



Sexual Ornaments, Body Morphology, and Swimming Performance in Naturally Hybridizing Swordtails (Teleostei: *Xiphophorus*)

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Abstract

Determining the costs of sexual ornaments is complicated by the fact that ornaments are often integrated with other, non-sexual traits, making it difficult to dissect the effect of ornaments independent of other aspects of the phenotype. Hybridization can produce reduced phenotypic integration, allowing one to evaluate performance across a broad range of multivariate trait values. Here we assess the relationship between morphology and performance in the swordtails *Xiphophorus malinche* and *X. birchmanni*, two naturally-hybridizing fish species that differ extensively in non-sexual as well as sexual traits. We took advantage of novel trait variation in hybrids to determine if sexual ornaments incur a cost in terms of locomotor ability. For both fast-start and endurance swimming, hybrids performed at least as well as the two parental species. The sexually-dimorphic sword did not impair swimming performance *per se*. Rather, the sword negatively affected performance only when paired with a sub-optimal body shape. Studies seeking to quantify the costs of ornaments should consider that covariance with non-sexual traits may create the spurious appearance of costs.

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Introduction

A pervasive view in the study of sexual selection is that sexual ornaments are costly to the bearer, and that these costs are integral in conveying information to choosers as to the “quality” of the signaler [1]. Yet sexually-selected ornaments occur as part of a suite of traits that interact to determine performance and fitness [2–5]. Just as the benefits of sexual ornaments depend on their integration with other traits, so do their costs [2,6]. In particular, exaggerated morphological structures like elongated feathers, fins, or horns are often assumed to impair locomotor performance; the evidence for such performance costs, however, is far from ubiquitous [2]. The lack of consensus likely emerges from the fact that it is ultimately the multivariate phenotype, including both sexually-selected and non-sexual traits, that affects locomotor performance [2,3]. As a result, the interactions between sexual traits, non-sexual traits, and locomotor performance can be complex, and may result in the compensation of sexual ornament costs by non-sexual traits [2,7]. Furthermore, this complexity could, in theory, result in exaggeration, rather than compensation, with sexual ornaments appearing more costly if paired with other traits which negatively influence locomotor performance.

To evaluate the fitness effects of sexually-selected traits in a multivariate context, we would ideally like to be able to decouple correlated traits to study an array of multivariate phenotypes in

which traits vary independently. Arguably few natural phenomena can alter multivariate phenotypes as dramatically as hybridization [8,9]. Hybridization can alter trait variances and covariances, thereby producing hybrid phenotypes that occupy a greater volume of phenotypic space relative to parental phenotypes [8–10]. This novel phenotypic variation can in turn affect ecologically relevant tasks such as locomotor performance [11–14]. We demonstrate that novel hybrid trait variation and its influence on locomotor performance can be used as a ‘natural laboratory’ [15] for evaluating functional relationships between traits and performance. For example, any assessment of the influence of a given trait on locomotor performance will be confounded by its co-expression with other trait values (i.e. trait correlations) [3,16,17]. Correlations among traits are often reduced in hybrids [9,18], thus expanding the range of trait combinations in which to evaluate locomotor performance.

Here we take advantage of novel trait variation in natural hybrids of the swordtail fishes *Xiphophorus birchmanni* and *X. malinche* [19,20] to evaluate the relationship between sexual ornaments, non-sexual traits and locomotor performance. *Xiphophorus malinche* males are similar to other swordtails and express an extension of the ventral rays of the caudal fin known as a sword [21]. *Xiphophorus birchmanni*, by contrast, have secondarily lost the sword; however, males bear an enlarged dorsal fin [21].

Xiphophorus birchmanni are also deeper-bodied, particularly in the mid-section, anterior region of the body, relative to *X. malinche*. Hybrids between the two species occupy a broad region of morphospace encompassing both parental species [19]. Thus, these fish differ radically with respect to not only sexual ornaments but also body morphology, which itself can have dramatic effects on swimming performance [22–25].

We evaluated the interaction between sexual ornaments, body shape and size on both unsteady swimming performance (fast-start velocity) and steady swimming performance (endurance swimming, time to fatigue). First, we determined if hybrids suffered reduced swimming performance relative to parentals. Second, we used novel phenotypic variation in a hybrid population to determine if body morphology is compensating for or exaggerating locomotor costs of sexual ornaments. Finally, we explored to what extent variation in hybrid performance is attributable to morphological and genetic similarities to parentals.

Materials and Methods

Specimen Collection

Animals were collected using funnel-traps between May–June 2010 from sites previously identified as *X. birchmanni*, *X. malinche* or hybrid [19,20]. None of the sampled species are endangered or threatened. Collection permits were obtained from the Government of Mexico and were in hand at the time of collection. Parental males were collected from two *X. birchmanni* populations, Río Garces (20.94°, –98.282°; n = 15) and Río Coacuilco (21.099°, –98.587°; n = 7) and two *X. malinche* populations, Chicayotla (20.925°, –98.577°; n = 13) and Tlatzintla (20.881°, –98.799°; n = 6). The hybrid population Tlatemaco (21.023°, –98.79°; n = 35) was chosen because it is highly admixed, with sampled alleles in Hardy-Weinberg equilibrium and with no significant linkage disequilibrium among marker loci [20]. In addition, this hybrid population is morphologically intermediate to both parentals (JBJ and GGR, unpublished data; see Results). Furthermore, Tlatemaco displays reduced phenotypic integration (reduced trait correlations) between body shape, body size, sword length and dorsal fin size relative to parental populations. Specifically, the relative standard deviation of the eigenvalues, a measure of phenotypic integration [26] was lowest in Tlatemaco hybrids ($SD_{rel}(\lambda) = 0.45$) followed by *X. malinche* ($SD_{rel}(\lambda) = 0.59$) and *X. birchmanni* ($SD_{rel}(\lambda) = 0.79$). Thus, this hybrid population provides a broad range of trait values to evaluate function relative to parental populations. Animals were transported to our laboratory facilities at Texas A&M University in College Station, Texas, USA. Individuals were housed by population in 76-l aquaria and maintained in the lab for three weeks on a 12:12 light cycle and on a diet of algae flake, decapsulated *Artemia* eggs, and bloodworms (*Glycera sp.*) prior to the start of swimming performance trials. Males were housed individually in 13-l aquaria between trials to track individual identity. Males were food-deprived for 48 hrs prior to the start of swimming performance trials to ensure they were in a post-absorptive state [27]. Research on animals was approved by the Institutional Animal Care and Use Committee of Texas A&M University and great care was taken to minimize animal suffering.

Fast-start swimming performance trials

Fast-start swimming performance trials were conducted by placing an individual fish in a 16.21 cm × 65 cm arena filled with filtered tap water to a depth of 4 cm. The test arena was illuminated using compact fluorescent lamps to minimize heating of the test arena. Water temperature was maintained at

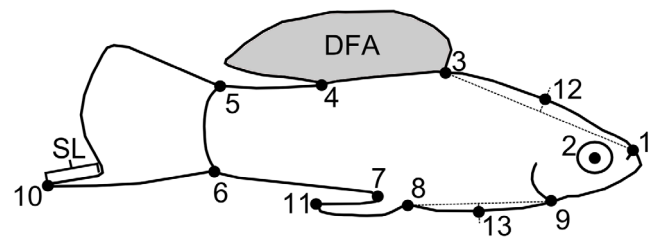


Figure 1. Landmark locations and measurements of sword length (SL) and dorsal fin area (DFA, shaded area).

