Repeated evolution of local adaptation in swimming performance: population-level trade-offs between burst and endurance swimming in *Brachyrhaphis* freshwater fish

SPENCER J. INGLEY[1]*, HENRY CAMARILLO[1], HANNAH WILLIS[1] and
JERALD B. JOHNSON[1,2]

[1]*Evolutionary Ecology Laboratories, Department of Biology, Brigham Young University, Provo, UT, 84602, USA*
[2]*Monte L. Bean Life Science Museum, Brigham Young University, Provo, UT, 84602, USA*

Specialization is fundamentally important in biology because specialized traits allow species to expand into new environments, in turn promoting population differentiation and speciation. Specialization often results in trade-offs between traits that maximize fitness in one environment but not others. Despite the ubiquity of trade-offs, we know relatively little about how consistently trade-offs evolve between populations when multiple sets of populations experience similarly divergent selective regimes. In the present study, we report a case study on *Brachyrhaphis* fishes from different predation environments. We evaluate apparent within/between population trade-offs in burst-speed and endurance at two levels of evolutionary diversification: high- and low-predation populations of *Brachyrhaphis rhabdophora*, and sister species *Brachyrhaphis roseni* and *Brachyrhaphis terrabensis*, which occur in high- and low-predation environments, respectively. Populations of *Brachyrhaphis* experiencing different predation regimes consistently evolved swimming specializations indicative of a trade-off between two swimming forms that are likely highly adaptive in the environment in which they occur. We show that populations have become similarly locally adapted at both levels of diversification, suggesting that swimming specialization has evolved rather rapidly and persisted post-speciation. Our findings provide valuable insight into how local adaptation evolves at different stages of evolutionary divergence. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, **119**, 1011–1026.

KEYWORDS: *Brachyrhaphis* – burst-speed swimming – endurance swimming – natural selection – path analysis – Poeciliidae – predation environment.

INTRODUCTION

Local adaptation has been a central topic in ecology and evolution because adaptive, specialized traits can allow species to expand into new environments, which in turn can help promote reproductive isolation and speciation (Funk, 1998; Schluter, 2000; Coyne & Orr, 2004; Rundle & Nosil, 2005; Sandoval & Nosil, 2005). Populations within a species that occur in different selective environments often become locally adapted to the environment in which they occur, sometimes resulting in phenotypic trade-offs (i.e. negative correlations among beneficial traits) in ecologically relevant, fitness determining traits (Joshi & Thompson, 1995; Schluter, 2000; Via,

Bouck & Skillman, 2000). Such trade-offs often result in the occurrence of closely-related populations that differ substantially in one or more traits. Where divergent traits are locally adaptive, but maladaptive in other selective regimes, gene flow among populations can be restricted by several reproductive isolating mechanisms. Although patterns of local adaptation and trade-offs between populations that occur in divergent selective regimes have been identified for numerous traits in several taxonomic groups (Joshi & Thompson, 1995; Schluter, 2000; Pfennig & Pfennig, 2005; Langerhans, 2009b; Agrawal, Conner & Rasmann, 2010; Franssen *et al.*, 2013; Martin, McGee & Langerhans, 2015), we still know relatively little about how consistent and repeatable these trade-offs are at different stages of

*Corresponding author. E-mail: sjingley@gmail.com

evolutionary divergence (e.g. at early vs. late stages of speciation). Understanding how predictably trade-offs evolve among lineages within the same clade has potentially important implications for variation in rates of diversification within and among lineages (Hendry & Kinnison, 1999; Kinnison & Hendry, 2001; Holzman *et al.*, 2012).

Members of the Neotropical livebearing fish genus *Brachyrhaphis* (Poeciliidae) have received increased attention in ecology and evolution research in recent years (Johnson & Zuniga-Vega, 2009; Ingley *et al.*, 2014a, 2015) and are useful for examining how trade-offs evolve in different lineages within the same clade, but that are found at different stages of divergence (Ingley, Rehm & Johnson, 2014a; Ingley & Johnson, 2016a, b). Several species within *Brachyrhaphis* contain populations that occur in divergent predation environments, and have repeatedly and independently evolved life-history (Johnson, 2001a; Jennions & Telford, 2002), morphological (Wesner *et al.*, 2011; Ingley *et al.*, 2014b), and behavioural (Archard & Braithwaite, 2011; Ingley *et al.*, 2014a, c) adaptations to their respective environments. Similar patterns have recently been documented at the between species level (Ingley *et al.*, 2014a, b), with sister species occurring primarily in divergent predation environments and showing similar behavioural and life-history adaptations to those seen within several species of *Brachyrhaphis* (e.g. *Brachyrhaphis rhabdophora*). This pattern suggests that divergent predation environments might be a primary driver of evolution in this group, and make it ideal for studying local adaptation and performance trade-offs at different stages of divergence. In the present study, we focus on evaluating patterns of local adaptation between populations of *B. rhabdophora* that occur in either high- ('Javilla') or low-predation ('Grande') environments, and between sister species of *Brachyrhaphis* that occur in high- (*Brachyrhaphis roseni*, hereafter *BR*) and low-predation (*Brachyrhaphis terrabensis*, hereafter *BT*) environments. We focus specifically on burst-speed and endurance swimming performance because these traits are likely under strong divergent natural selection, are ecological relevant (Domenici, 2010; Langerhans & Reznick, 2010), and are predicted to be tied to previously documented morphological differences observed in these species (Ingley *et al.*, 2014b).

High-predation environments have been shown to select for increased burst-speed ability because burst-speed is a strong predictor of predator escape ability (Walker *et al.*, 2005; Langerhans, 2009a; Domenici, 2010). By contrast, low-predation environments often select for increased endurance because these environments tend to have higher population densities, resulting in increased intraspecific competition for

resources and mates (Abrams, 1993; Langerhans, 2009b). Additionally, the low-predation environments that *Brachyrhaphis* experience also have higher flow-rates than high-predation environments. Theory predicts that a locomotor trade-off should occur between these two swimming gaits because a morphological arrangement that optimized one gait necessarily compromises the other, and vice versa (Webb, 1984; Langerhans *et al.*, 2004; Langerhans, 2009b). Although some studies have evaluated burst-speed and endurance swimming performance in fish and other taxa (Vanhooydonck, Van Damme & Aerts, 2001; Wilson, James & Van Damme, 2002; Langerhans, 2009b; Yan *et al.*, 2012, 2013; Fu *et al.*, 2015), to our knowledge, these traits have never been evaluated in pairs of populations that occur in similarly divergent environments but at different stages of evolutionary divergence, thus limiting our understanding of how predictable these trade-offs are within lineages.

The objectives of the present study are two-fold. First, we test for overall differences in burst-speed and endurance in populations that occur in different predation environments, both at early (Javilla vs. Grande) and late (*BR* vs. *BT*) stages of divergence, and attempt to identify what traits (e.g. body shape and size) drive these patterns. We predict that, at both stages of divergence, populations from high-predation environments will have higher burst-speeds than low-predation populations, and that low-predation populations will have higher endurance than high-predation populations. Finally, we expect that variation in body shape will be the primary driver of variation in swimming ability, as has been observed in other poeciliids (Langerhans, 2009a, b).

Second, as an extension of our first objective, we test for a negative correlation between burst and endurance among populations. We predict that, overall, populations will exhibit patterns indicative of a trade-off between burst and endurance swimming ability, namely a strong negative correlation between these swimming modes. We expect this relationship to be more pronounced between sister species *BR* and *BT* than between Javilla and Grande given that the former show deeper levels of genetic and morphological divergence than the latter (Ingley *et al.*, 2014b). Together, accomplishing these objectives will provide valuable insight into how performance trade-offs evolve at different stages of evolutionary divergence.

MATERIAL AND METHODS

STUDY SYSTEM AND SAMPLE POPULATIONS

BR and *BT*

BR and *BT* have recently emerged as a useful system for studying patterns of trait divergence in

recently diverged species that occur in different selective environments (Ingley, 2014, 2015; Ingley & Johnson, 2016a, b). Previous work has shown that these species diverge in numerous traits, such as behaviour and morphology, which correspond to different predation environments. For example, populations from high-predation environments tend to have more streamlined body shapes (Ingley *et al.*, 2014b) and are more bold, active, and prone to explore novel environments (Ingley *et al.*, 2014a, c). These sister species are co-distributed in Pacific slope streams throughout western Panama and south-eastern Costa Rica, although the majority of populations occur in allopatry and in divergent environments with respect to predation (Ingley *et al.*, 2014a, b). For the present study, we collected live fish from two streams in the Río Caño Seco drainage in Puntarenas, Costa Rica. *BR* were collected from a low-elevation tributary (N8.65427, W82.93489; elevation 70 m) and *BT* was collected from a high-elevation tributary (N8.81299, W82.97408; elevation 962 m). Both streams were characteristic of the respective species (Ingley *et al.*, 2014b), with the primary difference being the presence or absence of piscine predators and differences in population densities. Collection and transportation to Brigham Young University (BYU) took place in April 2014, and trials were conducted between April and July 2015. Fish were held in small group tanks (approximmately 10 fish per 38-L tank) until immediately before our trials began. Prior to beginning our trials, we removed a subset of males (approximmately 30 per species) and placed them in tanks as groups where individuals could be easily identified by standard length (SL). Males do not grow after reaching sexual maturity, and so individuals were easily identified when placed in tanks containing three or four individuals of different SL. Fish were allowed to acclimate to their new groupings for at least 1 week prior to testing. We held all tanks in an environmentally controlled laboratory with natural lighting (LD 12 : 12 h) and temperature conditions. We provided natural cover and aeration in each tank, and fed fish twice daily with TetraMin flakes (Tetra Europe) supplemented with brine shrimp and fruit flies.

