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SYMPOSIUM

Predicting Evolution with Generalized Models of Divergent Selection: A Case Study with Poeciliid Fish

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Synopsis Over the past century and half since the process of natural selection was first described, one enduring question has captivated many, "how predictable is evolution?" Because natural selection comprises deterministic components, the course of evolution may exhibit some level of predictability across organismal groups. Here, I provide an early appraisal of the utility of one particular approach to understanding the predictability of evolution: generalized models of divergent selection (GMDS). The GMDS approach is meant to provide a unifying framework for the science of evolutionary prediction, offering a means of better understanding the causes and consequences of phenotypic and genetic evolution. I describe and test a GMDS centered on the evolution of body shape, size of the gonopodium (sperm-transfer organ), steady-swimming abilities, fast-start swimming performance, and reproductive isolation between populations in *Gambusia* fishes (Family Poeciliidae). The GMDS produced some accurate evolutionary predictions in *Gambusia*, identifying variation in intensity of predation by piscivorous fish as a major factor driving repeatable and predictable phenotypic divergence, and apparently playing a key role in promoting ecological speciation. Moreover, the model's applicability seems quite general, as patterns of differentiation in body shape between predator regimes in many disparate fishes match the model's predictions. The fact that such a simple model could yield accurate evolutionary predictions in distantly related fishes inhabiting different geographic regions and types of habitat, and experiencing different predator species, suggests that the model pinpointed a causal factor underlying major, shared patterns of diversification. The GMDS approach appears to represent a promising method of addressing the predictability of evolution and identifying environmental factors responsible for driving major patterns of replicated evolution.

Introduction

Evolutionary biology has a wide reputation as a purely historical, descriptive science, in which scientists seek to uncover stories of the past. In reality, modern evolutionary science can be both experimental and predictive (Herre 1987; Schluter 1994; Reznick et al. 1997; Rainey and Travisano 1998; Losos et al. 2006; Meyer and Kassen 2007; Blount et al. 2008; Langerhans 2008; Langerhans and Reznick 2009; Stern and Orgogozo 2009), encompassing a broad range of investigative approaches to understanding life's ever-changing forms. Ever since the process of natural selection was originally described by Charles Darwin and Alfred Russell Wallace, one question has captivated the minds of many: "how predictable is evolution?" This is because natural selection inherently comprises deterministic components, and can yield apparently "well-designed" organismal traits. This fact opens the possibility for some level of predictability in the course of evolution but just how accurately might we predict evolutionary change? Some have argued for a highly stochastic, unpredictable course for evolution (Mani and Clarke 1990; Cadle and Greene 1993; Price et al. 2000; Gould 2002), while others have argued for highly deterministic, predictable evolutionary trajectories (Robinson and Wilson 1994; Conway

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Morris 1998; Losos et al. 1998; Schluter 2000; Wood et al. 2005; Hoekstra 2006). The truth probably lies somewhere between these extremes (Winemiller 1991; Travisano et al. 1995; Huey et al. 2000; Langerhans and DeWitt 2004; Langerhans et al. 2006; Ozgo and Kinnison 2008; Riopel et al. 2008; Langerhans and Makowicz 2009).

Here, I evaluate the utility of one particular approach to understanding the predictability of evolution: generalized models of divergent selection (GMDS). The approach is aimed toward accomplishing several goals: (1) assess the predictability and peculiarity of phenotypic change, (2) identify particular environmental gradients responsible for driving major evolutionary patterns within certain groups of organisms, (3) determine the role of predictable evolution in driving speciation, (4) uncover the genetic basis of phenotypic change and the predictability of genetic evolution, and (5) reveal the generality of these findings across multiple taxa. The present study is meant to provide one of the first direct appraisals of the GMDS approach, building a generalized model and testing the model's assumptions, predictions, implications for speciation, and generality.

