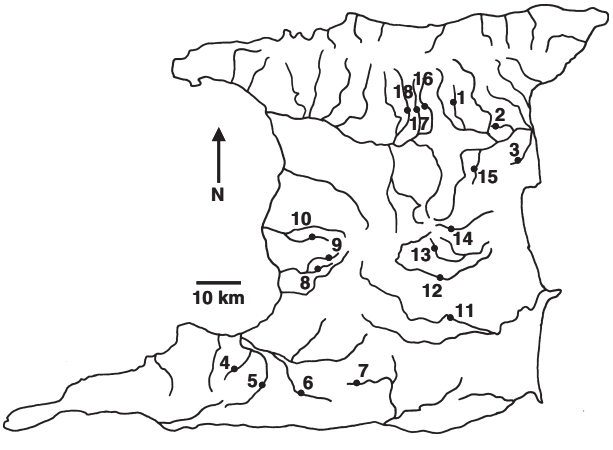


**Fig. 2** Major rivers and streams in Trinidad (after Kenny, 1995). Filled and numbered circles represent the location of the 18 populations studied here.

### Microsatellite analysis

Total genomic DNA was extracted using a conventional Chelex resin protocol. We first screened DNA samples of a subset of 15 individuals for cross amplification using 21 different microsatellite loci described from close relatives of *C. riisei* (see Calcagnotto *et al.*, 2001; Barroso *et al.*, 2003; Strecker, 2003; Beheregaray *et al.*, 2004, 2005; Sanches & Galetti, 2006; for primers and conditions for polymerase chain reactions). Four of these putative microsatellites were found to amplify well and be sufficiently polymorphic for our purposes. These were Ast1, Ast2 and Ast4 as described in Strecker (2003) and

Hb19 as described in Beheregaray *et al.* (2005). We then genotyped 20 individuals from each of the 18 populations for these four microsatellite loci. Polymerase chain reactions were performed as described by Strecker (2003) and Beheregaray *et al.* (2005), and sequencing was made using an ABI Prism® 377 automated DNA sequencer (PE Applied Biosystems, Foster City, CA, USA). Potential problems with the microsatellite data were assessed with MICRO-CHECKER version 2.2.3 (Van Oosterhout *et al.*, 2004). Data were then analysed using GENEPOP version 4.0.10 (Rousset, 2008), including the ISOLDE module for matrix comparisons (Mantel's tests were based on rank correlations and were tested using 10 000 random permutations), FSTAT version 2.9.3.2 (Goudet, 2001), SMOGD version 1.2.5 (Crawford, 2010) and Arlequin version 3.5.1.2 (Excoffier & Lischer, 2010).

### Morphological variation

To assess variation in male morphology within and across populations, we measured (i) fin and body lengths, (ii) body shape and (iii) opercular flag morphology of all 151 adult males collected. All metrics were acquired by placing each fish under a dissecting microscope (Leica® MZ10; Leica, Wetzlar, Germany) and projecting the image through a camera lucida onto a digitizing tablet (Summasketch® III; Summagraphics Corporation, Fairfield, CA, USA). This methodology is known to provide precise quantification of morphology in fish of this size (Arnqvist & Mårtensson, 1998).

We first recorded body length (tip of nose to caudal peduncle) and fin lengths (dorsal fin, upper lobe of tail fin, lower lobe of tail fin, anal fin). To enable analysis of variation in body shape, we then recorded the location of nine landmarks on the body of all individuals (see Fig. 1). Following a generalized orthogonal least-squares Procrustes superimposition of all landmark maps (Rohlf & Slice, 1990), retaining centroid size as an integrative measure of body size, shape variation was analysed using thin-plate spline relative warp analysis (TpsRelw version 1.45 and TpsRegr version 1.31 [Rohlf, 2006a,b]). The matrix of partial warp scores (i.e., the weight matrix; Bookstein, 1991) was joined with the matrix of the two uniform shape components (complement method; Rohlf & Bookstein, 2003). This matrix was then used as a body shape data matrix in subsequent statistical analyses of variation in body shape across the sample of 151 individual males. We tested for a difference in body shape between populations using a MANCOVA, with the body shape data matrix as a response variable matrix, body size as a covariate and population as a factor. The same basic inferential model was used to test for differences in opercular flag shape across populations.