High- and low-predation B. rhabdophora

We collected live *B. rhabdophora* from two streams in Guanacaste, Costa Rica in April 2014 and immediately transported them to BYU. High-predation fish were collected from the low-elevation Río Javilla (N10.40245, W85.07610; elevation 99 m; hereafter referred to as 'Javilla') and low-predation fish were collected from high-elevation Quebrada Grande (N10.44194, W84.98804; elevation 363 m; hereafter referred to as 'Grande'). These populations have been the subject of extensive study in the context of morphological (Wesner *et al.*, 2011; Ingley *et al.*, 2014b) and life-history (Johnson, 2001a, b, 2002; Johnson & Belk, 2001; Johnson & Zuniga-Vega, 2009) divergence in response to different predation environments. Both streams were characteristic of high- and low-predation populations, with the primary differences being the presence or absence of piscine predators and population density. We followed the same grouping protocol as that employed for *BR* and *BT*, and held fish under the same laboratory conditions. Because of logistical constraints, we were unable to test replicate populations of *B. rhabdophora* from high- and low-predation environments. Although this limits our scope of inference to these two populations for the traits at hand, previous work on *B. rhabdophora* has shown that other traits diverge in the same way repeatedly in independent population pairs (Johnson, 2001b; Johnson & Belk, 2001), suggesting that similar patterns might be expected in other traits as well.

GEOMETRIC MORPHOMETRICS

Following burst-speed performance trials, but prior to the endurance swimming trials (see below), we removed each fish from the test arena and anaesthetized it with tricaine methylsulphonate (MS-222). We then took a lateral photograph of each fish for geometric morphometric analyses in accordance with the methods of Ingley *et al.* (2014b). In brief, we digitized 13 morphological landmarks (or semi-landmarks) on lateral images of fish using TPSDIG (Rohlf, 2005). Landmarks were defined as: (1) anterior tip of the snout; (2), anterior extent of the eye; (3) semi-landmark midway between landmarks 1 and 4; (4) anterior insertion of the dorsal fin; (5) posterior insertion of the dorsal fin; (6) semi-landmark midway between landmarks 5 and 7; (7) dorsal origin of the caudal fin; (8) ventral origin of the caudal fin; (9) semi-landmark midway between landmarks 8 and 10; (10) posterior insertion of the gonopodium; (11) anterior insertion of the gonopodium; (12) semi-landmark midway between landmarks 11 and 13; and (13) intersection of the operculum with the ventral outline of the body (for a schematic of landmarks, see Supporting information, Appendix S1). To reduce variation among photos, a single researcher (SJI) photographed all of the fish and landmarked all images. We then summarized shape variation for all individuals tested in each comparison (i.e. Javilla–Grande and *BR*–*BT*) into relative warps (i.e. principal components; PC) using TPSRELW (Rohlf, 2003). We used generalized Procrustes analysis (Rohlf & Slice, 1990) to remove all nonshape variation as a result of position, orientation, and scale of the

specimens for each image. We then derived a canonical axis from the species effect (i.e. 'predation regime' effect) of each multivariate analysis of covariance (MANCOVA) that tested for differences in body shape, for which we used relative warps as response variables. These axes can serve as multivariate descriptions of morphological differences among predation regimes (which are predicted to correspond to locomotor trade-offs) because they represent sets of linear combinations of response variables. Thus, at one extreme of the axis are fish with 'high-predation' body shapes (i.e. streamlined head, enlarged caudal peduncle) and at the other extreme are fish with 'low-predation' body shapes (i.e. deeper head region, more narrow caudal peduncle). Each fish therefore fell somewhere along this continuous morphological axis of divergence. This allowed us to test for a relationship between swimming performance and body shape, with the prediction that more 'high-predation like' fish will have higher burst-speeds but lower endurance than more 'low-predation like' fish.

BURST-SPEED SWIMMING PERFORMANCE

We removed fish from grouping tanks and placed them in 11-L individual tanks 24 h prior to testing. Fish were fasted during this time to ensure that they were in a post-absorptive state (Niimi & Beamish, 1974). For each trial, we gently netted and removed the test fish from their individual tank and placed them in a clear cylinder (diameter 13 cm) that was contained within the burst-speed test arena. The test arena comprised an octagonal tank (width 60 cm) that was positioned within a 244-L (125 × 65 × 30 cm) buffer tank. The fish were allowed to acclimate for 5 min before lowering the acclimation cylinder remotely and eliciting an escape response. To elicit an escape response, we struck the arena with an acrylic hammer mechanism within approximately one body length of the fish. We filmed each trial with a high-speed Phantom v4.2 camera (Vision Research) at 400 fps, and analyzed videos using PHANTOM v630 software (Vision Research). Prior to each trial, we measured water temperature with a PCSTestr 35 probe (Eutech) because temperature has been found previously to influence burst-speed performance (Langerhans *et al.*, 2004). Temperature conditions in the experimental tanks did not differ from holding tanks. To quantify burst-speed from each trial video, we approximated the methods of Langerhans *et al.* (2004). In brief, we calculated burst-speed by digitizing the centre of mass for each frame of the fast-start response. We used measurement functions within PHANTOM v630 software to calculate the linear distance travelled and the speed of the fish from the time it initiated the C-start response to the

time when the fish was moving rapidly away from the probe just subsequent to the propulsive tail stroke. We repeated this protocol three times for each fish, allowing us to account for potential variability in response within each fish. For subsequent analyses, we used an average of the three burst-speed responses.

ENDURANCE SWIMMING TRIALS AND KINEMATICS

We investigated endurance swimming performance in the same fish described above, at least 1 week after testing burst-speed and anaesthetization with MS-222, in accordance with the methods of Ingley & Johnson (2016a). Our intent was to be able to directly compare performance in two swimming gaits (i.e. burst-speed and endurance swimming) in individual fish to test for trade-offs in locomotor performance. Individual level trade-offs are hypothesized to occur when propulsive mechanisms for different swimming types are linked (Webb, 1984; Langerhans, 2009b). Such trade-offs are often assumed, although they go untested. As with the burst-speed trials, we starved fish for 24 h prior to testing so that they were in a post-absorptive state (Niimi & Beamish, 1974). We conducted all swimming trials in a Loligo Systems swim tunnel, which consists of a 5-L swim chamber with flow straighteners on the upstream end and a steel mesh on the downstream end to prevent fish from escaping through either end. Additional details on the swim chamber are provided in Ingley & Johnson (2016a). The swim chamber was held in the same laboratory as the holding tanks, and therefore was subject to similar temperature and lighting conditions. Prior to and during all of the trials, we placed a biological aeration filter in the buffer tank of the flow system to ensure that the water was sufficiently oxygenated.

For each fish, we followed the methods of Ingley & Johnson (2016a). Briefly, we commenced by gently netting a single fish and placing it in the flow chamber, after which we allowed it to acclimate in still water for a period of 1 min. Following this acclimation period, we increased the flow rate gradually (over approximately 5 s) to 0.1 m s^{-1}. This speed was sufficient to force the fish to begin swimming with the flow (or be pushed to the downstream screen if unresponsive) but not so fast as to present an aerobically challenging effort. We allowed fish to acclimate at this low flow rate for 1 min before increasing the flow rate to 0.2 m s^{-1}. After 1 min of swimming at this flow rate, we captured a 5-s video at 400 frames per second using a Phantom v4.2 camera positioned directly above the chamber. We positioned a small mirror on the side of the chamber at a 45° angle so that we could capture both dorsal and

lateral views of the fish simultaneously. Where fish had not fatigued after a period of 15 min at 0.2 m s^{-1}, we gradually increased the flow rate to 0.32 m s^{-1} and, at 30 min, we further increased the flow to 0.38 m s^{-1} where it remained until the fish fatigued. For fish that swam beyond 15 min at 0.2 m s^{-1}, we captured high-speed videos at 0.32 and 0.38 m s^{-1}, although we do not report these results here. The speeds that we chose were based on typical flow rates found in low-predation sites and were not standardized to body size as is sometimes carried out in endurance studies. We used natural flow rates and not size standardized flow rates because a goal of the present study was to assess the extent to which populations were locally adapted.