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$21 \pm 0.2^\circ\text{C}$. Each fish was allowed 5 min to acclimate to the tank before the trial began. Fast-start behavior, i.e. c-starts [25] were elicited by startling the fish by striking the bottom of the test arena within 2 cm of the fish with one end of a wooden dowel (6.4 mm diameter) [28]. A high speed video camera (Casio Exilim Pro EX-F1, Casio Computer Co., Tokyo, Japan) recorded each fast-start event at 300 frames/s.

We measured fast-start velocity (v_{max}), on a standardized location on the fish's body, as follows. A line was fitted along the dorsal midsection of the fish in each frame starting at the tip of the snout to the end of the caudal peduncle. The point on the line which corresponded to half the standard length was used as a standard point for digitizing. Each video was digitized starting one frame before the fish's movement began to the 15th frame following movement (i.e. 53 ms of video was analyzed). Digitizing error was minimized using a mean-square quintic spline [29] executed in MATLAB 9.0 (The MathWorks, Natick, MA USA) [30].

Steady swimming performance trials

We performed endurance swimming performance (i.e. time to fatigue) trials using a Brett type swim tunnel [31,32]. The apparatus consisted of a flow-through tunnel with a test section (length = 45.7 cm, depth = 7.6 cm, width = 7.6 cm) with a matrix of plastic drinking straws upstream in order to minimize turbulent flow and a downstream grate to prevent the fish from leaving the test chamber. The apparatus was submerged in a 284-l aquarium and powered by a Leader Provort 540a propeller pump (Ladson SC, USA). Fish were tested using a modification of the protocol used by Royle et al. [33]. Water temperature was maintained at $21 \pm 1^\circ\text{C}$. Fish were placed individually in the test section and given 5 min to acclimatize. At the conclusion of the 5 min, flow was slowly increased to 20 cm/s for 5 min then increased to 30 cm/s for 1 min. Once the 30 cm/s interval was completed the flow was slowly increased to the test flow of 45 cm/s (~ 10.8 body lengths/s). Exhaustion was defined following the criterion used in other studies of steady swimming performance [33–35]. If a fish stopped swimming during the 45 cm/s test period, the fish would end up being impinged against the back grating that covers the water outflow channel. If the fish remained pinned for 5 s the test chamber was tapped once, and if the fish did not resume swimming, the trial was ended and the time recorded. If the fish failed to exhaust by 25 min, the trial was terminated and the exhaustion time was recorded as 1500 s. The 25 min cutoff was justified by a preliminary dataset (n = 61) using a separate sample of fish. Of the fish in this dataset which swam for 25 min (n = 13), 92% continued to swim for >60 min.

Trial order was randomized but to assure our results are not biased by order we performed two generalized linear models (GLMs) with either fast-start swimming performance (velocity) or endurance swimming performance (time to fatigue) as dependent

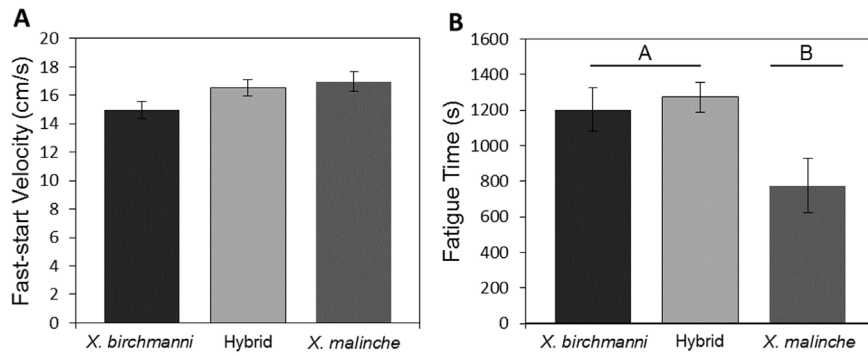


Figure 2. Means and standard errors for fast-start velocity (A) and time to fatigue (B) by species-type. Letters and lines indicate similarities between means determined using Tukey's HSD. doi:10.1371/journal.pone.0109025.g002

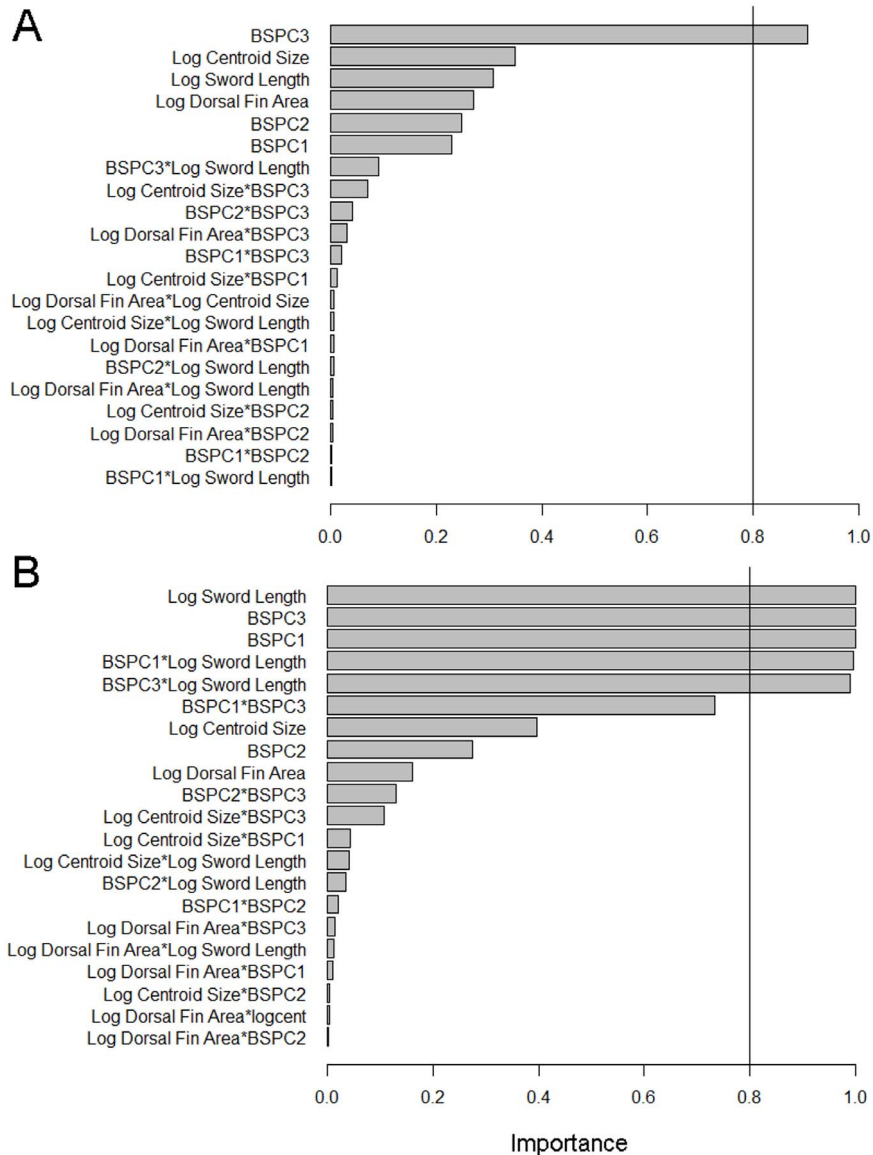


Figure 3. Importance of morphological variables in predicting fast-start velocity (A) and time to fatigue (B) in hybrids. doi:10.1371/journal.pone.0109025.g003

