

Shared and unique features of morphological differentiation between predator regimes in *Gambusia caymanensis*

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Abstract

When multiple groups of organisms experience similar environmental gradients, their patterns of differentiation might exhibit both shared and unique features. Here, we investigated the relative importance of three factors in generating body shape variation in a livebearing fish, *Gambusia caymanensis*, inhabiting the Cayman Islands: (i) shared patterns of divergent selection between predator regimes (presence/absence of piscivorous fish) driving replicated morphological differentiation, (ii) historical island effects yielding different morphologies across the three islands and (iii) unique effects of predation on morphological differentiation within each island. Shared effects of predation proved much more important than historical or unique effects. Populations coexisting with piscivorous fish exhibited larger caudal regions and smaller heads than conspecifics found in the absence of predatory fish. These results match *a priori* predictions, and mirror recent findings in a number of fish species, suggesting predation might often drive predictable morphological trends in disparate fishes. However, interestingly, the sexes achieved this morphological pattern through different means: head depth, caudal peduncle length and depth in males; head length, caudal peduncle depth in females. In *G. caymanensis*, we quantitatively confirmed that predation intensity represents a primary driver of body shape differentiation.

Introduction

To what extent do organisms experiencing similar environments exhibit similar phenotypic responses? Replicated patterns of divergence implicate selection as a driver of deterministic outcomes. Indeed, cases of convergent/parallel evolution provide some of the most remarkable demonstrations of the power of natural selection to produce predictable outcomes (Robinson & Wilson, 1994; Conway Morris, 1998; Losos *et al.*, 1998; Schluter, 2000; Wood *et al.*, 2005; Hoekstra, 2006; Ozgo & Kinnison, 2008). However, nonreplicated patterns of divergence also exist, revealing the unpredictability of organismal responses to selection. Differences in phenotypic responses to similar environments across groups of organisms can derive from a number of sources, including lineage-specific intrinsic factors (e.g. genetic vari-

ances and covariances among traits, mutation order), genetic drift and cryptic differences in selection (Mani & Clarke, 1990; Cadle & Greene, 1993; Schluter, 1996; Price *et al.*, 2000; Gould, 2002; McGuigan *et al.*, 2005; Blount *et al.*, 2008).

The combined influence of shared selective pressures and unique histories is expected to frequently produce both shared and unique features of phenotypic differentiation when multiple groups of organisms experience common environmental gradients (Winemiller, 1991; Travisano *et al.*, 1995; Huey *et al.*, 2000; Langerhans & DeWitt, 2004; Langerhans *et al.*, 2006; Ozgo & Kinnison, 2008; Riopel *et al.*, 2008). To illuminate the relative generality or peculiarity of phenotypic differentiation, evolutionary biologists must gain a better understanding of the relative importance of shared and unique responses to common environmental conditions in the wild (Matos *et al.*, 2002; Vanhooydonck & Irschick, 2002; Ruzzante *et al.*, 2003; Blackledge & Gillespie, 2004; Langerhans & DeWitt, 2004; Erokhmanoff *et al.*, 2009).

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Divergent selection – selection pulling trait means of two or more populations toward different adaptive peaks – is putatively the primary mechanism generating and maintaining phenotypic diversity (Rice & Hostert, 1993; Orr & Smith, 1998; Schluter, 2000). Organisms can respond to divergent selection with genetic divergence (differences in fixed, genetically determined phenotypes), phenotypic plasticity (environmentally contingent phenotype production), or some combination thereof; either source of adaptive phenotypic variation can facilitate microevolutionary change and speciation (Schlichting & Pigliucci, 1998; Pigliucci & Murren, 2003; West-Eberhard, 2003; Schlichting, 2004; Pigliucci *et al.*, 2006; Crispo, 2007; Ghalambor *et al.*, 2007).

For many fishes, an important source of divergent selection is thought to arise from the heterogeneous distribution of predation intensity from piscivorous fish. Selection is generally expected to favour steady-swimming performance in low-predation environments to increase competitive abilities, but instead favour unsteady-swimming performance (namely, fast-start escapes) in high-predation environments to increase survival (Walker, 1997; Domenici, 2003; Langerhans *et al.*, 2004, 2007; Walker *et al.*, 2005; Langerhans, 2009a,b; Langerhans & Reznick, 2009). Because these two swimming modes are optimized with different propulsor arrangements, and because many fish employ a mechanically coupled locomotor system – e.g. body and caudal fin propulsion for both steady and unsteady locomotion (see Webb, 1984; Blake, 2004) – selection is consequently expected to favour different body shapes in low- and high-predation environments. Specifically, fish are generally predicted to exhibit a larger caudal region (body region stretching from the dorsal and anal fins to the caudal fin base) and smaller anterior body/head region in environments with greater intensity of predation from piscivorous fish (Blake, 1983, 2004; Webb, 1984, 1986; Walker, 1997; Langerhans, 2009a,b; Langerhans & Reznick, 2009). This prediction derives from the functional morphology of fish locomotion, as these morphological features should act to maximize thrust and stability while minimizing drag and recoil energy losses during different swimming activities.

Livebearing fishes in the family Poeciliidae inhabit highly variable environments and often comprise important prey items for piscivorous fish. Recent work has uncovered morphological patterns in several poeciliid species that match the aforementioned predictions. Because of its apparent generality in poeciliids, the trend has been suggested to represent a sort of ecomorphological paradigm (e.g. Langerhans & DeWitt, 2004; Langerhans *et al.*, 2004, 2007; Hendry *et al.*, 2006; Gomes & Monteiro, 2008; Langerhans & Reznick, 2009). But just how predictable is morphological differentiation between predator regimes in poeciliid fishes?