Body shape was significantly related to male body size ($P = 0.002$; randomization test of multivariate regression based on 10 000 complete permutations using TpsRegr). Hence, to enable accurate and integrative ordinations of mean body shape across populations, we first generated a size-adjusted body shape data matrix where covariance between body size and shape was partialled out by means of ordinary multivariate regression. Following calculation of mean population values for all size-adjusted shape variables, we calculated Euclidean distances between all population-pairs in the multivariate space described by our shape variables. The resultant matrix of interpopulational distances represents a multivariate analogue of a univariate $P_{ST}$ matrix (Pujol *et al.*, 2008), in the sense that it describes relative differences in morphological shape between pairs of populations.

We quantified morphological variation in the opercular flag by recording the length of the entire extension (including the shaft) and the area and shape of the flag (Fig. 1) in both left and right flags for all individuals. The latter was achieved by first digitizing the outline of the proximal side of each opercular flag, after it had been placed between two microscope slides, using the method described earlier. Following reflection of left side outlines, all outlines were analysed using a conventional elliptic Fourier analysis (Lestrel, 1997). This standard geometric morphometric method involves fitting a nonlinear function to the outlines and subsequently analysing morphological shape variation among individuals as variance in the parameters of the fitted function (see Ferson *et al.*, 1985; Rohlf, 1992). All flag outlines of all individuals were included in a common elliptic Fourier analysis, using the software MORPHEUS *et al.* (Slice, 1998). The Fourier analysis was made invariant of size, position, rotation and used 20 harmonics (yielding 80 Fourier coefficients). These functions provided a near perfect fit to all flag outlines. To reduce the dimensionality of our shape descriptors, the 80 Fourier coefficients were treated as variables in principal component analyses based on the covariance matrix (Rohlf & Archie, 1984). The first nine principal components from these analyses collectively accounted for more than 98% of the total variation in opercular flag shape and were retained for subsequent analyses of shape variation. The square root of the area of the ellipse defined by the first harmonic was used as an integrative measure of opercular flag size (Rohlf, 1992).

Bilateral asymmetry in flag morphology can be considerable (Amcoff *et al.*, 2009). Our analyses revealed a high repeatability in overall opercular flag shape (whole set correlation $R = 0.99$, Rao's $F_{81,849.1} = 8.97$, $P < 0.001$) although repeatabilities of single components of shape variation were more moderate (PC1-9; $R = 0.3$–$0.7$; $P < 0.001$ in all cases). Thus, males vary significantly in opercular flag shape and all subsequent analyses were based on the mean value of any given metric for the left and right sides of each individual.

Flag shape was significantly related to the length of the total opercular flag and flag size (multivariate regression of flag shape on length and size; length: Wilk's $\lambda_{9,139} = 0.69$, $P < 0.001$; size: Wilk's $\lambda_{9,139} = 0.74$, $P < 0.001$) and to enable size-independent ordinations

of mean flag shape across populations, we, therefore, generated a size-adjusted flag shape data by partialling out covariance between total flag length and flag size on the one hand and flag shape on the other by means of ordinary multivariate regression. As for body shape, we then calculated Euclidean distances in mean flag shape between all population-pairs in the multivariate space described by these size-adjusted flag shape variables.

Finally, as a quantitative measure of phenotypic divergence between populations, we calculated $P_{ST}$ for all size-adjusted multivariate measures of male body and flag shape. This metric is basically analogous to divergence in genes coding for quantitative traits ($Q_{ST}$) but is potentially more sensitive to environmental and nonadditive genetic effects (Merilä & Crnokrak, 2001; Pujol *et al.*, 2008). Yet $P_{ST}$ provides a useful measure of population divergence when $Q_{ST}$ estimates are unachievable (Merilä *et al.*, 1997; Storz, 2002; Saint-Laurent *et al.*, 2003; Leinonen *et al.*, 2008). We estimated $P_{ST}$ as in Leinonen *et al.* (2008), assuming a heritability of $h^2 = 0.5$.