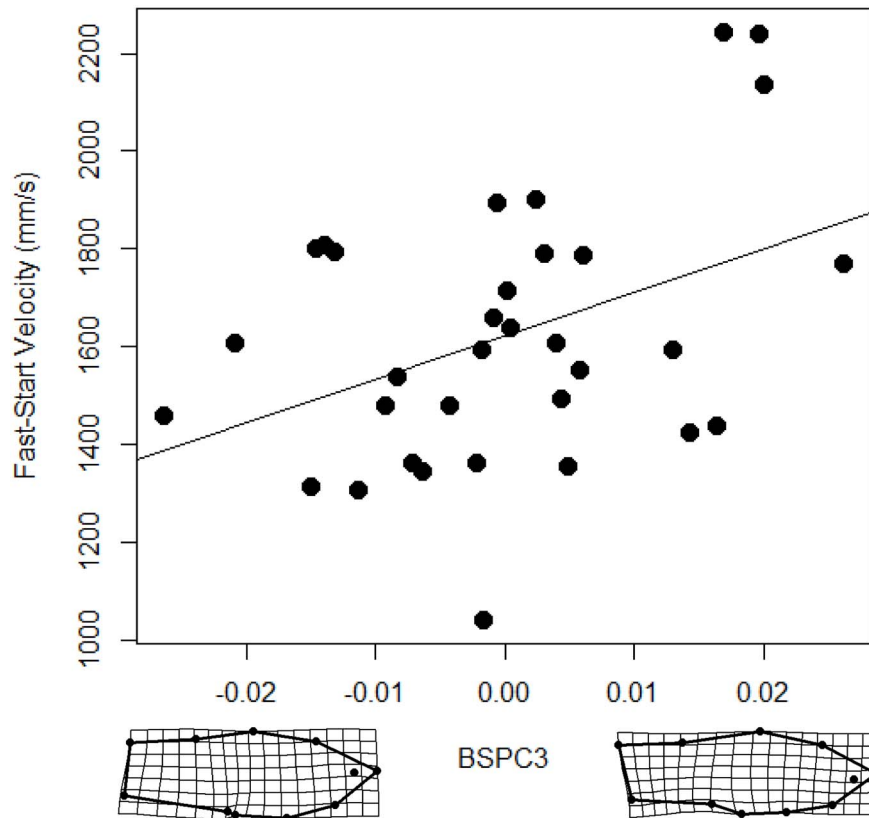


Figure 4. The relationship between body shape PC 3 (BSPC3) and fast-start velocity in hybrids.
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on which trial (fast-start or endurance trial) was first. Order of trial had no effect on swimming performance (GLM; fast-start: $F_{1, 74} = 0.02$, $P = 0.887$; endurance: $F_{1, 75} = 0.15$, $P = 0.695$).

Morphometrics

At the conclusion of both swimming performance trials, fish were anesthetized using tricaine methanesulfate (MS-222). We took a lateral image of the right side of the body using a Nikon D90 digital camera with a 50 mm Nikkor lens (Nikon, Tokyo, Japan) mounted to a copy stand, and removed a small portion of the upper caudal fin for genetic analysis (see below). From each image, 13 landmarks were digitized using tpsDig (Figure 1) [36]. Landmarks included (1) upper lip, (2) eye, (3) anterior insertion of the dorsal fin, (4) posterior insertion of the dorsal fin, (5) dorsal insertion of the caudal fin ray, (6) ventral insertion of the caudal fin ray, (7) posterior insertion of the gonopodium, or intromittent organ (8) anterior insertion of the gonopodium and (9) the ventral occlusion of the operculum cover (Figure 1). The sword (10) and gonopodium tip (11) landmarks were subject to idiosyncratic differences in orientation in the images, i.e. position of the sword or gonopodium in a given image. These differences were removed by rotating both the sword and gonopodium tip (landmarks 10 and 11, respectively) to 45° relative to the centerline of the body [37]. Semi-landmarks for the nuchal hump (12) and belly (13) were interpolated from right angles from half the distance (shown as dotted lines) between landmarks 1 and 3 for landmark 12 and 8 and 9 for landmark 13, respectively. These semi-landmarks were taken into account during landmark alignment [38,39]. Landmark coordinates were then subjected to generalized Procrustes superimposition where coordinates were translated, scaled and

rotated, i.e. aligned [38,39]. From the aligned coordinates, we calculated partial warps and uniform components (i.e. the weight matrix) which describe localized shape variation and uniform shearing in the X and Y dimensions, respectively [39,40]. A body size statistic, centroid size, was also calculated as the square root of the sum squared distances for each individual's landmark configuration to its centroid [39]. Alignment, calculation of the weight matrix and centroid size were performed using tpsRelw [41]. We also measured standard length (mm), sword extension length (mm) and dorsal fin surface area (mm^2 , Figure 1). Centroid size, sword length and dorsal fin area were log transformed.

Genetic analysis

To determine the genetic composition (*X. malinche* vs. *X. birchmanni*) of each hybrid individual we genotyped hybrid males after Culumber et al. [20] using one mitochondrial marker and three unlinked intron SNPs. Each hybrid male was assigned a hybrid score based on the number of *X. malinche* alleles it bore at the four marker loci, ranging from zero to seven (one allele at the mitochondrial marker and two at each of the nuclear markers). Based on allele frequencies sampled in 2007, and given that these are physically unlinked markers, the probabilities that a fish assigned a hybrid index of 7 (i.e. pure *X. malinche*) or 0 (pure *X. birchmanni*) is in fact a hybrid are 0.12 and 6×10^{-8} respectively. Thus, we believe that our marker-based methods provide a reasonable index of hybridization.

Statistical analysis

We used as series of generalized linear models (GLMs) to compare performance between species-types, order effects, hybrid

Table 1. Results for the best supported models ($\Delta AIC_c < 2$) predicting fast-start velocity.

Model	AIC _c	ΔAIC_c	Effects	SS	DF	F	P
1	463.37	0	Body Shape PC 3 Error	385834 1969327	1 31	6.07	0.019
2	464.83	1.46	Body Shape PC 3 Log Centroid Size Error	368464 67053 1902274	1 1 30	5.81 1.06	0.022 0.312
3	464.94	1.57	Body Shape PC 3 Log Dorsal Fin Area Error	424214 60377 1908949	1 1 30	6.67 0.95	0.015 0.338
4	465.36	1.99	Body Shape PC 3 Log Sword Length Error	396099 36223 1933104	1 1 30	6.15 0.56	0.019 0.459

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index and the relationship between morphology and performance in hybrids. For models where the dependent variable was fast-start velocity, the error distribution was specified as Gaussian, whereas for models evaluating time to fatigue, were specified as a gamma error distribution. For all models outliers and leverage points were evaluated by eye using R statistical software [42].