The Cayman Islands provide an exceptional opportunity to address this question. The Caymans comprise

three small, isolated islands in the western Caribbean, situated just over 200 km from the nearest shorelines of Cuba and Jamaica. A small poeciliid fish, *Gambusia caymanensis*, occurs on all three Cayman Islands, in each case inhabiting environments varying in predation intensity from piscivorous fish. Because of their geographical isolation from one another, *G. caymanensis* inhabiting different islands are likely relatively isolated. Indeed, gene flow appears more restricted among islands relative to genetic exchange among populations within islands (R.B. Langerhans & M.E. Gifford unpublished data). What is the relative importance of shared responses to predator regimes on each island, historical effects of isolation among islands, and unique responses to predation across islands? Here we address this question by evaluating the importance of these three factors in explaining body shape variation in *G. caymanensis*.

Methods

Study system

There is some disagreement regarding the taxonomic status of the focal study organism, i.e. whether the form represents an endemic species, *G. caymanensis*, or is synonymous with *G. puncticulata puncticulata* from Cuba (e.g. Rivas, 1963; Fink, 1971; Rauchenberger, 1989). Recent molecular work suggests the form indeed represents a recent colonization from Cuba (< 200 000 years ago; Lydeard *et al.*, 1995; R.B. Langerhans, M.E. Gifford, O. Domínguez-Domínguez, I. Doadrio unpublished data); however, considering the large amount of intervening ocean between Cuba and the Cayman Islands, levels of migration and gene flow between the two regions are probably very low. In any case, here we refer to these fish as *G. caymanensis*.

Gambusia caymanensis is a common inhabitant of ponds in the Cayman Islands (Abney & Rakocinski, 2004). We collected 970 adult *G. caymanensis* from nine ponds across the three Cayman Islands in March 2006 (Table 1, Fig. 1). To test *a priori* predictions regarding morphological differences between predator regimes, sites were

Table 1 Summary information for *Gambusia caymanensis* collections.

Island	Population	Predator regime	Salinity (ppt)	Males	Females
Grand Cayman	GC1	Low	23.19	2	8
Grand Cayman	GC2	Low	10.10	5	110
Grand Cayman	GC3	High	8.41	12	37
Grand Cayman	GC4	High	37.08	19	135
Little Cayman	LC1	Low	4.49	42	136
Little Cayman	LC2	Low	31.63	5	37
Little Cayman	LC3	High	13.66	2	83
Cayman Brac	CB1	Low	32.36	4	73
Cayman Brac	CB2	High	26.16	28	232

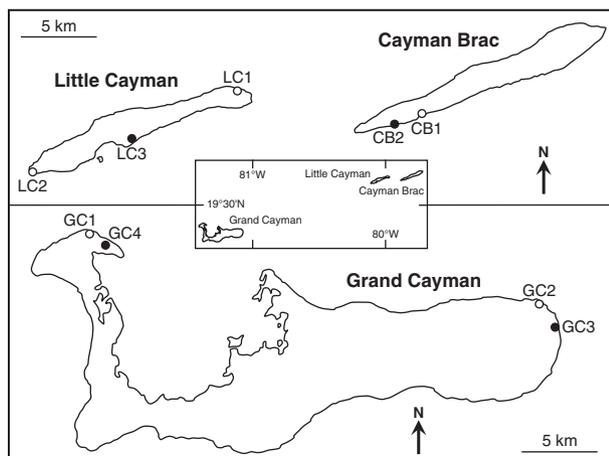


Fig. 1 Map of study sites in the Cayman Islands. Open circles: low predation; filled circles: high predation.

specifically chosen due to the apparent presence or absence of piscivorous fish. The presence of predatory fish was assessed using extensive surveys with seines, cast nets, dip nets and visual inspection. Potential predators included needlefish (*Strongylura* spp.), great barracuda (*Sphyrna barracuda*), tarpon (*Megalops atlanticus*) and sleepers (*Gobiomorus dormitor*, *Eleotris pisonis*). Avian predators might additionally represent important selective agents, and were present at most sites. Because we were interested in the effects of piscivorous fish, not birds, we did not quantify densities of bird predators here – although, bird densities are not expected to differ among sites with and without piscivorous fish. All ponds were brackish to saline, with similar average salinities in low- and high-predation sites (mean \pm standard error; low predation: 20.35 ± 5.7 ppt, high predation: 21.33 ± 6.36 ppt).

Morphometrics

A lateral photograph was taken of each fish using a digital camera (Canon EOS Digital Rebel XT_i with EF 50 mm f/2.5 macro lens; Tokyo, Japan). One of us (A.M.M.) digitized the following landmarks on digital images using tpsDig (Rohlf, 2006): (i) most anterodorsal point of premaxilla, (ii) indentation at the posterodorsal end of head, (iii) anterior insertion of dorsal fin, (iv) posterior insertion of dorsal fin, (v) dorsal insertion of caudal fin, (vi) ventral insertion of caudal fin, (vii) posterior insertion of anal fin, (viii) anterior insertion of anal fin, (ix) intersection of the operculum and ventral body profile, (x) dorsal insertion of pectoral fin, and (xi) centre of eye (Fig. 2). We used geometric morphometric methods to examine morphological variation (Rohlf & Marcus, 1993; Marcus *et al.*, 1996; Zelditch *et al.*, 2004). Using tpsRelw (Rohlf, 2007), we performed generalized

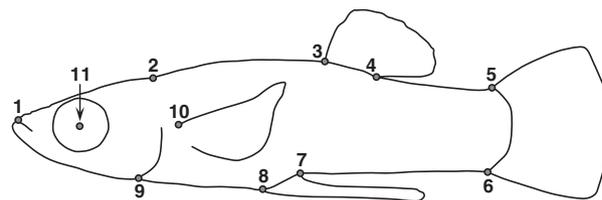


Fig. 2 Landmarks used for morphometric analysis (male depicted).