Predicting evolution with GMDS

Today, the science of evolutionary prediction is still in its infancy, with a scattering of researchers addressing the topic from varied perspectives (Ferguson et al. 2003; Bull and Molineux 2008; Langerhans 2008; Stern and Orgogozo 2008; Langerhans and Reznick 2009). So far these studies have lacked any unifying conceptual framework, which would promote the creation of a strong, cohesive field of inquiry. Recently, an approach to understanding the causes and consequences of phenotypic evolution was offered in the form of testing the predictability of trait-changes using GMDS (Langerhans 2008; Langerhans and Reznick 2009). GMDS might provide a unifying framework for the study of the predictability of evolution among populations, species, and higher taxa. Using this approach, a researcher explicitly derives *a priori* predictions of phenotypic or genetic change based on a specified set of assumptions for a particular system (e.g., see Table 1 in Langerhans 2008), and then tests the predictions using comparative and/or experimental data. The fundamental logic underlying the approach is that if we sufficiently understand the nature of selection acting on certain traits for a given set of organisms, then we should be capable of accurately predicting the course of evolution, assuming other factors do not overpower the signal of the focal evolutionary responses (e.g., genetic drift, genetic constraints, gene flow; all of these can be incorporated into the model). Previous research has used this logic to address the predictability of evolution, with certain fields making great strides, such as life history theory, sex-ratio theory, and the evolution of feeding apparatuses in fishes (e.g., Herre 1987; Reznick et al. 1990; Roff 2002; Wainwright et al. 2007). However, prior work has not provided a unifying framework applicable to alternative disciplines for the investigation of the predictability of evolution. In this study, I expand upon previous descriptions of GMDS and use recent work on the evolutionary ecology of a group of livebearing fishes (Family Poeciliidae) to assess the utility of this proposed unifying framework in gaining a better understanding of the causes and consequences of phenotypic evolution.

Understanding the intricacies of selection acting on all organismal traits is exceedingly challenging. Building a fully parameterized model that attempts to describe such an enormous amount of detail will almost certainly fail to provide much utility in understanding the predictability of evolution, identifying the primary selective agents driving evolutionary change, or yielding much generality beyond the particular system being modeled (see Fig. 1A for a hypothetical example). A more useful approach is to construct relatively simple models designed to capture major patterns of evolution for a broad set of organisms. When employing the GMDS approach, an investigator first builds a simplified, mechanistic model comprising a reduced version of the fully parameterized model mentioned above (see Fig. 1B and C for examples). This model describes how a system is believed to function (e.g., how morphology mediates performance, and how performance mediates fitness), and necessarily encompasses the critical hypothesis of divergent selection, which yields evolutionary predictions testable with comparative or experimental data. Divergent selection describes the situation whereby selection acts in contrasting directions across environments—either within a population (the special case of disruptive selection) or between populations (Rundle and Nosil 2005). Thus, at its core, the model contains hypotheses of natural/sexual selection pulling trait-values in different directions in different environments, either in the wild or in the laboratory (e.g., intensity of predation or habitat use in the wild; resource treatment or gene knockouts in the laboratory). Such divergent selection is widely believed to represent a major driver of phenotypic and lineage diversity across the planet (Endler 1977; Rice and Hostert 1993; Robinson and Wilson 1994; Schluter 2000; Thompson 2005;