Swimming performance is frequently expressed in units of body lengths (e.g. BL/s) in an effort to compare across animals of varying sizes. We did not perform size correction because when comparing species and populations we were explicitly interested in absolute differences in swimming performance, including body size. Further, we wished to evaluate the relationship between swimming performance and morphology, including size. We compared swimming performance among hybrids and parentals using two GLMs where swimming performance (either fast-start velocity or fatigue time) was dependent on species-type (*X. malinche*, *X. birchmanni* or hybrid). Differences between species-types were evaluated using Tukey's HSD. To account for population effects, we also included population nested in species-type. We also evaluated the relationship between both fast-start velocity and endurance using Kendall's rank correlation.

We evaluated the effect of hybrid morphological traits (sexual ornaments, body shape and size) on swimming performance (either fast-start velocity or time to fatigue) with model selection [43] using the *glmulti* package [44] in the R statistical software platform [42]. Because we were interested in body shape *per se*, we excluded landmarks 10 (sword tip) and 11 (gonopodium; Figure 1); otherwise, alignment and calculation of partial warps and uniform components were as presented above (see *Morphometrics*). Partial warps and uniform components were subjected to PCA to reduce dimensionality [39,45]. The first three principal components accounted for 22%, 19% and 14% of the variance in hybrid body shape, respectively, and were retained for further analysis. All possible models were considered, ranging from the full model (dorsal fin area, sword length, body shape PC 1 (BSPC1), PC 2 (BSPC2), PC 3 (BSPC3) and log centroid size and all pairwise interaction terms) to the null model (intercept only). If interaction terms were retained their respective main effects were likewise retained, i.e. the principle of marginality was observed [44]. Models were evaluated using Akaike Information Criteria (finite sample correction, AIC_c) [43]. In addition, we report the difference between the AIC_c score of a given model and the lowest AIC_c score (i.e. ΔAIC_c). Models which differ within 2 AIC_c units from the model with the lowest AIC_c ($\Delta AIC_c < 2$) are considered equally supported [46]. We also evaluated relative importance of main effects and interaction terms individually using the sum of the relative evidence weights for each model in which a given term appears, terms which exceed an importance value of 0.8 were considered important [44,47]. If sexual ornaments are costly *per se*, we expected that a negative relationship between ornament and locomotor performance would be retained in well supported models and will show greater relative importance. Furthermore, significant interaction terms between body shape and ornaments would suggest that ornaments are being compensated for or exaggerated by body shape and are not costly *per se*. Significant interaction terms were visualized using non-parametric thin-plate spline regression to create a performance surface [48,49]. Estimation of performance surfaces was performed in R [42] using the *fields* package [50] (smoothing parameter $\lambda = 0.001$).

To determine if observed differences in morphology and swimming performance between parentals were mirrored by variation among hybrids, we evaluated vectors describing morphological variation between both parental species, and between

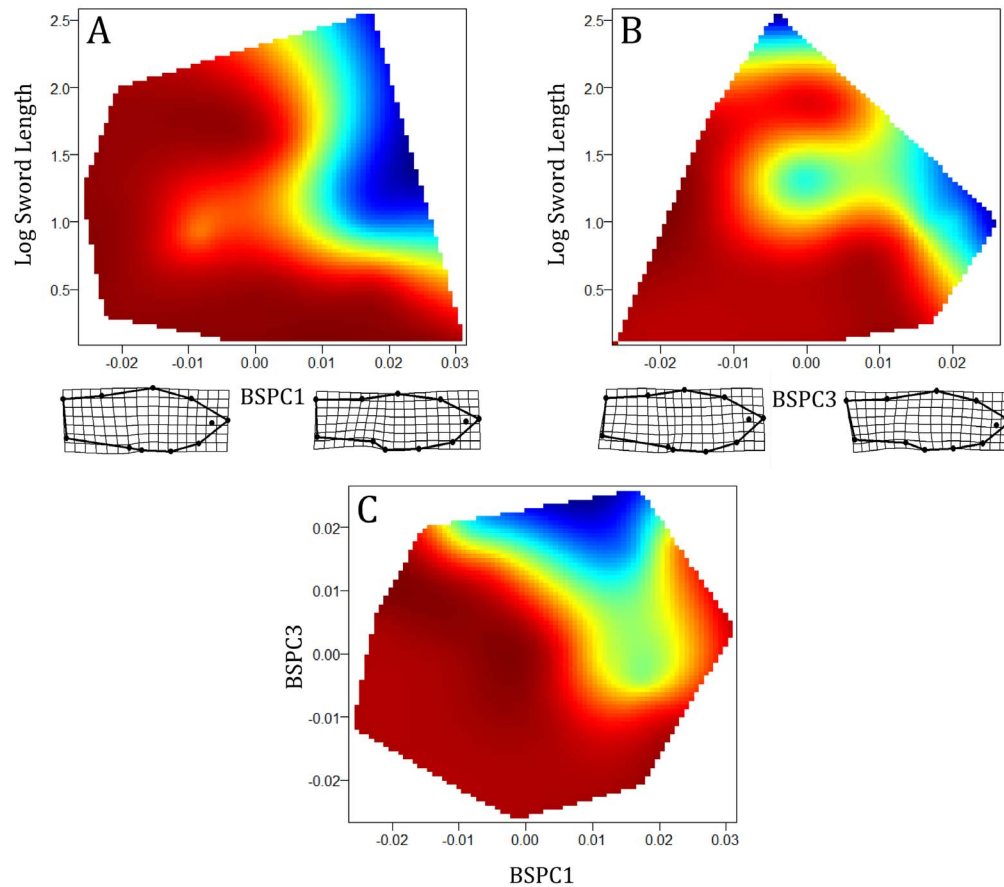


Figure 5. Performance landscapes for the significant interaction effects of log sword length by body shape PC 1 (BSPC1; A), log sword length by body shape PC 3 (BSPC3; B) and body shape PC 1 body shape by body shape PC 3 (C). Red represents longer endurance time.