Procrustes analysis for the entire dataset (i.e. align landmark coordinates by rotating, translating and scaling coordinates to remove positioning effects and isometric size effects; Bookstein, 1991; Marcus *et al.*, 1996) and obtained shape variables (relative warps) for statistical analysis.

Statistical analysis

Sexes are highly dimorphic in *G. caymanensis*. When pooling sexes for analysis, the large magnitude of differences between sexes relative to all other factors indicated that separate analyses would be most appropriate. Thus, all analyses, subsequent to the generalized Procrustes analysis, were conducted separately for males and females.

We employed a statistical approach that explicitly examines the relative importance of shared responses to similar predator regimes regardless of island history, unique histories among islands regardless of predator regime, and island-dependent responses to predator regimes in generating morphological variation (see Langerhans & DeWitt, 2004). Specifically, we conducted nested multivariate analysis of covariance (MANCOVA), where geometric shape variables (relative warps) served as dependent variables, centroid size served as the covariate (CS, controlling for multivariate allometry), and predator regime (PR, testing for shared selective regimes), island (ISL, testing for unique island histories), the interaction between predator regime and island (PR \times ISL, testing for unique effects of predator regime on different islands), and population nested within PR \times ISL (random effect; testing for variation among replicate populations) served as independent variables:

$$\text{Relative warps} = \text{Constant} + \text{CS} + \text{PR} + \text{ISL} + \text{PR} \\ \times \text{ISL} + \text{Pop}(\text{PR} \times \text{ISL}) + \text{Error}$$

It is true that populations could be feasibly treated as fixed effects, rather than random effects, since they are repeatable (i.e. populations could be re-sampled in the future), were deliberately selected based on their geographical and ecological characteristics, and differences among populations might be of direct interest (e.g. differences could indicate the importance of other selective agents). However, we feel that treating populations as random effects provides more robust statistical tests of

primary terms of interest (PR, ISL, PR \times ISL) by effectively using population as the unit of replication for those tests, which generalizes the results to other populations not yet examined. Moreover, the intended subject of this study is *G. caymanensis*, not these nine particular populations *per se*.

Random nested factors are not often possible in a MANOVA framework, as matrix determinants can become negative, making the term un-testable (see Rencher, 2002, p. 162). Thus, nested MANOVAs generally use fixed effects exclusively, which can cause tests of main effects to exhibit inflated type I error rates if the nested term is significant (i.e. treating individuals, rather than populations, as replicates). Because the nested term was significant here, we conducted mixed-model nested MANCOVA. In this study, the maximum number of dependent variables that would yield positive determinants for the three primary terms was three. Thus, statistical tests were conducted using only the first three relative warps as dependent variables. Because this mixed-model approach suffers low statistical power for the three primary terms, greater emphasis is placed on estimates of multivariate effect size and the nature of shape differentiation than on *P*-values.

To assess the relative importance of model terms, we used Wilks's partial η^2 (measure of partial variance explained by a particular term; multivariate approximation of $SS_{\text{effect}}/[SS_{\text{effect}} + SS_{\text{error}}]$, see appendix of Langerhans & DeWitt, 2004). Wilks's partial η^2 has recently garnered common usage in evolutionary studies as an estimate of effect size within a MANOVA framework (e.g. Langerhans & DeWitt, 2004; Hendry *et al.*, 2006; Langerhans *et al.*, 2006; Butler *et al.*, 2007; Karim *et al.*, 2007; Aguirre *et al.*, 2008; Sharpe *et al.*, 2008; Tobler *et al.*, 2008; Ward & McLennan, 2009). We calculated Wilks's partial η^2 using the full shape space (all 18 relative warps).

Body size distributions broadly overlapped between predator regimes and islands for both males and females. Heterogeneity of slopes (interaction between centroid size and main terms of interest) was nonsignificant for all terms in males (all *P* > 0.81), but was significant for all terms in females. However, these terms in females were of relatively minor importance (all Wilks's partial η^2 < 0.11, less than half as important as all terms of interest), and apparently largely reflected high statistical power. Further, this heterogeneity did not alter the nature or magnitude of shared, historical, or unique features of differentiation. All divergence vectors (see below) were highly correlated when including or excluding interaction terms with centroid size (all *r* > 0.98, *P* < 0.0001), and thus we only present analyses excluding the terms.

To associate the nature of morphological variation associated with terms of interest, we performed canonical analyses of the terms following Langerhans (2009b). Briefly, we performed a principal components analysis

(PCA) of the sums of squares and cross-products matrix of a given term from MANCOVA to derive eigenvectors of divergence. This procedure avoids scaling the multidimensional space by a matrix inverse, which can produce distortion of the shape space. These divergence vectors describe linear combinations of dependent variables exhibiting the greatest differences between groups, controlling for other factors in the model, in Euclidean space. Divergence vectors were calculated using the full shape space, and individuals were projected onto these vectors. Shape variation along relevant divergence vectors was visualized using the thin-plate spline approach (mapping deformations in shape from one object to another; Bookstein, 1991).