The protocol that we employed allowed us to accomplish two primary goals. First, by measuring time to fatigue in seconds (F_t; hereafter 'endurance') for each fish, we obtained an estimate of swimming endurance, which we defined as the time from initiation of high flow (i.e. 0.2 m s^{-1}) until the fish was unable to continue swimming and fell back against the downstream screen. Endurance swimming can be used as a metric of organism-level fitness, particularly in high-flow, high-competition environments, assuming that individuals that can swim at sustained speeds for longer can spend more time foraging and pursuing potential mates (Vogel, 1994; Plaut, 2001; Domenici, 2003; Blake, 2004; Langerhans, 2009b). Low-predation environments correspond to high population densities and increased intraspecific competition. Therefore, we predict that fish from low-predation environments will have higher endurance than high-predation fish to more efficiently acquire resources.

Second, by using high-speed video of sustained swimming behaviour, we were able to conduct frame-by-frame analyses of swimming behaviour to extract a series of kinematic variables. The kinematic variables that we examined were likely to have a direct relationship with overall endurance (all variables described below were extracted from videos using PHANTOM v630 software). Assuming a fairly simplistic model of undulatory swimming [i.e. where the fish is modeled as an actuator-driven, flexible body; (McHenry, Pell & Long, 1995; Langerhans, 2009b)], swimming speed can be controlled by modifying body stiffness, driving frequency, and driving amplitude. More efficient swimmers are predicted to have more stiff bodies, lower tail-beat frequencies, and decreased driving amplitude. Here, we followed the methods of Ingley & Johnson (2016a) to estimate the following kinematic variables over three complete tail beats: R (half the distance between right and left excursions of the anterior tip of the rostrum); driving frequency as tail-beat frequency, f (inverse of the average period of ten complete tail-beat cycles); body stiffness by measuring propulsive wavelength, λ (double the posterior half-wavelength); and driving amplitude as rostral amplitude. These three parameters together determine propulsive wave speed (calculated as $c = \lambda f$) and tail-beat amplitude, H (taken from video sequences), which consequently determines swimming speed, U (which was held constant in the flow chamber at the rates described above). Thus, if fish from populations that occur in different predation environments vary in their endurance swimming abilities, then at least one of these kinematic parameters should differ (Langerhans, 2009b). If fish from different predation environments do indeed differ in one of these traits, they will have to compensate by modifying one or more of the other kinematic variables to maintain a constant speed, which should in turn result in greater hydromechanical work produced by high-predation fish compared to low-predation fish swimming at the same speed. We therefore followed Langerhans (2009b) by calculating total hydromechanical power (P) as an overall summary of the magnitude of thrust production. To do so, we used Lighthill's elongated-body theory (Lighthill, 1975; Videler, 1993), which indicates that the mean thrust generated during swimming can be calculated from conditions at the trailing edge of the fin. Although this is not a perfect metric for all fish bodies, it provides a useful tool for comparing efficiency among populations. In addition to the above kinematic variables, we measured the caudal fin trailing-edge depth (B; mm) as the vertical distance between the dorsal-most and ventral-most points on the caudal fin. This allowed us to estimate power, or the mechanical rate of working, as $P \propto f^2 H^2 B^2 (1 - U/c)$. Given a constant swimming speed, a lower value of P would indicate greater overall locomotor efficiency (i.e. less power used to overcome drag forces). We predict that fish from low-predation environments will exhibit more efficient kinematics, which in turn will lower the power needed to maintain a constant speed and thus increase endurance.

STATISTICAL ANALYSIS

Body shape comparisons

We conducted a MANCOVA to test for overall differences in body shape between populations from different predation environments, with one MANCOVA per population pair. We used geometric shape variables (relative warps) as response variables, with centroid size as a covariate to control for multivariate allometry, and environment (i.e. high- or low-predation) as a main effect. Our sampling only included males, and so sex was not included as an effect. We also performed a discriminant function analysis

(DFA) for each taxonomic comparison to provide an intuitive metric of the magnitude of morphological divergence that occurs between each population pair. We used all 22 relative warps as predictor variables in the DFA to maximize the potential explanatory power of the model. To test the predictive power of the DFA, we conducted a leave-one-out cross-validation procedure.

Burst-speed comparisons

To test for overall differences in burst-speed among populations, we conducted an analysis of covariance (ANCOVA). Each population pair was tested separately. For each model, we used average burst-speed as the response variable, environment as a main effect, and SL as a covariate. We also conducted a multiple regression analysis to test for a relationship between burst-speed and body shape (i.e. the canonical axis score for each individual as described above for geometric morphometrics), both within and between populations. In each case, we used body shape and SL as predictor variables of burst-speed to test for the impact of each on burst-speed ability. We conducted the regression analysis in addition to the ANCOVA analysis to investigate the relationship between burst-speed and body shape within populations, in addition to comparing burst-speed between populations.

Endurance and kinematic variables

We tested for overall differences in kinematics by conducting a MANCOVA for each population pair with the five kinematic variables as dependent variables, body shape and SL as covariates, and population (i.e. predation environment) as a main effect. We then conducted separate univariate ANCOVAs for caudal fin depth, each of the five measured kinematic variables, power, and endurance time. This allowed us to test which specific traits differed among populations, in addition to testing for overall differences in kinematics among populations. We included environment as a main effect and SL as a covariate for each ANCOVA. We compared SL among population pairs using ANOVA. We used the following data transformations for all analyses to meet assumptions for parametric tests: for *BR–BT*, we used a natural log transformation for tail-beat frequency, power, and propulsive wave speed; log_{10} for rostral amplitude; and square root for endurance time; for Javilla–Grande, we used natural log transformation for tail-beat frequency, power, caudal fin depth, and propulsive wave speed; and log_{10} for rostral amplitude.

Path analysis and pairwise comparisons for endurance and kinematic variables

We conducted a series of path analyses to test for a relationship among morphology, kinematics, and endurance, in addition to the above analyses that allowed us to test for overall differences in these traits. Path analysis is a method that is frequently used to quantify how natural selection acts on traits (Arnold, 1983; Kingsolver & Schemske, 1991; Mitchell, 1992; Sinervo & DeNardo, 1996; Scheiner, Mitchell & Callahan, 2000). Path analysis is particularly useful for incorporating intermediate variables (kinematics in this case) between traits (e.g. morphology) and fitness (e.g. endurance), allowing researchers to clarify functional relationships between traits and fitness (Kingsolver & Schemske, 1991; Scheiner et al., 2000). In a classic study on this subject, Arnold (1983) provides a methodological approach that highlights performance as an intermediary between traits in the traditional sense (e.g. physiology or morphology) and measures of fitness (e.g. survival), suggesting that these traits do not directly determine fitness, with fitness instead being determined by various aspects of performance to which such traits contribute (Brodie & Ridenhour, 2003). Garland & Losos (1994) expanded the model of Arnold (1983) by including direct pathways between traits and fitness, thus allowing path models to test both direct and indirect pathways between traits, performance, and fitness. Further expansion of this approach by testing relationships between traits, performance, and fitness in a model selection framework (Johnson & Omland, 2004) provides a powerful tool for determining which traits are under the strongest selection.

To assess the effects of morphology on performance (i.e. kinematic variables) and fitness, as well as the effects of performance on fitness, we conducted path analyses using AMOS, version 19 (Arbuckle, 2010). We conducted path analyses that included either all individuals from both populations, or a population singly, conducting analyses for each population pair separately. This approach allowed us to test for significant relationships among morphology, performance (kinematics), and fitness (endurance), both within and among populations. We employed a bootstrapping method (5000 replicates) within AMOS to assess path significance. We used maximum likelihood methods to estimate path relationships. In addition to generating estimates of direct effects of each path, we also generated estimates of indirect effects. Indirect effects can be interpreted as the effect of a phenotypic trait (e.g. SL or body shape) on endurance mediated by kinematic variables. These indirect effects are in addition to any direct effects that body shape or SL have on endurance (Kline, 2005). To reduce dimensionality and multicolinearity, we conducted a PC analysis using correlation matrices for the five kinematic variables. We only retained the first two PC axes because they alone

explained > 99% of the variation, and were the only PC axes retained under a broken stick model. We used these PC axes to construct our path models. We did not include caudal fin depth in our analyses because it was highly correlated with SL ($R^2 > 0.5$, $P < 0.001$). For each path analysis, we generated models with direct and indirect paths or with indirect paths only. We compared these models and used the top model (based on Akaike information criterion) to generate path estimates (see Supporting information, Appendix S2).

Finally, to compare differences between paths for different populations, we conducted a critical ratio differences test (Hopwood, 2007; Byrne, 2010). This method allows for the comparison of the strength and direction (sign) of a path between models of different groups (e.g. the strength and direction of the relationship between SL and endurance in *BR* vs. *BT*). Using this method, we conducted two pairwise comparisons: *BR* vs. *BT* and Javilla vs. Grande.

Between population performance trade-offs
We assessed between population trade-offs in endurance and burst-speed ability by conducting multiple regression analyses. For our model, we included all four populations and used endurance as the response variable, and burst-speed, SL, and population of origin as predictor variables. We also included an interaction term between population and burst-speed, allowing us to test whether the trade-off was consistent across populations. With the exception of the path models, all statistical analyses were conducted in R (R Core Development Team, 2008).