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hybrids that differed in performance. Specifically, we tested whether morphological differences between hybrids that did and did not exhaust in the endurance swimming trials were consistent with morphological differences between *X. malinche* and *X. birchmanni*. We performed this analysis only for the endurance data, since parents and hybrids only marginally differed in fast-start performance (see Results). If the relationship between morphology and endurance in hybrids mirrors species differences (e.g. hybrids that did not exhaust are more morphologically similar to their better performing parental) we *a priori* expect the orientation between both vectors to be parallel. We included both the body and the sword ornament (landmark 10) and the gonopodium (landmark 11), which has been shown to influence swimming performance in other poeciliids [51]. Partial warps and uniform components were size-adjusted by taking residuals in a MANOVA model where the partial warp and uniform components were dependent on log centroid size ($F_{22, 54} = 6.07$, $P < 0.001$). Means of each size-adjusted partial warp and uniform component were calculated for *X. birchmanni*, *X. malinche*, hybrids that exhausted and hybrids that did not exhaust, and evaluated using MANOVA [52]. Using these means, two vectors were created: the first described variation between the parental species, *X. birchmanni* and *X. malinche* and the second described variation between hybrids that exhausted and hybrids that did not exhaust following the methods described in Collyer and Adams [52]. We determined if the two vectors were oriented similarly by calculating the angle between them [52]. Permutation tests (1000

iterations) were used to evaluate the significance of the observed angle between vectors using a residual randomization approach [52]. P -values were calculated to evaluate the null hypotheses that the two vectors are parallel [52]. Analysis of phenotypic vectors was performed in R [42] using a modification of the script provided in Collyer and Adams [52]. To visualize the differences between vectors we subjected the partial warps and uniform components to PCA [39,45,52]. The first two PC scores explained 75% and 7% of the variance respectively. Both PC1 and PC2 were size-adjusted by taking residuals using MANOVA ($F_{2, 74} = 30.98$, $P < 0.001$). Morphological vectors were visualized by plotting means and standard error of residual PC 1 and PC 2 scores for *X. birchmanni* and *X. malinche*, hybrids that exhausted, and hybrids that did not exhaust. These means were used to create vectors describing morphological change between parents and hybrids in residual PC space. In addition, we visualized a performance surface describing variation in performance in morphological space using non-parametric thin-plate spline regression (see above). The performance surface is provided for heuristic purposes and is independent of the calculation of the means, standard errors, vectors and contrasts.

To evaluate the relationship between fast-start swimming performance and genetic similarity of hybrids to parental species, we performed two GLMs where swimming performance (either fast-start velocity or fatigue time) was dependent on hybrid index.

Table 2. Results for the best supported model ($\Delta AIC_c < 2$) predicting time to fatigue.

Model	AIC _c	ΔAIC_c	Effects	SS	DF	F	P
1	486.51	0	Body Shape PC 1	0.18	1	1.06	0.313
			Body Shape PC 3	0.72	1	4.23	0.050
			Log Sword Length	3.25	1	19.08	0.000
			Body Shape PC 1*Body Shape PC 3	1.25	1	7.35	0.012
			Body Shape PC 1*Log Sword Length	2.58	1	15.14	0.001
			Body Shape PC 3*Log Sword Length	1.85	1	10.85	0.003
			Error	4.61	27		

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Results

Parentals and hybrid swimming performance

Fast-start velocity was marginally significantly different among species-types (*X. birchmanni*, *X. malinche*, or hybrids; $F_{2, 71} = 2.8$, $P = 0.068$). *Xiphophorus birchmanni* were slowest (mean = 14.95 cm/s, SE = 0.59), *X. malinche* fastest (mean = 16.97 cm/s, SE = 0.69) and hybrids intermediate (mean = 16.53 cm/s, SE = 0.55) in fast-start velocity (Figure 2A). Population nested in species-type was also marginally significant ($F_{2, 71} = 2.74$, $P = 0.071$).

We found significant variation between parentals and hybrids with respect to endurance swimming performance ($F_{2, 70} = 6.41$, $P = 0.003$). Hybrids showed the greatest endurance (mean fatigue time = 1273.17 s, SE = 85.79) and *X. malinche* the least (mean fatigue time = 776.28 s, SE = 153.86), with *X. birchmanni* intermediate in endurance (mean fatigue time = 1203.64 s, SE = 119.82; Figure 2B). Tukey's HSD post-hoc analysis suggests that fatigue time was not significantly different between hybrids and *X. birchmanni* ($z = -0.371$, $P = 0.925$) but that both hybrids ($z = 2.74$, $P = 0.016$) and *X. birchmanni* ($z = 2.354$, $P = 0.047$) had significantly greater endurance than *X. malinche*. Population nested in species-type was also significant ($F_{2, 70} = 31.41$, $P < 0.001$).

There was no detectable relationship between time to fatigue and fast-start velocity (Kendall's rank correlation, $\tau = -0.12$, $z = -1.33$, $P = 0.183$).

Hybrid morphology and performance

Model selection indicated that morphology had little association with fast-start swimming performance in hybrids. The model with the lowest AIC_c score consisted of only a minor component of body shape, body shape PC 3 (Table 1). Among other equally supported models (i.e. $\Delta AIC_c < 2$; Table 1) body shape PC 3 occurred in all models and was the only term which was statistically significant (Table 1). Furthermore, only body shape PC 3 exceeded the 0.8 threshold of relative importance (Figure 3A). Fish which were fastest were those with elongated midsections (Figure 4).

Unlike fast-start performance, endurance swimming performance in hybrids was strongly influenced by morphology. Only one model was supported (i.e. $\Delta AIC_c < 2$; Table 2) and consisted of body shape PC 1, body shape PC 3, sword length, and the interaction terms of body shape PC1 by body shape PC 3, log sword length by body shape PC 1 and finally, log sword length by body shape PC 3 (Table 2). Interaction terms of all morphological traits were significantly related to time to fatigue (Table 2). In addition, terms with importance values which exceeded the 0.8 threshold were body shape PC 1, body shape PC 3, log sword length and the interaction terms of body shape PC1 by sword length and body shape PC 3 by log sword length (Figure 3B). Surface visualizations of the significant interaction terms of body shape (PC 1 and PC 3) and sword length show that sword length had a negative effect on endurance swimming only when paired with a small anterior body shape (body shape PC 1) and elongated mid-section (body shape PC 3; Figure 5).

Parental and hybrid phenotypic and performance trajectories

MANOVA indicated significant differences in morphology between parental species and between exhausting and non-exhausting hybrids ($F_{66, 156.14} = 4.89$, $P < 0.001$). Vectors describing morphological variation between parental species and between exhausting and non-exhausting hybrids were parallel in

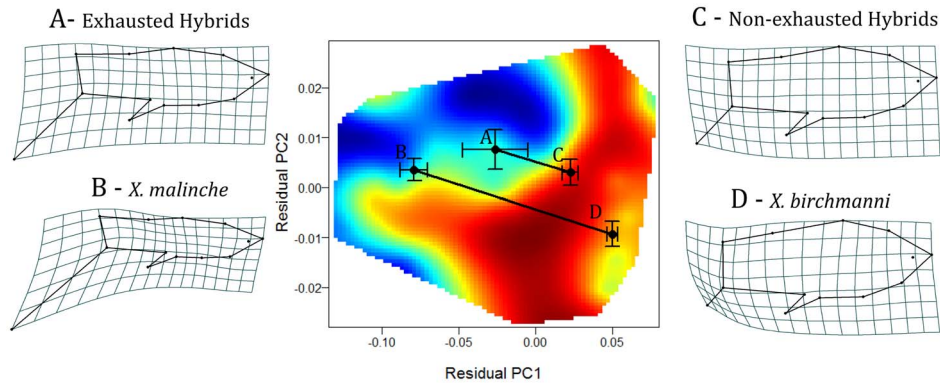


Figure 6. Performance landscape showing variation in morphology and swimming performance between parentals (*X. malinche* and *X. birchmanni*) and between hybrids that exhausted and hybrids that did not. Red represents longer endurance time. Means and standard errors of residual PC 1 and residual PC 2 for hybrids (A and C) and parentals (B and D) are shown. Morphometric visualizations were magnified by a factor of 3 [83–85].