For divergence vectors of interest, we assessed similarities in the nature of morphological responses among the sexes by calculating the angle between corresponding axes of divergence. For example, to evaluate whether differences in body shape between predator regimes was significantly similar for males and females, the angle between the divergence vectors derived from the predator regime term of each MANCOVA would be calculated. We tested whether two vectors were significantly associated by determining whether their angle was significantly smaller than 90° (i.e. null hypothesis of zero vector correlation). This was accomplished by constructing a 95% confidence interval for an observed angle based on 1000 bootstrapped samples. Specifically, we re-sampled MANCOVA residuals from each population (with replacement) separately by sex, assigned these residuals to predicted values from the original MANCOVA (to retain covariation among variables), re-performed MANCOVAs, and calculated the angle between the two divergence vectors derived from a particular model term for each sex. The one-tailed upper 95% confidence limit for the angle was estimated by the empirical upper 5.0 percentile of the bootstrapped angle distribution (Manly, 2007). The null hypothesis of vector independence was rejected when the upper confidence limit of the bootstrapped angle distribution was less than 90°.

Because we found that shared effects were much stronger than historical or unique effects for both sexes (see results below), we wished to determine whether variation in the presence of predatory fish was responsible for driving the major axis of between-population variation in morphology. That is, we asked whether shared responses to predation was not only more important than island histories and unique responses to predation, but whether it was also the primary driver of population differentiation. To accomplish this, we separately evaluated for each sex whether the shared divergence vector (\mathbf{d}_{PR} ; the divergence vector derived from the predator regime term of MANCOVA) was statistically indistinguishable from \mathbf{z} , the first principal component of variation among population means, which describes the major axis of phenotypic differentiation among populations (Schluter, 1996). We calculated \mathbf{z} by performing

PCA of the variance-covariance matrix of size-adjusted population means of geometric shape variables (least-squares means of relative warps from MANCOVA using centroid size as a covariate and population as the independent variable). We tested whether the observed angle between \mathbf{d}_{PR} and \mathbf{z} could be statistically distinguished from zero (i.e. null hypothesis of parallel vectors) by generating a null distribution of expected angles for parallel vectors resulting from sampling error. Specifically, we generated 999 pairs of bootstrapped samples of individuals within populations for each sex, and calculated the angle between the two estimates of \mathbf{z} . We tested significance of the observed angle by determining the number of bootstrapped angles that was greater than or equal to this angle.

We conducted two discriminant function analyses (DFA) for each sex to provide intuitive metrics regarding the distinctiveness of morphological differences between groups (i.e. percentage of fish correctly classified according to predator regime or island). Each DFA used the 18 relative warps as the dependent variables and either predator regime or island as the independent variable. DFAs were conducted using jackknife sampling as a cross-validation technique (i.e. each individual was sequentially removed from the dataset and classified according to a discriminant function derived with the remaining data).

To provide metrics of shape disparity comparable across geometric morphometric studies, and to evaluate overall shape differences between all population pairs, we also calculated Procrustes distance (D_P) between populations. D_P is the standard metric for shape dissimilarity in geometric morphometrics (Bookstein, 1996; Dryden & Mardia, 1998), and was calculated as a geodesic distance (angle in radians) using tpsSmall (Rohlf, 2003).

Because life-history variation (e.g. number and size of offspring) in females might affect body shape by producing extended abdominal regions during late stages of pregnancy (Langerhans & Reznick, 2009), and because

life-history traits are known to sometimes differ between predator regimes in poeciliid fishes (e.g. Reznick & Endler, 1982; Reznick *et al.*, 1990, 1997; Johnson & Belk, 2001; Jennions & Telford, 2002), we wished to avoid confounding effects of life histories on body shape variation in females. To this end, we visually examined each specimen, and identified those exhibiting apparent signs of pregnancy (obviously enlarged abdomens; $n = 137$). Re-performing analyses for females after excluding these pregnant individuals revealed that results were extremely similar to the full analyses, with all divergence vectors being virtually identical (all $r > 0.98$, $P < 0.0001$). This suggests that results for females unlikely reflect confounding effects of life-history variation, and thus only results using the full dataset are presented.

Results

The first three relative warps, which were used to determine significance in the mixed-model nested MANCOVAs, explained the majority of shape variance in both sexes (males: 71.8%, females: 67.6%). MANCOVAs revealed significant effects of centroid size (indicating multivariate allometry) and populations nested within the predator regime \times island interaction term (Table 2). Regarding the three terms of primary interest, predator regime was clearly the most important term in both sexes. Based on both Wilks's partial η^2 and statistical significance, predation had much stronger effects on body shape variation than island histories or island-specific responses to predation (Table 2). In both sexes, only allometry exceeded predation in explanatory ability. Examination of the divergence vector derived from the predator regime term in each MANCOVA indicated that males exhibited longer and deeper caudal peduncles, shallower heads, and a more ventrally positioned eye with predators, whereas females exhibited deeper caudal peduncles and shorter heads with predators (Figs 3 and 4). For females, unique effects approached statistical

Table 2 Results of MANCOVA examining body shape variation among *Gambusia caymanensis* populations.

Test for	Males					Females				
	Partial Variance	Relative Variance	Significance			Partial Variance	Relative Variance	Significance		
			F	d.f.	P			F	d.f.	P
Allometry (Centroid Size)	0.54	1.00	6.35	3, 107	0.0005	0.62	1.00	341.85	3, 839	< 0.0001
Shared Divergence (PR)	0.52	0.97	188.38	3, 1	0.0535	0.44	0.70	765.43	3, 1	0.0266
Island Histories (Isl)	0.29	0.54	7.87	6, 2	0.1170	0.27	0.44	5.04	6, 2	0.1748
Unique Divergence (PR \times Isl)	0.22	0.42	1.58	6, 2	0.4378	0.27	0.43	13.82	6, 2	0.0690
Replicate Variation (Pop[PR \times Isl])	0.27	0.51	3.07	9, 260.6	0.0016	0.21	0.33	6.79	9, 2042.1	< 0.0001

F-ratios were approximated using Wilks's Λ values for the island, predator regime \times island, and population nested within predator regime \times island terms. Partial variance explained by each term was estimated using Wilks's partial η^2 . Relative variance represents partial variance for a given term divided by the maximum partial variance value in the model.