## Results

### Genetic structure

In no case did any of the microsatellite loci show signs of large allele dropout or scoring errors (Van Oosterhout *et al.*, 2004). However, the allele frequencies of the Ast4 locus deviated significantly from Hardy–Weinberg proportions, because of homozygote excess, in 11 of 18 populations (at $\alpha = 0.05$ using Bonferroni adjusted *P*-values), and diagnostics suggested the presence of one or more null alleles at this locus. Further, allele frequencies of the Ast2 locus were dominated by a single allele and this locus was monomorphic in four populations. To assess the potential impact of the less well behaved loci, we ran all analyses presented below using all four loci as well as using a restricted marker data set including only Ast1 and Hb19. However, all results were quantitatively very similar and qualitatively identical, in terms of our ability or inability to reject null hypotheses at $\alpha$. Below we thus present the results of the multilocus analyses using all four markers (see Björklund & Bergek, 2009).

The structure of genetic variation across the 18 different populations is summarized in Table 2. The observed level of allelic diversity within populations was generally quite high but varied across loci. Although the magnitude of the global multilocus $F_{ST}$ value was low (0.025), the allelic distribution differed significantly across populations (Fisher exact test; $P < 0.001$ for all four loci). Moreover, pairwise tests between all population-pairs showed that the allelic distribution differed in the majority of comparisons (Fisher exact tests; 95 of 153 tests were significant at $\alpha = 0.05$ when using sequential Bonferroni adjusted *P*-values). Thus, the populations were clearly significantly differentiated. Because $F_{ST}$ can be a problematic measure of absolute differentiation, both in principle (Jost, 2008) and in practice (Heller & Siegismund, 2009), we also calculated the unbiased estimators of $G'_{ST\ est}$ (Hedrick, 2005) and $D_{est}$ (Jost, 2008). These estimates confirmed that absolute differentiation was, indeed, much higher than suggested by the global $F_{ST}$, although it varied across loci ($\approx 0.3$ at Ast1 and Hb19) (see Table 2). However, $F_{ST}$ does provide a sound measure of relative differentiation (Heller & Siegismund, 2009; Ryman & Leimar, 2009) and pairwise $F_{ST}$ and $D_{est}$ measures between *C. riisei* populations were indeed strongly correlated (rank correlation across all population pairs; $r_s = 0.88$). We thus based a cluster analysis, describing the genetic structure, on the $F_{ST}$ matrix and the results suggested that populations located in the same or in proximate drainages tended to be genetically similar, although clustering was not perfect (Fig. 3). This interpretation was confirmed in a hierarchical analysis of molecular variance (i.e., AMOVA; Excoffier & Lischer, 2010), based on the $R_{ST}$ matrix (Weir, 1996) and testing variance components with a permutation test using 10 000 random permutations. This analysis showed that 10.9% ($P < 0.001$) of the

**Table 2** The number of alleles, structure of genetic variation, mean unbiased within-population gene diversity and two unbiased measures of population differentiation of the four microsatellite loci across the sampled *Corynopoma riisei* populations.

| Locus | $N_a$ | $F_{is}$ | $F_{st}$ | $F_{it}$ | $H_e$ | $G'_{ST\ est}$ | $D_{est}$ |
|---|---|---|---|---|---|---|---|
| Ast1 | 28 | 0.25 | 0.05 | 0.28 | 0.82 | 0.31 | 0.27 |
| Ast2 | 12 | 0.33 | 0.02 | 0.35 | 0.17 | 0.04 | 0.01 |
| Ast4 | 46 | 0.52 | 0.01 | 0.52 | 0.83 | 0.10 | 0.09 |
| Hb19 | 35 | 0.22 | 0.02 | 0.23 | 0.93 | 0.33 | 0.32 |

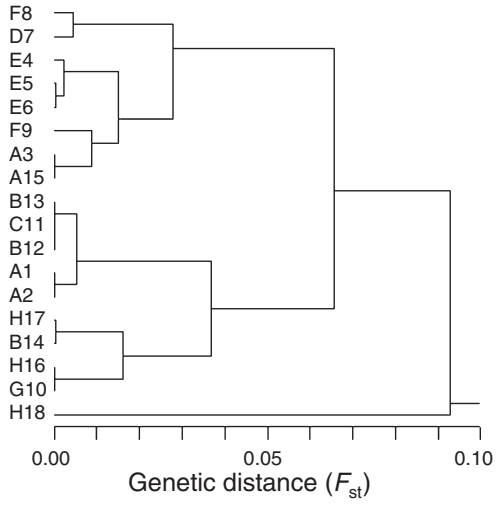


**Fig. 3** Hierarchical cluster tree, using the multilocus $F_{st}$ matrix, based on Johnsons's max method (Johnson, 1967). Letters denote drainages and numbers denote site number (see Fig. 2).