RESULTS
DIVERGENT BODY SHAPE
Body shape was significantly different between predation environments for both levels of comparison (Table 1). Patterns of morphological divergence matched those previously documented in these species (Ingley *et al.*, 2014b; Ingley & Johnson, 2016a). Specifically, populations from high-predation environments had more streamlined body shapes than populations from low-predation environments (see Supporting information, Appendix S3). The DFA provided additional evidence for morphological divergence between population pairs that occur in different predation environments. The DFA correctly assigned 22/22 *BR* (100%), 33/33 *BT* (100%), 25/26 Javilla (96.2%), and 29/29 Grande (100%). The cross-validation procedure that we conducted indicated that the model was robust to data removal, correctly assigning 22/22 *BR*, 33/33 *BT*, 21/26 Javilla, and 26/29 Grande. Thus, variation in body shape alone is

Table 1. Results for multivariate analysis of covariance comparing body shape between Javilla and Grande and between *Brachyrhaphis roseni* and *Brachyrhaphis terrabensis*

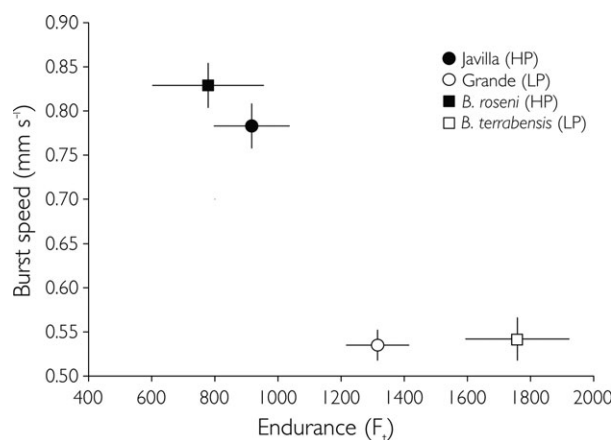| Comparison	Effect	d.f.	F	P
Javilla–Grande	Population	22,31	6.43	< 0.001
	Size	22,31	1.03	0.460
Brachyrhaphis roseni–Brachyrhaphis terrabensis	Species	22,31	47.8	< 0.001
	Size	22,31	7.1	< 0.001

Figure 1. Mean ± SE for endurance (F_t) as a function of burst-speed (mm s^{-1}) for each population tested.

sufficient for correctly assigning the vast majority of fish to their population of origin.

DIVERGENT BURST-SPEED
Our combined Javilla–Grande multiple regression analysis (overall model $R^2 = 0.184$, overall model $P = 0.005$) found that burst-speed increased with morphological axis of divergence ($F_{1,52} = 9.81$, $P = 0.004$) but not with size ($F_{1,52} = 2.50$, $P = 0.119$), such that faster fish had more streamlined bodies than slower fish. Our ANCOVA model found evidence that burst-speed was strongly associated with predation environment but not associated with size (Fig. 1, Table 2). However, our analysis of Javilla (overall model $R^2 = 0.169$, overall model $P = 0.119$) and Grande (overall model $R^2 = 0.042$, overall model $P = 0.572$) separately revealed that, within populations, burst-speed did not increase with morphological axis of divergence (Javilla: $F_{1,23} = 3.73$, $P = 0.066$; Grande: $F_{1,26} = 1.00$, $P = 0.33$), although,

Table 2. Results for analysis of covariance comparing burst-speed between Javilla and Grande and between *Brachyrhaphis roseni* and *Brachyrhaphis terrabensis*

Comparison	Effect	d.f.	F	P
Javilla–Grande	Population	1	63.17	< 0.001
	Size	1	0.04	0.84
	Residuals	52		
Brachyrhaphis	Species	1	63.13	< 0.001
roseni–Brachyrhaphis	Size	1	0.01	0.93
terrabensis	Residuals	52		

in Javilla, the relationship was only marginally non-significant. Size did not have an effect on burst-speed when populations were analyzed separately (Javilla: $F_{1,23} = 0.93$, $P = 0.344$; Grande: $F_{1,26} = 0.14$, $P = 0.71$).

Our combined *BR–BT* multiple regression analysis (overall model $R^2 = 0.494$, overall model $P < 0.001$) found that burst-speed increased with morphological axis of divergence ($F_{1,52} = 49.71$, $P < 0.001$) but not with size ($F_{1,52} = 0.95$, $P = 0.34$), such that faster fish had more streamlined bodies, which is common in low-predation environments. Our ANCOVA model found evidence that burst-speed was strongly associated with predation environment but not associated with size (Table 2). However, our multiple regression analysis of *BR* (overall model $R^2 = 0.106$, overall model $P = 0.344$) and *BT* (overall model $R^2 = 0.051$, overall model $P = 0.457$) separately revealed that, within populations, burst-speed did not increase with morphological axis of divergence (*BR*: $F_{1,19} = 1.33$, $P = 0.26$; *BT*: $F_{1,30} = 1.31$, $P = 0.26$), nor did it increase with size (*BR*: $F_{1,19} = 0.93$, $P = 0.35$; *BT*: $F_{1,30} = 0.30$, $P = 0.59$). In other words, the body shape differences that were driving burst-speed differences between populations did not appear to affect burst-speed within populations.

DIVERGENT KINEMATICS AND ENDURANCE PERFORMANCE

Overall, populations from different predation environments showed significant differences in swimming kinematics (Javilla–Grande: $F_{5,48} = 227.8$, $P < 0.001$; *BR–BT* $F_{5,48} = 588.6$, $P < 0.001$). Size also had a significant effect on kinematics (Javilla–Grande: $F_{5,48} = 498.3$, $P < 0.001$; *BR–BT* $F_{5,48} = 457.1$, $P < 0.001$). Based on univariate comparisons, we found significant differences in both SL and caudal fin depth (Table 3). Furthermore, univariate tests revealed that Javilla tended to have higher tail-beat frequency but significantly lower propulsive wavelength than Grande. The pattern was the same between *BR* and *BT*, although these

populations also differed in rostral amplitude and tail-beat amplitude, with *BR* having larger relative tail-beat and rostral amplitudes than *BT* (Fig. 2, Table 3). Hydromechanical power, which we used as a measure of energy efficiency (with higher power indicating lower efficiency), was significantly higher in high-predation than in low-predation populations (Fig. 3). Finally, endurance was significantly higher in low-predation than in high-predation populations (Fig. 1, Table 3). These results demonstrate that fish from divergent predation environments engage in distinctly different swimming styles, wherein low-predation fish produce thrust in a more energetically efficient way [i.e. with low-amplitude (rostral and tail-beat) and long-wavelength undulations] than high-predation fish. These kinematic differences result in dramatically higher endurance in low-predation populations relative to high-predation populations.

PATH ANALYSIS AND PAIRWISE COMPARISONS FOR ENDURANCE SWIMMING

Path analysis of the morphology-performance-fitness pathway allowed us to determine how morphology influenced kinematics, and how kinematics in turn influenced endurance. The results for the top models for each path analysis are shown in Figure 4, and the results for each pairwise path comparison are provided in the Supporting information (Appendix S4). Indirect path estimates and their significance are also provided in the Supporting information (Appendix S5). Overall, propulsive wavelength (λ) and tail-beat amplitude (H) loaded heavily on the first two PC axes and had the greatest effect on endurance. Body shape had a significant effect on both traits in the combined *BR–BT* model, with more 'high-predation like' individuals having shorter propulsive wavelengths. However, body shape did not have an indirect effect on endurance (Fig. 4; see also Supporting information, Appendix S5). Body shape did not affect kinematics in the *BT* or *BR* model, although it did have a strong direct effect on endurance in *BR*, with more high-predation like individuals having lower endurance. In *B. rhabdophora*, body shape only had an affect on PC1 in the Javilla model, with more high-predation like individuals having lower propulsive wavelengths. Body size did have a strong effect (either direct or indirect) in all populations except *BR*. Larger individuals consistently had higher values for propulsive wavelength and tail-beat amplitude (although tail-beat amplitude standardized by SL showed the opposite pattern, as expected), which resulted in higher endurance in all models except for *BR*. Our pairwise path comparisons revealed that

Table 3. Results for analysis of covariance and analysis of variance testing for variation in body size, kinematics, and endurance between Javilla and Grande and *Brachyrhaphis roseni* and *Brachyrhaphis terrabensis*