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orientation ($\theta = 20.8^\circ$, $P_{rand} = 0.99$) and differed in length ($D_{parental} = 0.13$, $D_{hybrid} = 0.05$, $P_{rand} = 0.001$). Hybrid fish that did not exhaust were predominantly *X. birchmanni*-like morphologically. These fish had, on average, deeper, more anterior-allocated bodies, shorter, tapered caudal peduncles, larger dorsal fins and very short or absent swords (Figure 6).

Genetic association with swimming performance in hybrids

Genetic similarity to the two parent species did not predict performance. There was no association between hybrid index for fast-start ($F_{1, 30} = 0.72$, $P = 0.401$) or endurance swimming performance ($F_{1, 28} = 0.02$, $P = 0.89$).

Discussion

Sexual ornaments are thought to encode information pertaining to the “quality” of the bearer [53–56], but see [57–59]. A central pillar of this argument is that sexual ornaments are often costly to express [56,60]. The honesty of sexual signals is then enforced by condition-dependence, whereby individuals in good “condition” are better equipped to bear these costs [61,62]. However, as Dobzhansky [63] famously stated, “no trait is an island”. Interactions between sexual and non-sexual traits may alter the burden of sexual ornaments [2,7]. Thus, to accurately evaluate the true costs of sexual ornaments we must consider how sexual and non-sexual traits interact to influence costs. Our data suggest that the apparent locomotor costs of the sword are a by-product of the sword being paired with a sub-optimal body shape. Fish that performed well in the endurance swimming predominantly had narrow, tapered caudal peduncle and increased anterior body depth (BSPC 1, Figure 5 and Figure 6) and reduced mid-sections (although this explained a rather small portion of the phenotypic variance, BSPC 3, Figure 5 and Figure 6). These results are congruent with theoretical and empirical findings describing the relationship between body morphology and steady swimming performance [22]. In short, a narrow, tapered caudal peduncle and increased anterior body depth, as found in *X. birchmanni* and *birchmanni*-like hybrids, should benefit endurance swimming performance by minimizing drag and maximizing thrust [22,24]. In line with our findings, Kruesi and Alcaraz [31] found that *X. montezumae* males with more rotund bodies had greater swimming performance, and incurred relatively little change in swimming performance after sword removal. Furthermore, while we did find

an association between a minor component of body shape (body shape PC 3) and fast-start velocity (Table 1, Figure 4 and Figure 5), we found no relationship between sexual ornaments and fast-start velocity (Table 1, Figure 4 and Figure 5). Fast-start velocity and time to fatigue represent measures of the two primary forms of fish swimming performance, unsteady and steady swimming performance, respectively [22,24,64]. We cannot exclude the possibility that sword and dorsal fin sexual ornaments are costly to an unmeasured aspect of performance. For example, the conspicuousness of the sword increases predation risk [65–67]. Furthermore, in *X. montezumae*, where the sword is exaggerated to the point that it exceeds body length, it increases metabolic demands [68]. However, our results strongly suggest that in this system, sexual ornaments by themselves incur little, if any, cost to swimming performance.

There have been mixed results on the relationship between swimming performance and the sword ornament in *Xiphophorus*: some studies have found a negative relationship between swimming performance and presence of the sword [31], others have found no relationship [34,69–72], and one has even found positive effects of the sword on swimming performance [73]. This lack of consensus may be the result of several factors. For example, experimental removal of the sword [31,69] can be informative [74] but may affect the relationship between other traits and swimming performance [70]. In addition, discrepancies among studies may be due to differing patterns of covariation between ornaments and non-sexual traits among species which, as our study suggests, can alter the locomotor costs of sexual ornaments [2,6]. These potential problems have been avoided in our study by taking advantage of the natural and novel phenotypic variation created via hybridization and highlight the importance of evaluating both the costs and the benefits of sexually selected traits from a multivariate perspective.

Fish morphology should have a strong association with swimming performance [22,24]. A wealth of empirical and theoretical work provides expectations with respect to both steady and unsteady swimming performance [22,24], some, but not all, of which were supported by our data. Steady swimming performance should be greater in fish with narrow, tapered caudal peduncles and an anteriorly shifted center of mass [22,24]. These morphological elements are seen in the robust body shapes of *X. birchmanni* and *X. birchmanni*-like hybrid males who, performed better in endurance swimming trials relative to *X. malinche* and *X. malinche*-like hybrids (Figure 5 and Figure 6). Conversely, a

reduced anterior body and large caudal may increase fast-start velocity [22,24]. We found a minor component of body shape (PC 3, 14%) was significantly related to fast-start velocity in hybrids (Table 1). PC 3 describes an increase in the body mid-section area (between the dorsal and anal fin insertions; Figure 4). This does not meet our expectations for morphological variation which would improve unsteady swimming performance. In addition, steady and unsteady swimming performance are expected to be negatively correlated as the morphological variation which maximizes one form of swimming is divergent [22,24]. Our results do not support this expectation as the correlation between fast-start velocity and time to fatigue was not significant.

Locomotor performance has a profound impact on the fitness of animals. In fishes, fast-start swimming performance is associated with predator evasion [28,75–77], whereas endurance swimming could influence competitive interactions, locating suitable microhabitat, and dispersal [23,78,79]. Furthermore, improved or compromised locomotor performance may indicate differential vulnerability to predators or dispersal ability of hybrid populations over parentals [11,13,80]. We observed that hybrids performed as well if not slightly better than the best-performing parental species in *both* fast-start and endurance swimming performance (Figure 2). These findings are consistent with previous observations in the *birchmanni-malinche* hybrid system. For example, we observed that fish with a *X. birchmanni*-like body shape performed better in endurance swimming trials (Figure 5 and Figure 6). Previous work indicates that the *X. birchmanni*-like morphology is overrepresented

in the hybrid zone [19]. Thus the prevalence of *X. birchmanni*-like morphology among hybrid populations may result from improved endurance swimming performance and thus dispersal ability of *X. birchmanni* like hybrids. Furthermore, hybrid male phenotypes are not costly with respect to sexual selection; indeed, females of both parental species fail to prefer conspecifics over hybrids in mate-choice trials, and express preferences for some hybrid phenotypes [81]. Thus, hybrids in this system do not appear to suffer costs in either attractiveness or locomotor performance, and may in fact have greater fitness at the intermediate elevations where they are found [82].

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Author Contributions

Conceived and designed the experiments: JBJ DCM CNP GGR. Performed the experiments: JBJ DCM CNP GGR. Analyzed the data: JBJ DCM CNP GGR. Contributed to the writing of the manuscript: JBJ DCM CNP GGR.