variance could be ascribed to variation among drainages, whereas 1.3% ($P = 0.089$) of the genetic variance was ascribed to variation among populations within drainages. Consequently, the scaled within-population variance component was 87.8% ($P < 0.001$). Our analysis further suggested the presence of two main clusters (Fig. 3): populations inhabiting drainages in the southwest tended to cluster together and were genetically distinct from populations in drainages in the north and east. This pattern was corroborated by isolation-by-distance analyses [$F_{ST}$ against ln (distance)]: the genetic distance between populations was positively related to the geographical distance between populations ($\beta = 0.005$; Mantel's test, $P_{\alpha/2} = 0.008$) and, if anything, even more strongly related to the nearest water-way distance between populations ($\beta = 0.007$; Mantel's test, $P_{\alpha/2} = 0.003$) (Fig. 4).

## Allometry

Although the length of all appendages (i.e., fins and opercular flag) scaled positively with male body length, we note that these sexually dimorphic traits (Bushmann & Burns, 1994) showed a distinct pattern of covariation with body size: an apparent upper boundary region was present on the allometric relationship, with a concentration of observation within this region and with scattered observations under it (exemplified in Fig. 5). We tested for such a pattern by first fitting linear regressions, using body length as the independent variable ($P < 0.001$ in all cases) and appendage length as the dependent variable, and then asking whether the variance of the absolute value of negative residuals was equal to that of positive residuals. These analyses confirmed that the variance of negative residuals was, indeed, generally much larger than that of positive residuals (dorsal fin length: $F_{57,92} = 3.5$; length of upper lobe of tail fin: $F_{63,86} = 5.97$; length of lower lobe of tail fin: $F_{62,87} = 8.42$; anal fin length: $F_{61,88} = 4.03$; opercular flag length: $F_{48,101} = 5.81$; $P < 0.001$ in all cases).

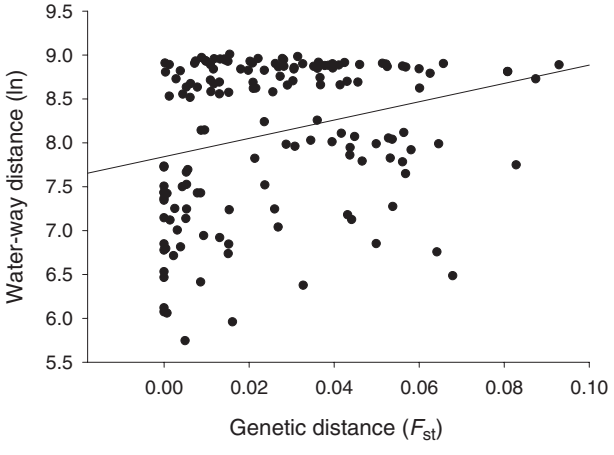


**Fig. 4** Bivariate plot showing the relationship between genetic distance and the nearest water-way distance (including oceanic distance for different drainages) between all population pairs. Line represents conventional LS regression.

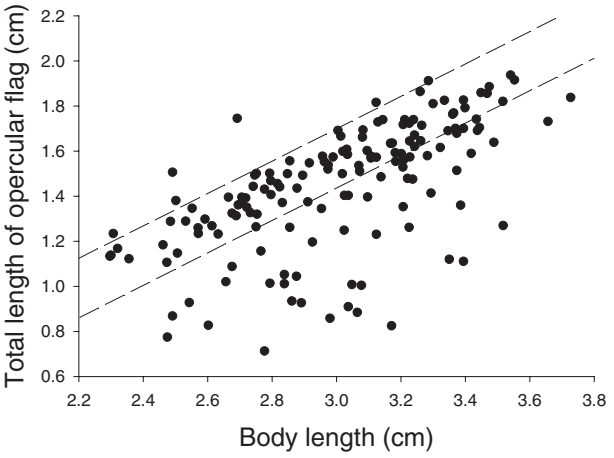


**Fig. 5** The relationship between body length and total opercular flag length, showing an apparent upper boundary region and scattered observations below this. Dashed lines represent a subjective delineation of an upper boundary region (see text).