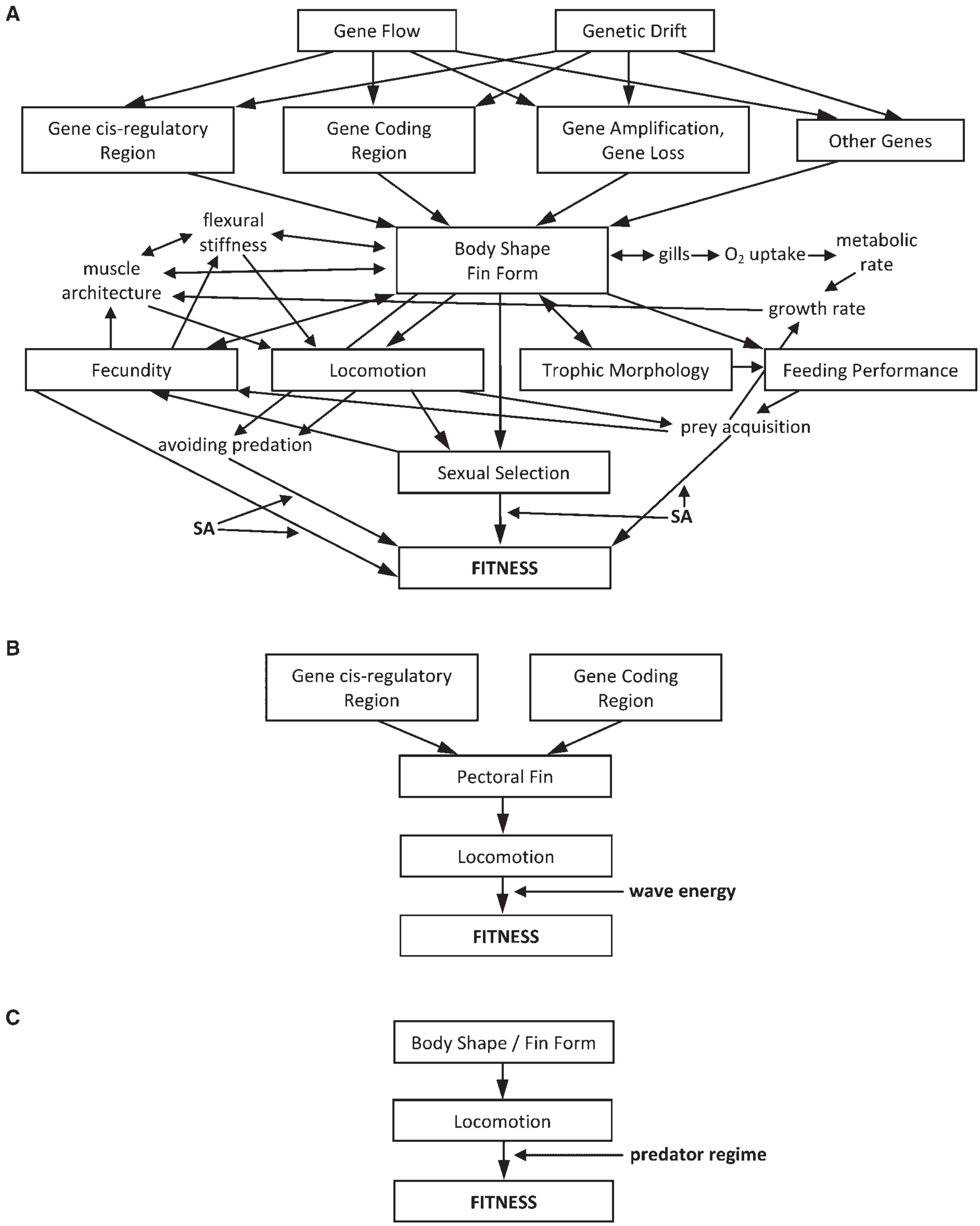

Fig. 1 Three alternative models for predicting the evolution of fish morphology. (A) A highly parameterized model, attempting to capture virtually all potential pathways involved in the evolution of body shape and fin form in fish (SA = selective agents). (B) A generalized model of divergent selection (GMDS) predicting the evolution of pectoral-fin morphology and its genetic basis, ultimately based on a hypothesis of divergent selection on locomotor abilities across wave-energy environments (e.g., Wainwright et al. 2002; Walker and Westneat 2002; Fulton et al. 2005). Predictions derived from this model include a higher pectoral-fin aspect ratio in environments with greater wave energy, largely caused by *cis*-regulatory changes rather than by changes in the coding regions of the primary underlying genes. (C) A GMDS predicting the evolution of fish morphology ultimately based on a hypothesis of divergent selection on locomotor abilities across predator regimes (e.g., presence versus absence of predators). Predictions derived from this model include larger mid-body/caudal regions and greater capability for acceleration in the presence of predators and more streamlined bodies and greater swimming endurance in the absence of predators (e.g., see Langerhans and Reznick 2009).