References

- Mead LS, Arnold SJ (2004) Quantitative genetic models of sexual selection. *Trends Ecol Evol* 19: 264–271.
- Oufiero CE, Garland T Jr (2007) Evaluating performance costs of sexually selected traits. *Funct Ecol* 21: 676–689.
- Arnold SJ (1983) Morphology, performance and fitness. *Am Zool* 23: 347–361.
- Lailvaux SP, Irschick DJ (2006) A functional perspective on sexual selection: insights and future prospects. *Anim Behav* 72: 263–273.
- Ballentine B (2006) Morphological adaptation influences the evolution of a mating signal. *Evolution* 60: 1936–1944.
- Husak JF, Swallow JG (2011) Compensatory traits and the evolution of male ornaments. *Behaviour* 148: 1–29.
- Wilson RS, Condon CH, David G, FitzGibbon S, Niehaus AC, et al. (2010) Females prefer athletes, males fear the disadvantaged: different signals used in female choice and male competition have varied consequences. *Proc R Soc B* 277: 1923–1928.
- Riesberg LH, Archer MA, Wayne RK (1999) Transgressive segregation, adaptation and speciation. *Heredity* 83: 363–372.
- Seehausen O (2004) Hybridization and adaptive radiation. *Trends Ecol Evol* 19: 198–207.
- Grant PR, Grant BR (1994) Phenotypic and genetic-effects of hybridization in Darwin's Finches. *Evolution* 48: 297–316.
- Semlitsch RD, Pickle J, Parris MJ, Sage RD (1999) Jumping performance and short-term repeatability of newly metamorphosed hybrid and parental leopard frogs (*Rana sphenoccephala* and *Rana blairi*). *Can J Zool* 77: 748–754.
- Johnson JR, Johnson BB, Shaffer HB (2010) Genotype and temperature affect locomotor performance in a tiger salamander hybrid swarm. *Funct Ecol* 24: 1073–1080.
- Fitzpatrick BM (2008) Dobzhansky-Muller model of hybrid dysfunction supported by poor burst-speed performance in hybrid tiger salamanders. *J Evol Biol* 21: 342–351.
- Hawkins DK, Quinn TP (1996) Critical swimming velocity and associated morphology of juvenile coastal cutthroat trout (*Oncorhynchus clarki clarki*), steelhead trout (*Oncorhynchus mykiss*), and their hybrids. *Can J Fish Aquat Sci* 53: 1487–1496.
- Hewitt GM (1988) Hybrid zones - natural laboratories for evolutionary studies. *Trends Ecol Evol* 3: 158–167.
- Ghalambor CK, Walker JA, Reznick DN (2003) Multi-trait selection, adaptation, and constraints on the evolution of burst swimming performance. *Integr Comp Biol* 43: 431–438.
- Ghalambor CK, Reznick DN, Walker JA (2004) Constraints on adaptive evolution: the functional trade-off between reproduction and fast-start swimming performance in the Trinidadian guppy (*Poecilia reticulata*). *Am Nat* 164: 38–50.
- Parsons KJ, Son YH, Albertson RC (2011) Hybridization promotes evolvability in African Cichlids: connections between transgressive segregation and phenotypic integration. *Evol Biol* 38: 306–315.
- Rosenthal GG, de La Rosa Reyna XF, Kazianis S, Stephens MJ, Morizot DC, et al. (2003) Dissolution of sexual signal complexes in a hybrid zone between the swordtails *Xiphophorus birchmanni* and *Xiphophorus malinche* (Poeciliidae). *Copeia*: 299–307.
- Culumber ZW, Fisher HS, Tobler M, Mateos M, Barber PH, et al. (2011) Replicated hybrid zones of *Xiphophorus* swordtails along an elevational gradient. *Mol Ecol* 2: 342–356.
- Rauchenberger M, Kallman KD, Morizot DC (1990) Monophyly and geography of the Rio Panuco basin swordtails (genus *Xiphophorus*) with descriptions of four new species. *Am Mus Novit* 2975: 1–41.
- Langerhans RB, Reznick DN, editors (2010) Ecology and evolution of swimming performance in fishes: predicting evolution with biomechanics. New York, NY: Enfield, Science Publishers. 200–248 p.
- Blake RW (2004) Fish functional design and swimming performance. *J Fish Biol* 65: 1193–1222.
- Blake RW (1983) Fish Locomotion. Cambridge, UK: Cambridge University Press. 228.
- Domenici P, Blake RW (1997) The kinematics and performance of fish fast-start swimming. *J Exp Biol* 200: 1165–1178.
- Pavlicev M, Cheverud JM, Wagner GP (2009) Measuring morphological integration using eigenvalue variance. *Evol Biol* 36: 157–170.
- Niimi AJ, Beamish FWH (1974) Bioenergetics and growth of largemouth bass (*Micropterus salmoides*) in relation to body weight and temperature. *Can J Zool* 52: 447–456.
- Langerhans RB (2009) Morphology, performance, fitness: functional insight into a post-Pleistocene radiation of mosquitofish. *Biol Lett* 5: 488–491.
- Walker JA (1998) Estimating velocities and accelerations of animal locomotion: a simulation experiment comparing numerical differentiation algorithms. *J Exp Biol* 201: 981–995.
- Tytell ED, Lauder GV (2008) Hydrodynamics of the escape response in bluegill sunfish, *Lepomis macrochirus*. *J Exp Biol* 211: 3359–3369.
- Kruesi K, Alcaraz G (2007) Does a sexually selected trait represent a burden in locomotion? *J Fish Biol* 70: 1161–1170.
- Langerhans RB (2009) Trade-off between steady and unsteady swimming underlies predator-driven divergence in *Gambusia affinis*. *J Evol Biol* 22: 1057–1075.
- Royle NJ, Lindstrom J, Metcalfe NB (2006) Effect of growth compensation on subsequent physical fitness in green swordtails *Xiphophorus helleri*. *Biol Lett* 2: 39–42.
- Ryan MJ (1988) Phenotype, genotype, swimming endurance and sexual selection in a swordtail (*Xiphophorus nigrensis*). *Copeia* 1988: 484–487.
- Ojanguren AF, Braña F (2000) Thermal dependence of swimming endurance in juvenile brown trout. *J Fish Biol* 56: 1342–1347.
- Rohlf FJ (2010) TpsDig v.2.16. Department of Ecology and Evolution, State University of New York, Stony Brook, New York.