## Body shape

Overall, there were highly significant differences in male body shape across populations (MANCOVA; population: Wilk's $\lambda_{224,1303} = 0.087$, $P < 0.001$; body size: Wilk's $\lambda_{14,120} = 0.809$, $P = 0.021$). However, the magnitude of the difference in mean body shape between pairs of populations was not positively related to their relative genetic distance (Euclidean distance matrix against $F_{st}$ matrix: Mantel's test, $P_{\alpha/2} = 0.97$) or to their geographical distance (Euclidean distance matrix against nearest water-way distance matrix: Mantel's test, $P_{\alpha/2} = 0.70$). Thus, the degree of body morphology differences was not significantly related to the magnitude of genetic differentiation across populations. This suggests that body shape variation across populations is dictated by factors unrelated to the degree of genetic differentiation. The values of $P_{ST}$ for our size-adjusted body shape indices ranged between 0.004 and 0.087 (mean $P_{ST} = 0.052$).

We investigated the relationship between body shape and environment by first conducting a discriminant function analysis of the size-adjusted body shape data matrix, using population as the grouping factor. This analysis yielded four discriminant functions with scores that differed significantly between populations (at $\alpha = 0.05$). Mean score per population on these four discriminant functions were then collectively used as independent variables in multiple regression models

where our composite measures of habitat characteristics were treated as the dependent variable. These analyses showed that average body shape was significantly related to the first ($F_{4,12} = 3.61$, $P = 0.038$) but not the second ($F_{4,12} = 0.25$, $P = 0.903$) principal component of environmental variation across sites. Further analyses revealed that this pattern was primarily caused by a covariation between the third discriminant function and the degree of shading vegetation and stream width in habitats. Visualizations (see Fig. 1) showed that fish in populations inhabiting wide streams with much shading vegetation tended to have more elongated and shallow bodies with a more upwards pointed mouth compared to fish in narrow streams with little shading vegetation.

### Opercular flag shape

As the shape of the opercular flag varied with its total length, we tested for a difference in opercular flag shape between populations using a MANCOVA, with the PCs of the elliptic Fourier coefficients as the response variable matrix, entire flag length and flag size as covariates and population as a factor. This analysis showed that flag length and size had independent effects on flag shape (Wilk's $\lambda_{9,123} = 0.622$, $P < 0.001$ and Wilk's $\lambda_{9,123} = 0.716$, $P < 0.001$, respectively) and that populations differed significantly in flag shape (Wilk's $\lambda_{144,987} = 0.253$, $P = 0.014$). As was the case for variation in body shape, the magnitude of the difference in mean flag shape between populations was not positively related to their genetic distance (Euclidean distance matrix against $F_{st}$ matrix: Mantel's test, $P_{\alpha/2} = 0.95$) or to their geographical distance (Euclidean distance matrix against nearest water-way distance matrix: Mantel's test, $P_{\alpha/2} = 0.71$). In contrast, the magnitude of body shape differences was positively related to the magnitude of opercular flag shape differences across populations (Mantel's test, $P_{\alpha/2} = 0.001$), such that populations that were more different in mean body shape were also more different in flag shape. To test directly for a covariation between body shape and opercular flag shape, we first reduced the number of size-adjusted body shape variables by means of a principal component analysis based on the covariance matrix. We then related size-adjusted body shape PCs to our size-adjusted measures of opercular flag shape (EFA) by means of a canonical correlation analysis. This analysis revealed a significant covariation both across all individuals (PC1-5 versus EFA1-8; Rao's $F_{40,595.6} = 1.46$, $P = 0.035$) and across population means (PC1-4 versus EFA1-4; Rao's $F_{16,28.1} = 2.53$, $P = 0.015$). Collectively, these analyses strongly suggest that the same factors affect variation in body and flag shape across populations in male *C. riisei* but that these factors are independent of genetic differentiation at neutral loci. The values of $P_{ST}$ for our size-adjusted opercular flag shape indices ranged between 0.003 and 0.110 (mean $P_{ST} = 0.060$).