Dependent variable	Cohen's d (95% CI)	Predation regime		Standard length		Means ± SE	
		F	P	F	P	Javilla	Grande
Standard length (SL, mm)	1.243 (0.639, 1.848)	21.18	< 0.001			31.8 ± 1.2	38.6 ± 0.9
Caudal fin depth (log B)	0.605 (0.040, 1.169)	7.883	0.007	31.224	< 0.001	11.087 ± 0.349	12.277 ± 0.405
Tail-beat frequency (log f)	−0.503 (−1.063, 0.058)	3.652	0.062	3.876	0.054	1.098 ± 0.039	1.010 ± 0.031
Rostral amplitude ($\log_{10} R$)	−0.150 (−0.703, 0.403)	0.311	0.579	1.404	0.241	2.217 ± 0.123	2.132 ± 0.124
Tail-beat amplitude (H)	0.391 (−0.167, 0.948)	2.483	0.121	10.880	0.002	10.613 ± 0.259	11.136 ± 0.251
Propulsive wavelength (λ)	1.562 (0.929, 2.195)	76.90	< 0.001	69.91	< 0.001	16.843 ± 0.604	21.283 ± 0.486
Propulsive wave speed (log c)	−0.236 (−0.789, 0.318)	0.963	0.331	14.846	< 0.001	0.583 ± 0.021	0.557 ± 0.018
Power (log P)	−1.104 (−1.698, −0.511)	30.53	< 0.001	44.76	< 0.001	0.0215 ± 0.003	0.009 ± 0.001
Fatigue time (F_t)	0.698 (0.129, 1.267)	7.027	0.012	3.736	0.059	916.8 ± 119.0	1315.7 ± 99.8

						Means ± SE	
						Brachyrhaphis roseni	*Brachyrhaphis terrabensis*
Standard length (SL)	−2.946 (−2.946, −1.514)	65.64	< 0.001			29.2 ± 0.9	40.7 ± 1.0
Caudal fin depth (B)	−1.111 (−1.716, −0.508)	26.85	< 0.001	35.24	< 0.001	10.250 ± 0.379	12.182 ± 0.298
Tail-beat frequency (log f)	0.943 (0.350, 1.536)	13.73	< 0.001	10	0.003	1.040 ± 0.041	0.899 ± 0.022
Rostral amplitude ($\log_{10} R$)	1.245 (0.631, 1.859)	20.45	< 0.001	0.98	0.327	3.378 ± 0.261	2.288 ± 0.082
Tail-beat amplitude (H)	−1.857 (−2.529, −1.185)	57.26	< 0.001	14.66	< 0.001	8.793 ± 0.265	11.436 ± 0.267
Propulsive wavelength (λ)	−2.382 (−3.117, −1.647)	195.33	< 0.001	86.24	< 0.001	13.518 ± 0.521	19.988 ± 0.502
Propulsive wave speed (log c)	0.415 (−0.153, 0.983)	2.693	0.107	10.77	0.002	0.481 ± 0.020	0.445 ± 0.015
Power (log P)	1.286 (0.669, 1.904)	38.45	< 0.001	41.32	< 0.001	0.014 ± 0.002	0.007 ± 0.0004
Fatigue time ($\sqrt{F_t}$)	−1.316 (−1.936, −0.696)	25.364	< 0.001	6.806	0.012	778.6 ± 175.5	175 ± 163.6

Cohen's d is the standardized effect size for the population (i.e. predation environment) term. Data are means ±SE untransformed values. CI, confidence interval.

the strength and direction (sign) of paths did not differ between *BR* and *BT*. However, the paths connecting SL to PC2 (on which propulsive wavelength and tail-beat amplitude loaded heavily) differed significantly between Javilla and Grande (see Supporting information, Appendix S4).

BETWEEN POPULATION TRADE-OFFS

Our multiple regression analyses indicated that, between populations, there was a significant negative correlation between burst-speed and endurance ($R^2 = 0.310$, $F_{8,100} = 5.624$, $P < 0.001$). Overall, endurance increased as burst-speed decreased. The
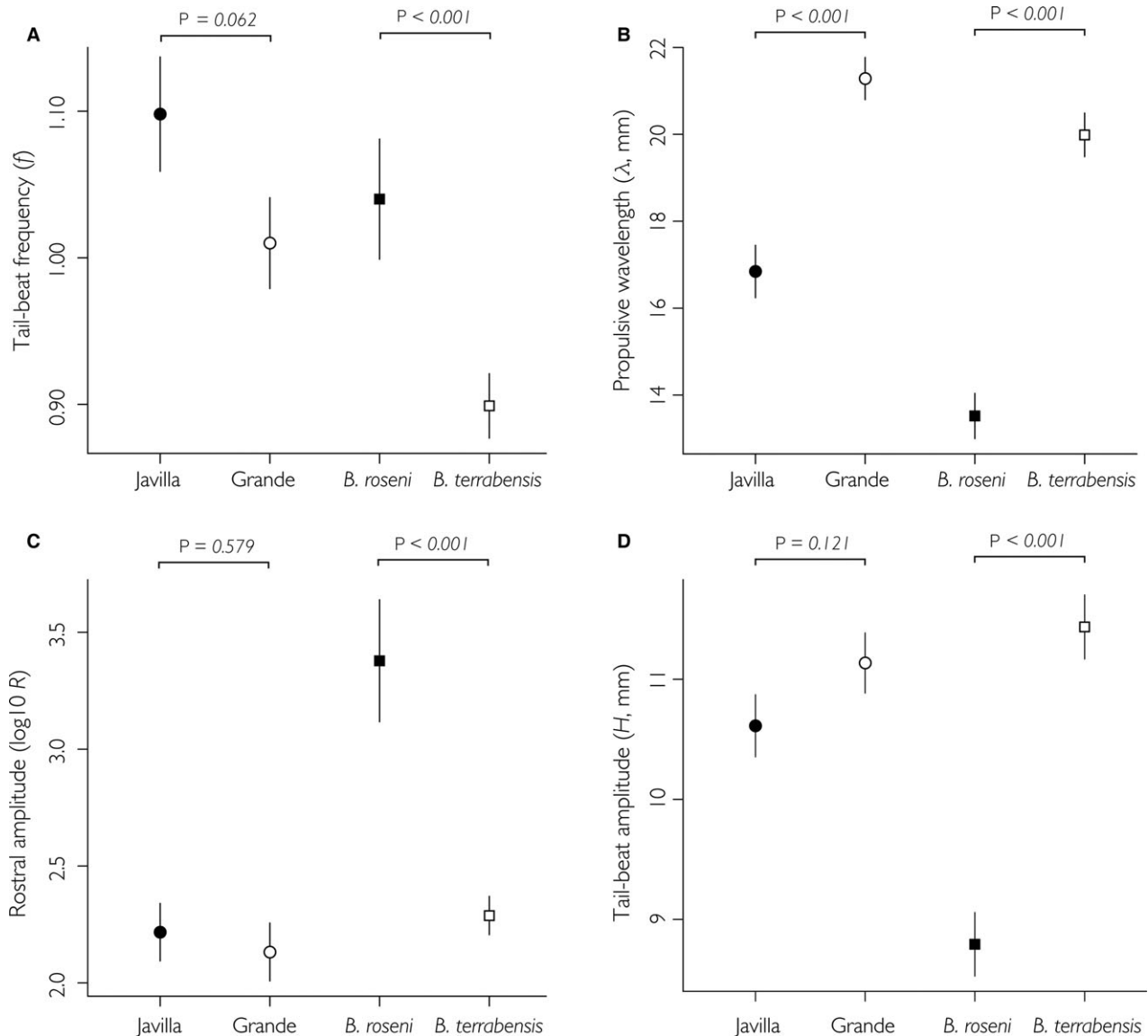
Figure 2. Univariate comparisons of kinematic variables representing population means and standard errors. Kinematics variables shown are: (A) tail-beat frequency (f); (B) standardized propulsive wavelength (λ, mm); (C) standardized rostral amplitude ($\log_{10} R$); and (D) standardized tail-beat amplitude (H, mm). Figure 2 uses the same symbols as Fig. 1 (i.e. open and closed symbols correspond to low- and high-predation populations, respectively).

interaction between burst-speed and population was nonsignificant ($F_{3,100} = 0.482$, $P = 0.695$, indicating that there was no difference in this relationship among populations (i.e. endurance consistently increased as burst-speed decreased).

DISCUSSION

The results of the present study suggest that different predation environments have driven the evolution of divergent swimming behaviours in *Brachyrhaphis* fishes, with low-predation environments favouring high endurance, and high-predation environments favouring high burst-speeds. Although between population differences in swimming performance were strong and consistent across our comparisons, the traits that affected differences in swimming performance were less consistent. We also found that the magnitude of change at different stages of divergence was similar for burst-speed but less exaggerated for endurance swimming between Javilla and Grande compared to *BR* and *BT*. Our results suggest that local adaptation, in terms of
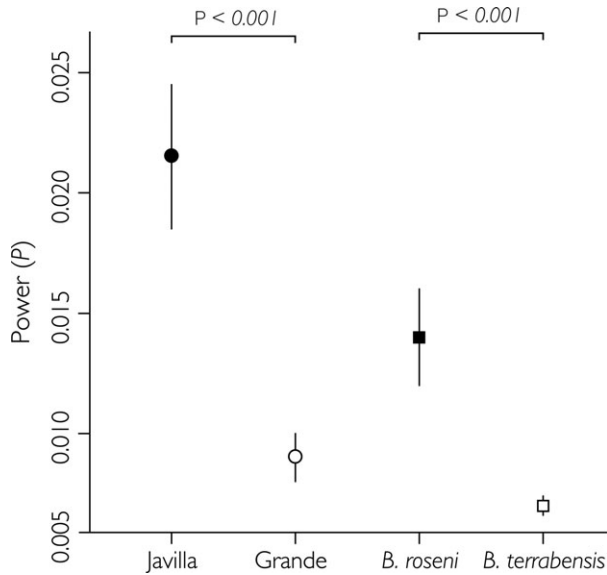
Figure 3. Population means and standard errors for power (P). Power was calculated as $P \propto f^2 H^2 B^2 (1 - U/c)$. High values for power indicate less efficient swimming kinematics. Figure 3 uses the same symbols as in Figs 1 and 2 (i.e. open and closed symbols correspond to low- and high-predation populations, respectively).

performance, can be remarkably consistent in closely-related lineages evolving in similarly divergent selective regimes, even when functional relationships underlying performance differences are less predictable.