PR, predator regime; Isl: island; Pop: population.

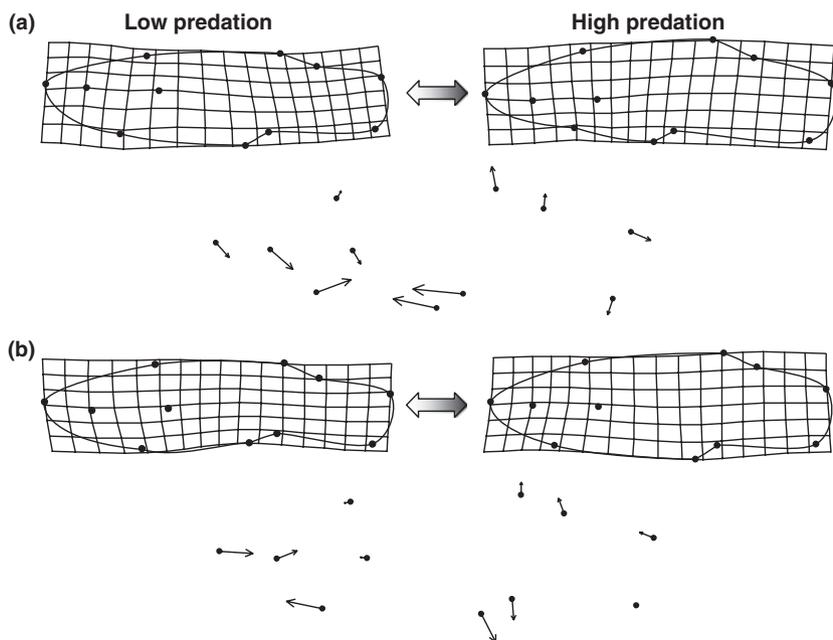


Fig. 3 Morphological differentiation between low-predation (left) and high-predation (right) populations of (a) male and (b) female *Gambusia caymanensis*. Body shape variation described by the divergence vector derived from the predator regime term of each MANCOVA, illustrated using thin-plate spline transformations relative to mean landmark positions for each sex. Grids depict observed range of variation (i.e. no magnification). Landmark vectors presented beneath each set of grids convey the direction and relative magnitude of change in the location of each landmark (arrows point toward values characteristic of high-predation populations). Solid lines connecting outer landmarks in grids are drawn to aid interpretation.

significance. Examination of the divergence vector derived from the interaction term of the MANCOVA revealed that the magnitude of divergence between predator regimes was lower on Little Cayman compared to the other two islands, although the nature of divergence was highly similar across each island. For males, there was some suggestive evidence for historical effects, as its effect size was larger than unique effects, and it was marginally insignificant. Inspection of the divergence vector derived from the island term of the MANCOVA suggested that males on Cayman Brac generally exhibited shorter and narrower caudal peduncles relative to the other two islands. Other historical and unique effects received little support in the MANCOVAs.

Because shared effects of predation were more important than historical or unique effects in both sexes, we exclusively examined the shared divergence vector (\mathbf{d}_{PR}) to evaluate the similarity of differentiation between predator regimes across the sexes. While the angle between male \mathbf{d}_{PR} and female \mathbf{d}_{PR} was fairly high, 62.9° , it was significantly less than 90° , as the upper 95% confidence limit determined from 1000 bootstraps was 75.6° . Thus, shared vectors of divergence, while certainly not identical, were nevertheless significantly associated among males and females.

The principal axis of phenotypic differentiation among populations, \mathbf{z} , explained about half of the between-population variance for both males (56.0%) and females (49.8%). Evaluating whether predation serves as the major driver of population differentiation, we found that the angle between \mathbf{d}_{PR} and \mathbf{z} was very low for both sexes (males: 8.3° , females: 12.1°). This resulted in \mathbf{d}_{PR} being

indistinguishable from \mathbf{z} , as the null hypothesis of parallel vectors could not be rejected for males ($P = 0.97$) or females ($P = 0.65$).

Discriminant function analyses revealed that 84% of males ($F_{18,100} = 10.84$, $P < 0.0001$) and 85% of females ($F_{18,832} = 57.91$, $P < 0.0001$) were correctly assigned to their predator regime of origin based on morphology. Regarding island of origin, 63% of males ($F_{36,198} = 4.50$, $P < 0.0001$) and 77% of females ($F_{36,1662} = 40.83$, $P < 0.0001$) were assigned correctly.

The magnitude of total body shape differences between populations, as measured by D_p , was larger for males (0.042 ± 0.002) than females (0.031 ± 0.001) (see Table 3). For both sexes, D_p was greater between predator regimes (males: 0.046 ± 0.003 , females: 0.034 ± 0.002) than within predator regimes (males:

Table 3 Procrustes distances (D_p) between populations for males (below diagonal) and females (above diagonal).