A canonical correlation analysis between the set of nine size-adjusted opercular flag shape variables on the one hand and the two principal components of environmental variation on the other revealed a significant covariation between flag shape and environment across populations (Rao's $F_{18,12} = 2.986$, $P = 0.029$). There was a strong and significant canonical correlation between the first ($R_1 = 0.97$, $\chi^2 = 34.02$, d.f. = 18, $P = 0.013$) but not the second ($R_2 = 0.65$, $\chi^2 = 5.49$, d.f. = 8, $P = 0.704$) pair of canonical variables (Fig. 6). Further analyses revealed that this pattern of covariation was caused primarily by males from shallow streams with more shading vegetation having opercular flags that tended to be relatively narrow with a more convex curvature.

## Discussion

Our study represents the first study of population differentiation in *C. riisei* and several insights can be gained from our analyses of geographical variation in morphology in this remarkable fish. We address three main points below. First, there was extensive genetic structuring of *C. riisei* populations both at a larger scale across Trinidad but also within single river drainages. Second, general body morphology covaried with habitat characteristics across populations. Third, and most importantly, male opercular flag shape differed between populations and covaried with both environmental variables and with general body morphology in a manner that is consistent with sensory drive contributing to population differentiation of this sexual signal.

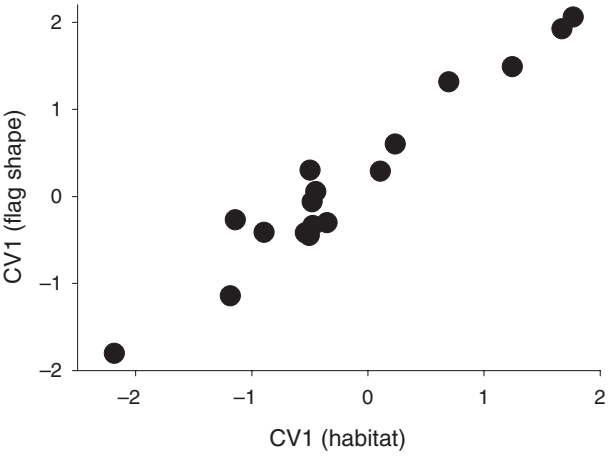


**Fig. 6** Ordination of populations in the space described by the first canonical variable, from an analysis of covariation between habitat characteristics and the shape of the opercular flag across populations. A positive loading on CV1 for the habitat variables was associated with relatively shallow streams with more shading vegetation. A positive loading on CV1 for flag shape describes relatively narrow opercular flags with a more convex curvature.

The fact that we found substantial genetic subdivision among populations of *C. riisei* is perhaps not surprising: fine-scaled genetic structure is common in freshwater fish, because of a combination of limited gene flow and divergent natural selection (e.g., Bermingham & Martin, 1998; Lu & Bernatchez, 1999; Lovejoy & de Araujo, 2000; Sivasundar *et al.*, 2001; Ogden & Thorpe, 2002; Crispo *et al.*, 2006). Interestingly, our results show four similarities with the structure of the most intensely studied freshwater fish of Trinidad, the guppy (*Poecilia reticulata*). *Corynopoma riisei* is fairly similar to the guppy both in terms of distribution and ecology (Kenny, 1995). First, as in guppies, we found an east-west subdivision in line with the hypothesis that Trinidad has been colonized from the mainland of South America by two different main routes during periods of glacial maxima (Carvalho *et al.*, 1991; Shaw *et al.*, 1991, 1994; Fajen & Breden, 1992; Alexander *et al.*, 2006). We note, however, that the major subdivision between the Caroni (site 16, 17 and 18) and the Oropuche (site 1,2,3 and 15) river drainages found in guppies is apparently not paralleled in *C. riisei*. Second, we found an overall pattern of isolation-by-distance indicative of restricted amounts of gene flow, as earlier documented in guppies (Alexander *et al.*, 2006; Crispo *et al.*, 2006). Third, the pattern of genetic structure in *C. riisei* was not as simple as might be expected from hypotheses based on a few distinct vicariant events. This parallels recent findings suggesting a geographically more complex pattern of genetic differentiation across Trinidad (Barson *et al.*, 2009; Suk & Neff, 2009), reflecting a moderate amount of more recent colonization events perhaps even related to anthropogenic activities (e.g., translocations for mosquito control purposes). In *C. riisei*, the fact that the south western populations formed a distinct group also opens up the possibility of a relatively recent colonization event from the South American mainland. Fourth, as in *C. riisei*, genetic distances between populations based on neutral markers are not correlated with morphological distances in guppies despite significant genetic structuring within and among river drainages (Alexander *et al.*, 2006). In the guppy, this has been widely interpreted as supporting a major role for divergent selection and local adaptation (Reznick & Bryga, 1996; Reznick *et al.*, 2001; Magurran, 2005; Alexander *et al.*, 2006).