BODY SHAPE, BURST-SPEED, AND ENDURANCE DIFFER AMONG PREDATION ENVIRONMENTS

As in previous studies of *Brachyrhaphis*, we found that body shape differed consistently among predation environments. Patterns of body shape divergence in our current samples were similar to those documented previously (Ingley *et al.*, 2014b), in that fish from high-predation environments had more streamlined bodies than fish from low-predation environments (see Supporting information, Appendix S3). Although these patterns were similar to those observed in other poeciliids, where they had a significant effect on swimming ability (Langerhans, 2009a, b), body shape differences within populations of *Brachyrhaphis* did not have a strong effect on swimming ability. Instead, these body shape differences did correlate strongly with burst-speed swimming between populations. This lack of within population effects could be a result of limited body shape variation within populations, or it could result from some unmeasured trait that better accounts for individual differences in burst-speed. For example,

differences in red–white muscle ratios (which has not been evaluated frequently) could account for inter-individual variation in burst-speed because this ratio often corresponds to differences in aerobic vs. anaerobic swimming performance (Goolish, 1989) and is not necessarily expected to vary with body shape.

Although our measured morphological traits did not correlate with burst-speed performance, among population divergence in burst-speed was strong both at early and late stages of divergence (Fig. 1). Indeed, burst-speed values for populations from the same predation environments, regardless of their stage of divergence, were indistinguishable. Burst-speed is under strong selection by predators in high-predation environments, with faster individuals consistently out-surviving slower individuals in the presence of a predator (Ingley & Johnson, 2016a). This pattern is present both within and among species of *Brachyrhaphis* from different predation environments, and is consistent with previous work reporting that faster burst-speeds increase predator escape ability (Domenici, 2010). Although we have not evaluated how selection acts on burst-speed in low-predation environments, our results show that populations quickly lost their burst-speed swimming ability (i.e. fish from Grande) and that this loss has persisted in late stages of divergence (i.e. *BT*). The loss of burst-speed swimming ability in low-predation environments suggests that a trade-off between these swimming gaits is present. Alternatively, this loss could be associated with strong selection on some other trait that is negatively correlated with burst-speed. This difference could also be a result of environmental factors, with low-predation fish simply falling 'out of practice' when found in habitats lacking predators. Environmental conditioning does have the potential to influence swimming performance (Goolish, 1989) and this could result in among individual or population differences in traits that could underlie swimming trade-offs (Young & Cech, 1993). However, all fish used in the present study were held under common conditions (no-flow) for 1 year prior to testing, suggesting that their current level of conditioning was equivalent. In either case, burst-speed performance appears to diverge predictably with predation environment in the populations of *Brachyrhaphis* that we examined at early and late stages of divergence. Future work could benefit from examining multiple populations pairs, which would strengthen inferences regarding the agents and targets of selection in this system.

Endurance differed significantly among populations of *Brachyrhaphis* from divergent predation environments, with low-predation fish having higher endurance than high-predation fish (Fig. 1, Table 3).
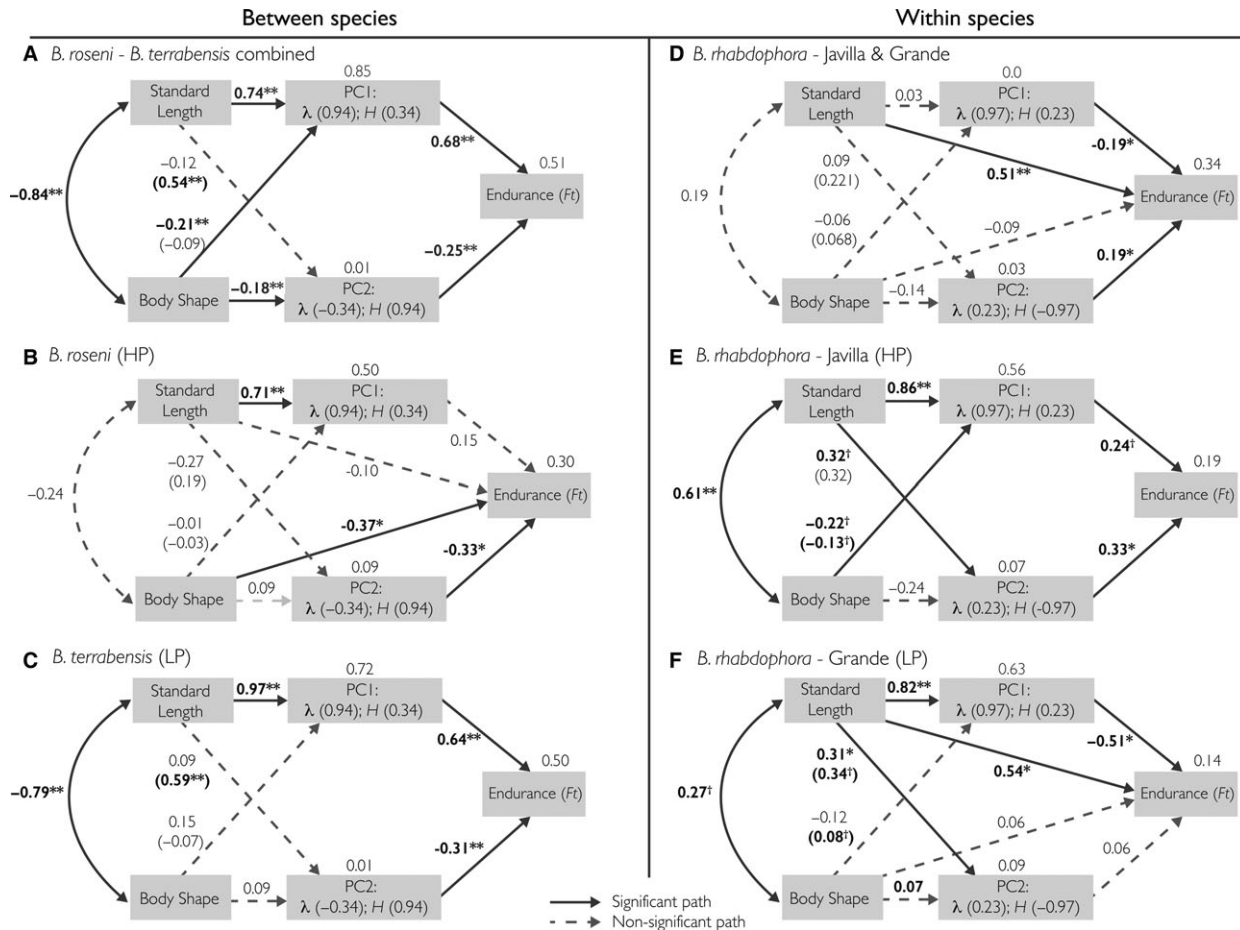
Figure 4. Path analysis results for top models with populations analyzed together (A, D) and individually (B, C, E, F). Significant paths, assessed through bootstrapping (5000 replicates) are indicated by solid arrows, whereas nonsignificant paths are indicated by broken arrows. Double-headed arrows represent correlations between body-shape and SL. Values near each arrow represent regression coefficients, and values above each rectangle represent squared correlation coefficients. Indirect effects of SL and body-shape on survival are shown in parentheses below regression coefficients. Indirect effects can be interpreted as mediated effects that occur in addition to any direct effect that these kinematics variables have on endurance. $^\dagger P < 0.10$; $^* P < 0.05$; $^{**} P < 0.01$.

Endurance is considered to be a good indicator of organism level fitness in areas with high population-densities because fish with higher endurance can spend more time foraging and pursuing potential mates (Langerhans, 2009b). Given that low-predation environments tend to have higher intraspecific competition as a result of higher population densities, individuals that have higher endurance can spend more time engaging in these competitive interactions, and thus increase their overall fitness (Vogel, 1994; Plaut, 2001; Domenici, 2003; Blake, 2004; Langerhans, 2009b). Similarly, higher-flow rates common in low-predation environments could select for increased endurance and select against high-predation fish that immigrate into low-predation environments. Although the difference was less

exaggerated between Javilla–Grande, we found consistent differences in endurance between populations at both stages of divergence. Furthermore, we found consistent differences in kinematic variables, although fewer traits differed between Javilla and Grande than between *BR* and *BT*, with measured traits varying among predation environments in line with our predictions. Tail-beat frequency, rostral amplitude, and tail-beat amplitude were higher in *BR* compared to *BT* (Fig. 2, Table 3), and propulsive wavelength was significantly higher in *BT* compared to *BR*. Similar patterns were found within *B. rhabdophora*, although they did not differ in either rostral amplitude or tail-beat amplitude. Therefore, on average low-predation fish had less flexible bodies and used longer, lower amplitude undulations during

swimming. As predicted, these differences resulted in between population variation in the amount of power used to maintain a constant speed, with both high-predation populations using significantly more power than their low-predation counterparts (Fig. 3, Table 3). These patterns were remarkably consistent at different stages of divergence within *Brachyrhaphis* (Figs 2, 3) and they are also parallel to patterns observed in distantly related poeciliids (Langerhans, 2009b). Further work within *Brachyrhaphis* (e.g. examining swimming performance in repeatedly divergent lineages within *B. rhabdophora*) would help determine how consistently these traits evolve in response to different predation environments.