37. Adams DC (1999) Methods for shape analysis of landmark data from articulated structures. *Evol Ecol Res* 1: 959–970.
38. Bookstein FL (1991) *Morphometric tools for landmark data: geometry and biology*. New York: Cambridge Univ. Press.
39. Zelditch M, Swinderski D, Sheets D, Fink W (2004) *Geometric morphometrics for biologists: a primer*. Amsterdam: Elsevier Academic Press. 443 p.
40. Rohlf FJ, Loy A, Corti M (1996) Morphometric analysis of Old World Talpidae (Mammalia, Insectivora) using partial-warp scores. *Syst Biol* 45: 344–362.
41. Rohlf FJ (2010) *TpsRelw v.1.49*. Department of Ecology and Evolution, State University of New York, Stony Brook, New York.
42. R Core Development Team (2014) *R: A language and environment for statistical computing*, 3.1.1 ed. Vienna, Austria: R Foundation for Statistical Computing.
43. Johnson JB, Omland KS (2004) Model selection in ecology and evolution. *Trends Ecol Evol* 19: 101–108.
44. Calcagno V, de Mazancourt C (2010) *glmulti: An R package for easy automated model selection with (generalized) linear models*. *J Stat Soft* 34: 1–29.
45. Rohlf FJ (1993) Relative warp analysis and an example of its application to mosquito wings. In: Marcus LF, Bello E, Garcia-Valdecasas A, editors. *Contributions to morphometrics*. Madrid: CSIC. 134–159.
46. Burnham KP, Anderson DR (2002) *Model selection and multimodel inference*; 2nd, editor. New York, NY: Springer-Verlag.
47. Buckland ST, Burnham KP, Augustin NH (1997) Model selection: an integral part of inference. *Biometrics* 53: 603–618.
48. Arnold SJ (2003) Performance surfaces and adaptive landscapes. *Integr Comp Biol* 43: 367–375.
49. Lee KP, Simpson SJ, Clissold FJ, Brooks R, Ballard JWO, et al. (2008) Lifespan and reproduction in *Drosophila*: new insights from nutritional geometry. *Proc Natl Acad Sci USA* 105: 2498–2503.
50. Nychka D, Furrer R, Sain S (2014) *Fields: Tools for spatial data*. 7.1 ed.
51. Langerhans RB, Layman CA, DeWitt TJ (2005) Male genital size reflects a tradeoff between attracting mates and avoiding predators in two live-bearing fish species. *Proc Natl Acad Sci USA* 102: 7618–7623.
52. Collyer ML, Adams DC (2007) Analysis of two-state multivariate phenotypic change in ecological studies. *Ecology* 88: 683–692.
53. Cotton S, Small J, Pomiankowski A (2006) Sexual selection and condition-dependent mate preferences. *Curr Biol* 16: R755–R765.
54. Bonduriansky R (2007) The evolution of condition-dependent sexual dimorphism. *Am Nat* 169: 9–19.
55. Kokko H, Jennions MD, Brooks R (2006) Unifying and testing models of sexual selection. *Annu Rev Ecol Evol Syst* 37: 43–66.
56. Zahavi A (1975) Mate selection—A selection for a handicap. *J Theor Biol* 53: 205–214.
57. Rendall D, Owren MJ, Ryan MJ (2009) What do animal signals mean? *Anim Behav* 78: 233–240.
58. Prum RO (2010) The Lande–Kirkpatrick mechanism is the null model of evolution by intersexual selection: implications for meaning, honest, and design in intersexual signals. *Evolution* 64: 3085–3100.
59. Prum RO (2012) Aesthetic evolution by mate choice: Darwin's really dangerous idea. *Phil Trans R Soc B* 367: 2253–2265.
60. Andersson M (1994) *Sexual Selection*. Princeton, NJ: Princeton University Press.
61. Cotton S, Fowler K, Pomiankowski A (2004) Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proc R Soc Lond B* 271: 771–783.
62. Hill GE (2011) Condition-dependent traits as signals of the functionality of vital cellular processes. *Ecol Lett* 14: 625–634.
63. Dobzhansky T (1956) What is an adaptive trait? *Am Nat* 90: 337–347.
64. Sfakiotakis M, Lane DM, Davies JBC (1999) Review of fish swimming modes for aquatic locomotion. *IEEE J Ocean Eng* 24: 237–252.
65. Rosenthal GG, Martinez TYF, de León FJG, Ryan MJ (2001) Shared preferences by predators and females for male ornaments in swordtails. *Am Nat* 158: 146–154.
66. Basolo AL, Wagner WE (2004) Covariation between predation risk, body size and fin elaboration in the green swordtail, *Xiphophorus helleri*. *Biol J Linn Soc* 83: 87–100.
67. Hernandez-Jimenez A, Rios-Cardenas O (2012) Natural versus sexual selection: predation risk in relation to body size and sexual ornaments in the green swordtail. *Anim Behav* 84: 1051–1059.
68. Basolo AL, Alcaraz G (2003) The turn of the sword: length increases male swimming costs in swordtails. *Proc R Soc Lond B* 270: 1631–1636.
69. Baumgartner A, Coleman S, Swanson B (2011) The cost of the sword: escape performance in male swordtails. *Plos ONE* 6: e15837.
70. Oufiero CE, Jugo K, Tran P, Garland T (2012) As the sword grows: individual variation and ontogenetic effects of a sexually selected trait on locomotor performance in *Xiphophorus helleri*. *Physiol Biochem Zool* 85: 684–693.
71. Oufiero CE, Meredith RW, Jugo KN, Tran P, Chappell MA, et al. (2014) The evolution of the sexually selected sword in *Xiphophorus* does not compromise aerobic locomotor performance. *Evolution* 68: 1806–1823.
72. Oufiero CE, Jugo K, Garland T (2014) Swimming with a sword: tail beat kinematics in relation to sword length in *Xiphophorus*. *Funct Ecol* 28: 924–932.
73. Royle NJ, Metcalfe NB, Lindstrom J (2006) Sexual selection, growth compensation and fast-start swimming performance in Green Swordtails, *Xiphophorus helleri*. *Funct Ecol* 20: 662–669.
74. Sinervo B, Basolo AL (1996) Testing adaptation using phenotypic manipulations. In: Rose MR, editor. *Adaptation*. San Diego, CA: Academic Press.
75. Walker JA, Ghalambor CK, Griset OL, McKenney D, Reznick DN (2005) Do faster starts increase the probability of evading predators? *Funct Ecol* 19: 808–815.
76. Webb PW (1986) Effect of body form and response threshold on the vulnerability of four species of teleost prey attacked by largemouth bass (*Micropterus salmoides*). *Can J Fish Aquat Sci* 43: 763–771.
77. Katzir G, Camhi JM (1993) Escape response of black mollies (*Poecilia shenops*) to predatory dives of a pied kingfisher (*Ceryle rudis*). *Copeia* 1993: 549–553.
78. Plaut I (2001) Critical swimming speed: its ecological relevance. *Comp Biochem Phys A* 131: 41–50.
79. Domenici P (2003) Habitat, body design and the swimming performance of fish. In: Bels VL, Gasc JP, Casinos A, editors. *Vertebrate Biomechanics and Evolution*. Oxford: BIOS Scientific Publishers Ltd.
80. Rosenfield JA, Nolasco S, Lindauer S, Sandoval C, Kodric-Brown A (2004) The role of hybrid vigor in the replacement of Pecos pupfish by its hybrids with sheepshead minnow. *Conserv Biol* 18: 1589–1598.
81. Culumber ZW, Ochoa OM, Rosenthal GG (2014) Assortative mating and the maintenance of population structure in a natural hybrid zone. *Am Nat* 184: 225–232.
82. Culumber ZW, Shepard DB, Coleman SW, Rosenthal GG, Tobler M (2012) Physiological adaptation along environmental gradients and replicated hybrid zone structure in swordtails (Teleostei: *Xiphophorus*). *J Evol Biol* 25: 1800–1814.
83. Langerhans RB, Layman CA, Shokrollahi AM, DeWitt TJ (2004) Predator-driven phenotypic diversification in *Gambusia affinis*. *Evolution* 58: 2305–2318.
84. Ward JL, McLennan DA (2009) Historical and ecological correlates of body shape in the brook stickleback, *Culaea inconstans*. *Biol J Linn Soc* 96: 769–783.
85. Haas TC, Blum MJ, Heins DC (2010) Morphological responses of a stream fish to water impoundment. *Biol Lett* 6: 803–806.