Much of what we know about adaptation is rooted in studies of the pattern of covariation between the environment and mean phenotypes across populations or higher order taxa. In freshwater fish, a major general axis of morphological adaptation is body depth. Fish inhabiting structurally complex habitats in stagnant waters tend to show deep-bodied morphologies, whereas fish in pelagic or lotic habitats tend to show more streamlined body morphologies (Webb, 1984; Webb & Weihs, 1986). This is true both across and within species (e.g., Malmquist *et al.*, 1992; Walker, 1997; Brinsmead & Fox, 2002; Svanbäck & Eklöv, 2004; Langerhans, 2008) and is no doubt because of the fact that deep bodies are associated with better manoeuvring ability while streamlined morphologies show a lower drag and, thus, are better for persistent cruising or swimming in running water (Webb, 1984; Langerhans, 2008). The pattern of covariation between body morphology and habitat characteristics across populations of *C. riisei* documented here is consistent with local adaptation. The swordtail characin feeds almost exclusively on invertebrates (Alkins-Koo, 2000), representing both truly aquatic prey caught in the mid water column (e.g. aquatic insect larvae) and terrestrial prey items caught by surface drift feeding (e.g. ants and beetles) and the diet of *C. riisei* varies markedly across populations (N. Kolm & G. Arnqvist, unpubl.). Wider streams with much overhanging and shading vegetation generally offer more opportunities for profitable foraging by surface drift feeding in areas with relatively strong current. The body morphology of *C. riisei* associated with these habitat characteristics (slender bodies with a more upwards pointing mouth) is a close fit with predictions based on functional morphology as well as with those based on a large extant body of empirical work in other systems (see Langerhans, 2008). Although we cannot separate the relative roles of phenotypic plasticity and additive genetic effects in causing this covariation in the absence of common garden rearing experiments, we note that this type of variation in body morphology across populations has been found to have a sizeable genetic component in many other freshwater fish (e.g., Baumgartner, 1995; Langerhans, 2008; Sharpe *et al.*, 2008; Janhunen *et al.*, 2009). Further, the fact that the genetic distances between populations based on neutral markers were not correlated with morphological distances in *C. riisei* is at least consistent with divergent selection and local adaptation (Alexander *et al.*, 2006) as is the fact that our estimates for $P_{ST}$ for body morphology were about twice as large as the estimated global $F_{ST}$ (but see Hendry, 2002; Jost, 2008; Leinonen *et al.*, 2008). Yet these inferences are indirect and are also consistent with phenotypic plasticity: a solid case for a role of additive genetic factors would thus need to rely on direct experimental data.

The pattern of allometry of the male secondary sexual traits measured here (fins and opercular flags) provides a field validation of a previous finding in a laboratory population. Swordtail characins live in small schools (Alkins-Koo, 2000), and Bushmann & Burns (1994) showed that sexual maturation in males is socially controlled such that the presence of one or more sexually mature males in a social group inhibits sexual maturation among younger males. Once mature males are removed, sexual maturation (including the growth of secondary sexual traits) is rapid. The pattern of allometry of the secondary sexual traits in the field (see Fig. 5) indicates that the onset of growth of secondary sexual traits occurs at a range of different male sizes. This is consistent with social control such that males mature when social

suppression is released, which may occur at varying male ages.