Our path analysis allowed us to determine which traits directly and/or indirectly affected overall endurance. Propulsive wavelength and tail-beat amplitude had the greatest and most consistent effects on endurance in all our comparisons, with higher values of these variables generally leading to higher endurance (Fig. 4; although SL standardized values for tail-beat amplitude had the opposite relationship) but there were some exceptions. Body shape affected kinematics and endurance in the models that included both *BR–BT*, and the models that included *BR*, Grande and Javilla singly. More 'high-predation like' individuals (i.e. more streamlined body shape) had smaller propulsive wavelengths, although this only manifested itself as a significant indirect effect on endurance in the Javilla and Grande models. By contrast to body shape, which had equivocal effects on endurance, body size was a strong predictor of endurance, either directly or indirectly, in almost all populations. Overall, larger fish had higher endurance. This pattern suggests that selection for higher endurance could be an additional factor leading to divergence in body size among populations occurring in different predation environments and differing in their levels of intraspecific competition and flow rates. These results suggest that, even with the lack of consistent functional relationships underlying performance differences, local adaptation can be remarkably consistent in closely-related lineages evolving in similarly divergent selective regimes. Future work would benefit from evaluating more lineages within this genus, particularly within *B. rhabdophora*, to determine whether functional relationships underlying performance differences remain unclear, or whether a more definite pattern emerges.

LOCAL ADAPTATION AND PERFORMANCE SPECIALIZATION

Brachyrhaphis fishes appear to have evolved divergent swimming strategies in response to different predation environments, both within *B. rhabdophora* and between *BR* and *BT*. Our combined regression analyses indicated that, at the among population level, increased performance in one swimming gait compromises performance in the other. Our comparative approach further revealed that this pattern could evolve rather quickly, although studies of additional population pairs within *B. rhabdophora* would be needed to conclusively identify the speed with which performance evolves. Javilla and Grande show low levels of genetic divergence (Johnson, 2001b; Ingley *et al.*, 2014b), yet they have achieved the same magnitude of divergence in burst-speed performance as observed between *BR* and *BT*, and almost the same as that observed in endurance swimming (Fig. 1). Trade-offs at the population level are common in nature (Schluter, 2000), and ecological divergence and local adaptation appear to be significant drivers of speciation (Funk, Nosil & Etges, 2006; Nosil, 2012). Indeed, population level patterns of local adaptation in swimming ability appear to be common, with different predation environments favouring different locomotor adaptations in a diversity of taxa (Webb, 1986; Katzir, 1993; Johnson, Burt & DeWitt, 2008; Arendt, 2009; Fu *et al.*, 2015). Our results suggest that divergent predation environments select for divergent swimming modes, and that this could result in a population level trade-off between endurance and burst-speed swimming.

CONCLUSIONS

The results of the present study provide further evidence that divergent predation environments favour the evolution of locally adapted swimming performance traits. However, we show that the traits underlying these patterns are less predictable than often assumed. Across *Brachyrhaphis*, low-predation populations are considered to have evolved from high-predation populations, as individuals moved up stream drainages from low-elevation, high-predation sites to high-elevation streams lacking predators (Johnson, 2001b). Thus, populations from different lineages appear to have become locally adapted to divergent predation environments in remarkably similar ways. Given that similar patterns were observed both at early (Javilla–Grande) and late (*BR–BT*) stages of divergence, these patterns of local adaptation appear to have evolved rather quickly. Future work comparing population pairs at additional levels of divergence would provide valuable insight into the repeatability and temporal progression of local adaptation.

ACKNOWLEDGEMENTS

REFERENCES

Abrams PA. 1993. Does increased mortality favor the evolution of more repid senescence. *Evolution* **47:** 877–887.

Agrawal AA, Conner JK, Rasmann S. 2010. Tradeoffs and adaptive negative correlations in evolutionary ecology. In: Bell M, Eanes W, Futuyma D, Levinton J, eds. *Evolution after Darwin: the first 150 years*. Sunderland, MA: Sinauer Associated Inc., 243–268.

Arbuckle JL. 2010. *IBM SPSS Amos 19 user's guide, 19th edn*. In: Corporation AD, ed. Crawfordville, FL: IBM SPSS.

Archard GA, Braithwaite VA. 2011. Increased exposure to predators increases both exploration and activity level in *Brachyrhaphis episcopi*. *Journal of Fish Biology* **78:** 593–601.

Arendt JD. 2009. Influence of sprint speed and body size on predator avoidance in New Mexican spadefoot toads (*Spea multiplicata*). *Oecologia* **159:** 455–461.

Arnold SJ. 1983. Morphology, performance and fitness. *American Zoologist* **23:** 347–361.

Blake RW. 2004. Fish functional design and swimming performance. *Journal of Fish Biology* **65:** 1193–1222.

Brodie ED, Ridenhour BJ. 2003. Reciprocal selection at the phenotypic interface of coevolution. *Integrative and Comparative Biology* **43:** 408–418.

Byrne BM. 2010. *Structural equation modeling with AMOS: basic concepts, applications, and programming*. New York, NY: Routledge, Taylor & Francis Group.

Coyne JA, Orr HA. 2004. *Speciation*. Sunderland, MA: Sinauer Associates Inc.

Domenici P. 2003. Habitat, body design and the swimming performance of fish. In: Bels VL, Gasc J-P, Casinos A, eds. *Vertebrate biomechanics and evolution*. Oxford: BIOS Scientific Publishers Ltd, 137–160.

Domenici P. 2010. *Escape responses in fish: kinematics, performance, and behavior Fish locomotion: an etho-ecological perspective*. Enfield, NH: Enfield Science Publishers, 123–170.

Franssen NR, Harris J, Clark SR, Schaefer JF, Stewart LK. 2013. Shared and unique morphological responses of stream fishes to anthropogenic habitat alteration. *Proceedings of the Royal Society of London Series B, Biological Sciences* **280:** 20122715.

Fu C, Fu SJ, Yuan XZ, Cao ZD. 2015. Predator-driven intra-species variation in locomotion, metabolism and water velocity preference in pale chub (*Zacco platypus*) along a river. *Journal of Experimental Biology* **218:** 255–264.

Funk DJ. 1998. Isolating a role for natural selection in speciation: host adaptation and sexual isolation in *Neochlamisus bebbianae* leaf beetles. *Evolution* **52:** 1744–1759.

Funk DJ, Nosil P, Etges WJ. 2006. Ecological divergence exhibits consistently positive associations with reproductive isolation across disparate taxa. *Proceedings of the National Academy of Sciences of the United States of America* **103:** 3209–3213.

Garland T, Losos JB. 1994. Ecological morphology of locomotor performance in squamate reptiles. In: Wainwright PC, Reilly SM, eds. *Ecological morphology*. Chicago, IL: University of Chicago Press, 240–302.

Goolish EM. 1989. The scaling of aerobic and anaerobic muscle power in rainbow-trout (*Salmo gairdneri*). *Journal of Experimental Biology* **147:** 493–505.

Hendry AP, Kinnison MT. 1999. Perspective: the pace of modern life: measuring rates of contemporary microevolution. *Evolution* **53:** 1637–1653.

Holzman R, Collar DC, Price SA, Hulsey CD, Thomson RC, Wainwright PC. 2012. Biomechanical trade-offs bias rates of evolution in the feeding apparatus of fishes. *Proceedings of the Royal Society of London Series B, Biological Sciences* **279:** 1287–1292.

Hopwood CJ. 2007. Moderation and mediation in structural equation modeling: applications for early intervention research. *Journal of Early Intervention* **29:** 262–272.

Ingley SJ. 2014. Elevational range expansion in a neotropical live-bearing fish from Panama: implications for adaptive evolution. *The Southwestern Naturalist* **59:** 396–399.

Ingley SJ. 2015. Sympatric populations of sister species of *Brachyrhaphis*. *Journal of Fish Biology* **86:** 1163–1170.

Ingley SJ, Johnson JB. 2016a. Divergent natural selection promotes immigrant inviability at early and late stages of evolutionary divergence. *Evolution* **70:** 600–616.

Ingley SJ, Johnson JB. 2016b. Selection is stronger in early-versus-late stages of divergence in a Neotropical live-bearing fish. *Biology Letters* 12: 20151022.