	GC1	GC2	GC3	GC4	LC1	LC2	LC3	CB1	CB2
GC1	–	0.025	0.035	0.031	0.019	0.029	0.023	0.019	0.033
GC2	0.045	–	0.048	0.047	0.030	0.039	0.026	0.019	0.052
GC3	0.056	0.053	–	0.017	0.026	0.040	0.034	0.040	0.027
GC4	0.049	0.059	0.027	–	0.027	0.031	0.034	0.038	0.022
LC1	0.031	0.031	0.038	0.039	–	0.029	0.020	0.019	0.028
LC2	0.035	0.036	0.037	0.036	0.021	–	0.034	0.026	0.030
LC3	0.043	0.051	0.041	0.039	0.043	0.034	–	0.024	0.039
CB1	0.051	0.038	0.073	0.074	0.042	0.045	0.069	–	0.041
CB2	0.030	0.043	0.035	0.032	0.019	0.019	0.040	0.051	–

Site abbreviations follow Table 1, Fig. 1.

0.037 ± 0.002 , females: 0.027 ± 0.002). However, D_P was similar whether between islands (males: 0.041 ± 0.003 , females: 0.030 ± 0.002) or within islands (males: 0.044 ± 0.004 , females: 0.033 ± 0.003).

Discussion

Shared, historical, and unique features of shape differentiation

A fundamental goal of evolutionary biology is to understand the relative importance of deterministic, replicated responses to common selective pressures, unique histories among groups of organisms experiencing this shared selection, and idiosyncratic responses to similar environmental conditions in generating phenotypic diversity (e.g. Travisano *et al.*, 1995; Huey *et al.*, 2000; Matos *et al.*, 2002; Blackledge & Gillespie, 2004; Langerhans & DeWitt, 2004; Langerhans *et al.*, 2006; Eroukhmanoff *et al.*, 2009). Here, we quantified the relative magnitudes of shared selective regimes, historical island effects, and the interaction between these factors – island-specific responses to common selective regimes – in generating morphological variation in *G. caymanensis*. We found that replicated morphological differentiation between predator regimes was of greatest importance in both sexes. However, suggestive effects of island histories and island-specific responses to predation were also apparent, albeit of lesser magnitude. The observed shared response across predator regimes is consistent with *a priori* predictions of divergent selection and previous empirical work demonstrating the importance of predation in the evolution of body shape in poeciliid fishes. In contrast to the predictable and deterministic effects of predation, it is unclear what underlying factors generated the observed historical and unique effects.

First, island effects appeared relatively small in magnitude: partial variance explained in MANCOVAs indicated marginal effects relative to predation, and D_P among populations revealed no clear island effects on overall body shape differences; although DFA suggested that islands did tend to exhibit characteristic mean shape differences. Thus, while previous work revealed no island effects on life-history traits of *G. caymanensis* (Abney & Rakocinski, 2004), we found evidence for minor differences among islands in body shape. Based on divergence vectors derived from the island term of MANCOVAs, island effects in both sexes reflected slightly unique body shapes in Cayman Brac populations relative to the other islands: shorter and narrower caudal peduncle in males; shorter caudal peduncle, longer midsection (region between landmarks 8 and 9) in females. These effects were most apparent in males. The relatively low-predation shape seen in Cayman Brac males (e.g. see negative displacement of \mathbf{d}_{PR} for Cayman Brac sites in Fig. 4) could reflect overall greater levels of resource competition and/or lower predation intensities on this island, although

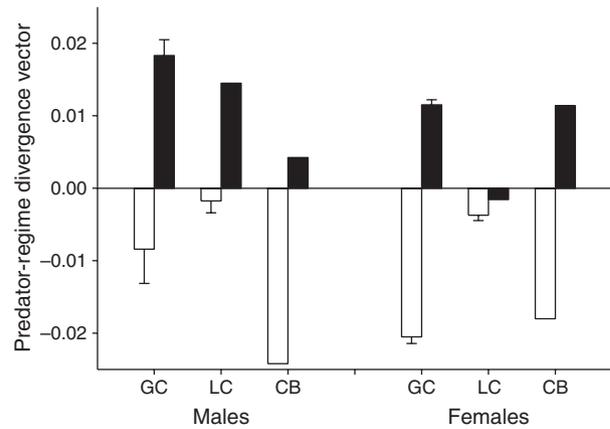


Fig. 4 Morphological differentiation between low-predation (open bars) and high-predation (filled bars) populations of *Gambusia caymanensis* across each island as depicted by the divergence vector derived from the predator regime term of each MANCOVA (mean \pm 1 SE). Sexes analysed separately. GC, Grand Cayman; LC, Little Cayman; CB: Cayman Brac.

quantification of such factors have not yet been performed. Interestingly, the high-predation locality on Cayman Brac appeared to be the only such site lacking tarpon as a potential predator. Whether this contributed to the unique body shape observed in Cayman Brac males is unknown, as other factors such as alternative selective agents, genetic drift, or island-specific intrinsic factors (e.g. **G** matrix) could have additionally played important roles.

Second, island-specific effects of variation in predation threat from piscivorous fish were of lesser importance than predation, and only observed in females. Both MANCOVA and D_P results revealed that females exhibited a lower magnitude of differences between predator regimes on Little Cayman compared to the other islands. The underlying cause of this finding is unknown, as predation intensity appears just as divergent across predator regimes on Little Cayman as on the other islands, and males exhibited strong morphological differences on the island. Possible causes of reduced differentiation for Little Cayman females include sex-specific selective regime variation on the island (i.e. convergence of fitness surfaces across predator regimes for females, perhaps related to selection on life-history traits), sex-specific intrinsic constraints to responses to selection (e.g. **G** matrix), population mixing and/or gene flow, and genetic drift.