The primary aim of this study was to assess the degree to which male sexual signals have diverged across populations and whether such divergence is consistent with a sensory drive scenario (see Introduction). We found that populations had indeed diverged significantly in morphology of the opercular flag, if anything to an even larger extent than in general body shape ($P_{ST}$ = 0.060 and $P_{ST}$ = 0.052, respectively). Differentiation in flag shape was, however, unrelated to both genetic and geographical distances between populations. Instead, three facts are consistent with the hypothesis that population divergence in flag morphology should reflect differences in local abundance of prey across populations. First, opercular flag shape covaried significantly with those environmental variables that should reflect local prey abundance. As argued earlier, deep streams with little shading vegetation should offer relatively more aquatic prey items (as opposed to terrestrial surface drift) compared to populations at the other extreme of this environmental gradient. Notably, the amount of shading/overhanging vegetation is a key predictor of both flag shape and diet (N. Kolm & G. Arnqvist, unpubl.) in *C. riisei*. Second, the magnitude of population differences in flag shape was positively related to the magnitude of population differences in body shape across populations, consistent with the tenet that greater environmental differences between populations are manifested both as large differences in trophic morphology and as greater differences in the sexual signal. Third, body shape covaried with opercular flag shape across populations, suggesting that much of the same factors affect variation in body and flag shape across populations of *C. riisei*. As argued earlier, we suggest that these factors relate to local prey abundance.

However, there are alternative explanations for the variation in the shape of the opercular flag seen here. These include the following: (i) nonadaptive effects of varying degrees of genetic similarity, (ii) a nonadaptive genetic correlation with body shape because of genes with pleiotropic effects and (iii) phenotypic plasticity in flag shape. The fact that genetic differentiation was unrelated to morphological differentiation refutes the first of these possibilities whereas the second is made unlikely by the fact that the opercular flag is a key courtship trait (Kutaygil, 1959; Nelson, 1964; Amcoff *et al.*, 2009; M. Amcoff & N. Kolm, unpubl.) that should be under direct and strong sexual selection. Further, the possibility that the shape of the opercular flag on one the hand and general body shape on the other should become strongly correlated because of pleiotropy seems unlikely: the operculum and the body do not belong to the same developmental module and should thus not be tightly morphologically integrated (Janvier, 1996; Gong & Korzh, 2004). Although phenotypic plasticity in flag shape cannot be ruled out given our data, we note that the fact that the same environmental factors affected variation in body and flag shape across populations is consistent with local adaptation of this sexually selected signal.

In summary, all of our *a priori* predictions were supported, and we suggest that the pattern of population differentiation in the remarkable and unique sexual signal of male swordtail characins provides at least provisional support for sensory drive at this geographical scale. Fish are well known to rapidly become habituated to different food items and have been shown to establish a search image matching the most common prey items (Kieffer & Colgan, 1992). We suggest that differences in female search images reflecting differences in prey abundance translates into differences in female preferences for male opercular flags, favouring males with flags that best mimic prey items common in any given population. Given the fact that female *C. riisei* store viable sperm internally for at least several months (Kutaygil, 1959; Alkins-Koo, 2000) and most likely mate with several different males during their life, this should result in strong sexual selection on opercular flag shape in males. Under this hypothesis, evolution would track food-related shifts in female sensory biases across populations, by adaptive fine-tuning of the male sensory exploitation device to match local female search images. We note that this scenario is perhaps not a classic sensory drive scenario (Boughman, 2002), as it is not caused by direct environmental effects on the visibility of the sexual signal but rather on indirect environmental effects on the draw of the signal.

To date, the extent to which the documented differences in male sexual signals may contribute to any incipient reproductive isolation, or assortative mating, between populations of *C. riisei* is unknown. Other male sexual signals, in particular the release of pheromones from caudal glands (Atkins & Fink, 1979) and gill glands (Burns & Weitzman, 1996), are likely to play an additional role in mate attraction and sexual isolation in *C. riisei*. Controlled laboratory crosses would be required to assess reproductive isolation between populations and to clearly separate genetic and environmental sources of trait differentiation. Interestingly, a certain amount of reproductive isolation, both premating (Alexander & Breden, 2004) and post-mating (Ludlow & Magurran, 2006; Russell & Magurran, 2006), has evolved in guppies over the same geographical scale. Finally, we note that the heart of the sensory drive scenario suggested here could be verified in a laboratory setting, where females are first trained by foraging on different prey items and then tested for diversification in preference for opercular flags. Sensory drive predicts that female attraction to male flags from different populations should vary with female food regime in the laboratory and should match the prey items most common in different populations.

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