Ingley SJ, Rehm J, Johnson JB. 2014a. Size doesn't matter, sex does: a test for boldness in sister species of *Brachyrhaphis* fishes. *Ecology and Evolution* 4: 4361–4369.

Ingley SJ, Billman EJ, Belk MC, Johnson JB. 2014b. Morphological divergence driven by predation environment within and between species of *Brachyrhaphis* fishes. *PLoS ONE* 9: e90274.

Ingley SJ, Billman EJ, Hancock C, Johnson JB. 2014c. Repeated geographic divergence in behavior: a case study employing phenotypic trajectory analyses. *Behavioral Ecology and Sociobiology* 68: 1577–1587.

Ingley SJ, Reina RG, Bermingham E, Johnson JB. 2015. Phylogenetic analyses provide insights into the historical biogeography and evolution of *Brachyrhaphis* fishes. *Molecular Phylogenetics and Evolution* 89: 104–114.

Jennions MD, Telford SR. 2002. Life-history phenotypes in populations of *Brachyrhaphis episcopi* (Poeciliidae) with different predator communities. *Oecologia* 132: 44–50.

Johnson JB. 2001a. Adaptive life-history evolution in the livebearing fish *Brachyrhaphis rhabdophora*: genetic basis for parallel divergence in age and size at maturity and a test of predator-induced plasticity. *Evolution* 55: 1486–1491.

Johnson JB. 2001b. Hierarchical organization of genetic variation in the Costa Rican livebearing fish *Brachyrhaphis rhabdophora* (Poeciliidae). *Biological Journal of the Linnean Society* 72: 519–527.

Johnson JB. 2002. Divergent life histories among populations of the fish *Brachyrhaphis rhabdophora*: detecting putative agents of selection by candidate model analysis. *Oikos* 96: 82–91.

Johnson JB, Belk MC. 2001. Predation environment predicts divergent life-history phenotypes among populations of the livebearing fish *Brachyrhaphis rhabdophora*. *Oecologia* 126: 142–149.

Johnson JB, Omland KS. 2004. Model selection in ecology and evolution. *Trends in Ecology & Evolution* 19: 101–108.

Johnson JB, Zuniga-Vega JJ. 2009. Differential mortality drives life-history evolution and population dynamics in the fish *Brachyrhaphis rhabdophora*. *Ecology* 90: 2243–2252.

Johnson JB, Burt DB, DeWitt TJ. 2008. Form, function, and fitness: pathways to survival. *Evolution* 62: 1243–1251.

Joshi A, Thompson JN. 1995. Trade-offs and the evolution of host specialization. *Evolutionary Ecology* 9: 82–92.

Katzir G. 1993. Escape response of black mollies (*Poecilia shenops*) to predatory dives of a pied kingfisher (*Ceryle rudis*). *Copeia* 2: 549–553.

Kingsolver JG, Schemske DW. 1991. Path analyses of selection. *Trends in Ecology & Evolution* 6: 276–280.

Kinnison MT, Hendry AP. 2001. The pace of modern life II: from rates of contemporary microevolution to pattern and process. *Genetica* 112: 145–164.

Kline RB. 2005. *Principles and practice of structural equation modeling*. New York, NY: The Guildford Press.

Langerhans RB. 2009a. Morphology, performance, fitness: functional insight into a post-Pleistocene radiation of mosquitofish. *Biology Letters* 5: 488–491.

Langerhans RB. 2009b. Trade-off between steady and unsteady swimming underlies predator-driven divergence in *Gambusia affinis*. *Journal of Evolutionary Biology* 22: 1057–1075.

Langerhans RB, Reznick D. 2010. Ecology and evolution of swimming performance in fishes: predicting evolution with biomechanics. In: Domenici P, Kapoor BG, eds. *Fish locomotion: an etho-ecological perspective*. Enfield, NH: Enfield Science Publishers, 200–248.

Langerhans RB, Layman CA, Shokrollahi AM, DeWitt TJ. 2004. Predator-driven phenotypic diversification in *Gambusia affinis*. *Evolution* 58: 2305–2318.

Lighthill MJ. 1975. *Mathematical biofluiddynamics*. Philadelphia, PA: Society for Industrial and Applied Mathematics.

Martin RA, McGee MD, Langerhans RB. 2015. Predicting ecological and phenotypic differentiation in the wild: a case of piscivorous fish in a fishless environment. *Biological Journal of the Linnean Society* 114: 588–607.

McHenry MJ, Pell CA, Long JH. 1995. Mechanical control of swimming speed – stiffness and axial wave-form in undulating fish models. *Journal of Experimental Biology* 198: 2293–2305.

Mitchell RJ. 1992. Testing evolutionary and ecological hypotheses using path-analysis and structural equation modeling. *Functional Ecology* 6: 123–129.

Niimi AJ, Beamish FWH. 1974. Bioenergetics and growth of largemouth bass (*Micropterus salmoides*) in relation to body-weight and temperature. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 52: 447–456.

Nosil P. 2012. *Ecological speciation*. New York, NY: Oxford University Press.

Pfennig KS, Pfennig DW. 2005. Character displacement as the 'best of a bad situation': fitness trade-offs resulting from selection to minimize resource and mate competition. *Evolution* 59: 2200–2208.

Plaut I. 2001. Critical swimming speed: its ecological relevance. *Comparative Biochemistry and Physiology A-Molecular and Integrative Physiology* 131: 41–50.

R Core Development Team. 2008. *R: a language and environment for statistical computing*. In: Computing RFfS, ed. Vienna: R Foundation for Statistical Computing.

Rohlf F. 2003. *tpsRelw, relative warps analysis, Version 1.36*. Stony Brook, NY: Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf F. 2005. *TpsDig, digitize landmarks and outlines, Version 2.05*. Stony Brook, NY: Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf FJ, Slice D. 1990. Extensions of the procrustes method for the optimal superimposition of landmarks. *Systematic Zoology* 39: 40–59.

Rundle HD, Nosil P. 2005. Ecological speciation. *Ecology Letters* 8: 336–352.

Sandoval CP, Nosil P. 2005. Counteracting selective regimes and host preference evolution in ecotypes of two species of walking-sticks. *Evolution* **59:** 2405–2413.

Scheiner SM, Mitchell RJ, Callahan HS. 2000. Using path analysis to measure natural selection. *Journal of Evolutionary Biology* **13:** 423–433.

Schluter D. 2000. *The ecology of adaptive radiation.* Oxford: Oxford University Press.

Sinervo B, DeNardo DF. 1996. Costs of reproduction in the wild: path analysis of natural selection and experimental tests of causation. *Evolution* **50:** 1299–1313.

Vanhooydonck B, Van Damme R, Aerts P. 2001. Speed and stamina trade-off in lacertid lizards. *Evolution* **55:** 1040–1048.

Via S, Bouck AC, Skillman S. 2000. Reproductive isolation between divergent races of pea aphids on two hosts. II. Selection against migrants and hybrids in the parental environments. *Evolution* **54:** 1626–1637.

Videler JJ. 1993. *Fish swimming.* London: Chapman & Hall.

Vogel S. 1994. *Life in moving fluids.* Princeton, NJ: Princeton University Press.

Walker JA, Ghalambor CK, Griset OL, McKenney D, Reznick DN. 2005. Do faster starts increase the probability of evading predators? *Functional Ecology* **19:** 808–815.

Webb PW. 1984. Body form, locomotion, and foraging in aquatic vertebrates. *American Zoologist* **24:** 107–120.

Webb PW. 1986. Effect of body form and response threshold on the vulnerability of 4 species of Teleost prey attacked by Largemouth Bass (*Micropterus salmoides*). *Canadian Journal of Fisheries and Aquatic Sciences* **43:** 763–771.

Wesner JS, Billman EJ, Meier A, Belk MC. 2011. Morphological convergence during pregnancy among predator and nonpredator populations of the livebearing fish *Brachyrhaphis rhabdophora* (Teleostei: Poeciliidae). *Biological Journal of the Linnean Society* **104:** 386–392.

Wilson RS, James RS, Van Damme R. 2002. Trade-offs between speed and endurance in the frog *Xenopus laevis*: a multi-level approach. *Journal of Experimental Biology* **205:** 1145–1152.

Yan GJ, He XK, Cao ZD, Fu SJ. 2012. The trade-off between steady and unsteady swimming performance in six cyprinids at two temperatures. *Journal of Thermal Biology* **37:** 424–431.

Yan GJ, He XK, Cao ZD, Fu SJ. 2013. An interspecific comparison between morphology and swimming performance in cyprinids. *Journal of Evolutionary Biology* **26:** 1802–1815.

Young PS, Cech JJ. 1993. Improved growth, swimming performance, and muscular development in exercise-conditioned young-of-the-year striped bass (*Morone saxatilis*). *Canadian Journal of Fisheries and Aquatic Sciences* **50:** 703–707.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1. Geometric morphometric landmark locations.
Appendix S2. Path model comparisons.
Appendix S3. Thin plate spline deformation grids representing body shape for populations used in the present study.
Appendix S4. Pairwise path comparisons.
Appendix S5. Indirect path estimates.