Combined with recent studies, a general picture is now emerging which suggests that shared responses to predation typically outweigh unique responses for body shape differentiation in poeciliid fishes. Based on partial variance explained from MANCOVAs, the importance of shared patterns of body shape differentiation between predator regimes exceeded that of unique responses by

72% for different sexes of Trinidadian guppies (*Poecilia reticulata*; Hendry *et al.*, 2006), by 64% for different genera of poeciliid fishes (Langerhans & DeWitt, 2004), and by 136% and 63% for male and female *G. caymanensis* across different islands respectively (this study). While shared responses are generally greater in magnitude, the occurrence of unique responses is fairly common – although, unique responses primarily involved differences in the magnitude, not direction, of morphological differentiation in this study.

Predation intensity and morphological differentiation in fishes

A general morphological prediction exists for fish experiencing higher levels of predation from piscivorous fish: larger caudal region and smaller anterior body/head region (e.g. Walker, 1997; Langerhans, 2009a,b; Langerhans & Reznick, 2009). Results for both male and female *G. caymanensis* matched these predictions. Calculating caudal peduncle area and head area to provide relatively intuitive metrics (convex polygon of interconnected landmarks in those regions; see Langerhans *et al.*, 2007), high-predation populations in this study exhibited, on average, a larger caudal peduncle (males: 13%, females: 8%) and smaller head (males: 7%, females: 6%) than conspecifics in low-predation populations. These patterns mirror those observed in blue hole populations of *G. hubbsi* in the Bahamas (caudal peduncle area: 13%; head area: 5%; Langerhans *et al.*, 2007) and pond populations of *G. affinis* in Texas, USA. (caudal peduncle area: 3%; head area: 10%; Langerhans *et al.*, 2004). Moreover, the overall level of shape differences (measured as D_p) between predator regimes observed here was similar to findings in these other two previously studied *Gambusia* species (data from Langerhans *et al.*, 2007; Langerhans, 2009b).

The analogous morphological patterns observed in other *Gambusia* species are known to produce locomotor consequences (Langerhans *et al.*, 2004; Langerhans, 2006, 2009a,b). While not yet tested in *G. caymanensis*, based on empirical equations linking body morphology and locomotor performance in *Gambusia* fishes (Langerhans, 2009a,b), morphological differences between predator regimes observed here are indeed predicted to result in differences in locomotor performance: high-predation fish are predicted to exhibit higher maximum acceleration during fast-start escape bursts (17% higher in males, 20% higher in females) and lower endurance (time before fatigue) during steady swimming at 0.20 m s^{-1} (24% lower in males, 8% lower in females). Thus, consistent with *a priori* predictions, results suggest that divergent selection on locomotor performance between predator regimes has played a primary role in driving morphological differentiation.

This phenomenon of divergent selection on locomotor abilities across predator regimes may be relatively

common, as similar phenotypic patterns have now been uncovered in several distantly related fishes: e.g. *Brachyrhaphis rhabdophora* (Langerhans & DeWitt, 2004), *Carassius carassius* (Domenici *et al.*, 2008); *Culaea inconstans* (Zimmerman, 2007); *G. affinis* (Langerhans *et al.*, 2004; Langerhans, 2009b); *G. caymanensis* (this study); *G. hubbsi* (Langerhans *et al.*, 2007; Langerhans, 2009a); *Gasterosteus aculeatus* (Walker, 1997; Walker & Bell, 2000); *Poecilia reticulata* (Langerhans & DeWitt, 2004; Hendry *et al.*, 2006). These findings in multiple taxa across various geographical regions, and involving different predator species, suggests a high degree of predictability in both the nature of divergent selection between predator regimes, and the phenotypic responses to that selection.

In this study, geographical variation in the density of piscivorous fish appears to represent the principal axis of shape differentiation. In both sexes, the vector of body shape differentiation between predator regimes was statistically indistinguishable from **z**, the first principal component of between-population shape variance. As our collections spanned the geographical range of the species, this suggests that ecological interactions associated with the presence of predatory fish, namely resource competition and predation, form the primary drivers of morphological diversity in *G. caymanensis*.

Phenotypic differences among populations result from some combination of genetic divergence and phenotypic plasticity. The relative contributions of these sources of variation are currently unknown in this system. While morphological differences between predator regimes in other *Gambusia* species have been shown to have a genetic basis (e.g. Langerhans *et al.*, 2004, 2005; Langerhans, 2009a,b), other fishes are known to induce morphological differences depending on the presence of piscivorous fish (Brönmark & Miner, 1992; Eklöv & Jonsson, 2007; Januszkiewicz & Robinson, 2007; Chivers *et al.*, 2008). In *G. caymanensis*, migration levels or temporal variability of predation intensity in some localities might present a scenario where selection favours some level of phenotypic plasticity. It is perhaps most likely that both genetic divergence and phenotypic plasticity play important roles in generating the morphological differences observed here.

Variation among sexes in predator-regime differentiation

Patterns of body shape differentiation between predator regimes were functionally similar among the sexes (i.e. larger caudal region, smaller anterior body/head region with predators). However, sex-specific features of predator-regime differentiation were observed: (i) only females exhibited island-specific responses to predation, (ii) males exhibited a greater overall magnitude of shape differences than females, and (iii) the nature of morphological differences varied between the sexes. In light of the high degree of sexual dimorphism in this species,

perhaps sex-specific responses to variation in predation intensity was to be expected. Yet previous work on this topic has found mixed results in other sexually dimorphic poeciliid fishes. In *G. affinis* and *G. hubbsi*, sexes exhibit highly similar patterns of morphological differences between predator regimes (Langerhans *et al.*, 2004, 2007; Langerhans, 2009a,b). In *P. reticulata*, sexes exhibit a significant degree of independence in their responses to predation (Hendry *et al.*, 2006). While the island-specific responses to predation observed in female *G. caymanensis* were already discussed above, here we examine possible causes of the other two ways that sexes responded differently to the threat of predation.

Males exhibited greater levels of shape differentiation among populations than females. This suggests that either males are under stronger divergent selection than females, or are less constrained in their phenotypic responses to selection. Males might indeed experience stronger divergent selection than females, as mortality rates are known to be greater for males in some livebearers (Seghers, 1973; Reznick *et al.*, 1996; Macías García *et al.*, 1998) – which, if accurate, could explain the apparently strong female-biased sex ratios in *G. caymanensis*. In high-predation localities, sex-biased predation rates are currently unknown in this system, but could result from greater conspicuousness due to male coloration (males have orange unpaired fins), sex-specific habitat use, or reduced vigilance and antipredator behaviours in males (common in Trinidadian guppies; see Magurran & Macías García, 2000). Alternatively, females might experience similarly strong divergent selection on locomotor capacities, but possess greater constraints due to the reproductive demands of viviparity. That is, selection on reproductive traits (e.g. offspring size, number) might indirectly affect body shape by opposing selection on locomotor performance. This would effectively shift the overall fitness surfaces experienced by females in low- and high-predation sites in a manner that reduces their level of divergence (e.g. contracts the phenotypic distance between fitness peaks). Morphological differentiation between predator regimes in females would then largely reflect a balance between selection on locomotion and selection on reproduction, whereas male differentiation would almost exclusively reflect selection on locomotion. Further investigation is required to uncover the underlying causes of variation in the magnitude of shape differentiation among the sexes.

Although both sexes exhibited smaller heads and larger caudal peduncles in the presence of piscivorous fish, they accomplished this pattern through different morphological adjustments. Smaller heads in high-predation localities were achieved by a decrease in head depth in males (ventral shift of point 9; see Fig. 3a), and a shorter head length in females (posterior shift of point 1, anterior shift of point 9; see Fig. 3b). Larger caudal regions in high-predation sites were realized by a lengthening and deepening of the caudal peduncle in

males (anterior shift of points 7 and 8, dorsal shift of points 3 and 4, posterior shift point 5, ventral shift of point 6; see Fig. 3a), and only a deepening in females (ventral shift of points 7 and 8, dorsal shift of points 3 and 4; see Fig. 3b). Why might the sexes respond in such different manners to variation in predation intensity?

It is possible that the sexes experience similar patterns of selection on locomotor performance across predator regimes, but differ with respect to other forms of selection that affect body shape. For instance, females must additionally contend with the physical constraints of viviparity, where fecundity selection likely favours large abdominal regions for carrying embryos. This is probably especially true in high-predation localities, where selection for increased reproductive allocation is expected based on life-history theory and prior empirical work in a number of poeciliid fishes (e.g. Reznick *et al.*, 1997; Johnson & Belk, 2001; Jennions & Telford, 2002). Thus females might avoid producing shallower head regions and longer caudal regions in high-predation sites (as seen in males) because these changes would reduce the size of the abdominal region. This could explain why females only exhibit changes in *length* of the head and *depth* of the caudal peduncle across predator regimes. That is, females might have produced adaptive morphologies within the constraints of viviparity.

Alternatively, selection on locomotor performance might differ among the sexes. One possibility is that selection in females favouring steady-swimming abilities might remain strong even in high-predation localities due to the greater energetic requirements of embryo production (i.e. more time spent foraging) and/or the avoidance of harassment by males. This seems plausible, as female Trinidadian guppies spend much more time foraging than males, and spend more time avoiding male harassment in high-predation localities (Dussault & Kramer, 1981; Magurran & Seghers, 1994). If true, then female body shape would experience different patterns of selection than males: selection in high-predation sites would favour morphologies that increase fast-start performance, while simultaneously maintaining relatively high steady-swimming capacities. Indeed, our findings are consistent with this hypothesis, as the nature of shape differences in female *G. caymanensis* is predicted to do just that (see calculations of swimming performance above). Specifically, females deepened their caudal regions and shortened their heads in the presence of predators, which should increase fast-start performance; but they also maintained relatively deep anterior/head regions and short caudal regions, which are trait values associated with increased steady-swimming performance (e.g. Langerhans, 2009b; Langerhans & Reznick, 2009).

Males exhibited a more ventrally positioned eye in the presence of piscivorous fish, whereas female eye position did not differ across predator regimes. A ventrally located eye in the presence of predators mirrors patterns observed in some poeciliid species (Langerhans & DeWitt,

2004; Langerhans *et al.*, 2004; Gomes & Monteiro, 2008; Langerhans, 2009b), although other some studies have found no association between predator regime and eye position (Hendry *et al.*, 2006; Langerhans *et al.*, 2007). Position of the eye might be related to predator or prey detection, but the causes of sex-specific variation in the nature of eye-location differentiation between predator regimes is currently unknown.

Conclusions

Results of this study suggest that variation in the density of piscivorous fish plays a critical role in generating morphological diversity in *G. caymanensis*, and is consistent with divergent natural selection on locomotor performance being the primary causal mechanism. This adds to the growing evidence that predation drives predictable phenotypic outcomes in a variety of fishes. Despite the principal role of predation in producing generally parallel patterns of differentiation across the islands, unexpected idiosyncrasies coloured the finer details of these morphological patterns: e.g. island effects were evident (primarily in males), females exhibited minimal differences on Little Cayman, the magnitude of divergence was greater in males than females, and sexes exhibited separate morphological trajectories to achieve similar functional consequences. This suggests that historical contingencies and/or variation in the nature of divergent selection across predator regimes has produced variations on the general theme of repeated patterns of morphological differentiation matching *a priori* predictions.

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