Langerhans and Reznick 2009). Hypotheses of divergent selection lead to predictions for the course of evolution in alternative environments. The ideal GMDS is simple, applies to multiple groups of organisms, and makes clear, testable evolutionary predictions of phenotypic/genetic divergence between multiple, commonly encountered types of habitat.

Generalized models can be built from the understanding of genetics, biochemistry, development, biomechanics, behavior, and ecology. The model should ideally be grounded in a quantitative framework such that a conceptual visualization of the model (Fig. 1B and C) can also be expressed in mathematical terms (e.g., see Fig. 1 in Langerhans 2008). Often, GMDS will be based on the Lande equation (Lande 1979; Lande and Arnold 1983), the functional-constraints equation (Ghalambor et al. 2003; Walker 2007), and non-linear extensions of these equations. Placing the models in a mathematical and theoretical framework can aid in identifying regions of parameter space that yield particular evolutionary predictions (see Langerhans 2008), determining optimal phenotypic values for maximizing certain performance traits, or parameterizing the model with empirical data that refines assumptions and predictions.

Predictions generated with GMDS will typically take the form of probabilistic predictions, rather than precise evolutionary endpoints. For example, common predictions might comprise expected phenotypic trajectories across environments (e.g., larger body size in Environment A, smaller body size in Environment B), or the relative probability of *cis*-regulatory versus coding mutations observed in particular genes. However, predictions could also be highly specific, such as predictions of particular trait-values (e.g., based on empirically-derived fitness surfaces or maximizing certain functions) or particular changes in certain gene regions (e.g., missense change in a specified gene). Importantly, GMDS can apply to various "scales" of enquiry, such as within-population changes (e.g., before and after treatments, sexual dimorphism, resource polymorphism), between-population divergence (e.g., different selective regimes across conspecific populations), differences among species (e.g., testing evolutionary patterns in a phylogenetic context), and patterns at higher taxonomic levels (e.g., differences in speciation rates or morphological disparity among clades). The form of predictions will depend on the goals and state of knowledge of the researcher. In addition to the generation of testable evolutionary predictions, GMDS can be further used to test the role of predictable evolution in driving additional processes/patterns—such as speciation and population demographics—and assess the generality of models' predictions by performing tests in multiple organismal groups.

In some sense, researchers have been employing this approach for a number of years. That is, many investigators have generated and tested hypotheses of differential adaptation across environments. However, the GMDS approach offers a number of advantages over many previous studies by simultaneously offering (1) a quantitative foundation underlying predictions, (2) explicit descriptions of evolutionary predictions as well as their reasoning and support, (3) an emphasis on generalizability and multidisciplinary integration, and most importantly (4) a common framework and terminology for the investigation of the predictability of evolution. This latter point is a critical advance of the GMDS approach, providing a unified framework for researchers in different disciplines addressing similar questions.

Testing the utility of GMDS: a case study

Poeciliid fishes provide a useful model system for assessing the value of the GMDS approach. These fish inhabit a wide breadth of habitats, exhibit considerable variation in the form and intensity of sexual selection, possess substantial phenotypic diversity, are amenable to laboratory experimentation, contain many well-resolved phylogenetic relationships, and a wealth of genetic, developmental, functional, behavioral, ecological, and evolutionary knowledge exists for the group (reviewed by Meffe and Snelson 1989; Houde 1997; Magurran 2005; Evans et al. 2011). Here, I will use recent work investigating phenotypic divergence between low- and high-predation environments in fish of the genus *Gambusia* (mosquitofishes) to illustrate how the GMDS approach might be used to better understand the causes and consequences of phenotypic evolution.

First, we must develop a generalized model. In this case, the model is based on an hypothesis of divergent selection between predator regimes. Throughout this article, predator regimes refer to two alternative types of environments: those in which predatory fish are absent or in low densities (low predation) and those in which piscivorous fish are present or in high densities (high predation). The model is designed to strengthen our understanding of the evolution of body shape, size of the gonopodium (modified anal fin used by male poeciliids for transfer of sperm), locomotor abilities, and reproductive isolation between populations. Representing an extension of the model depicted in Fig. 1C, the model examined in this study is illustrated in Fig. 2. The model is

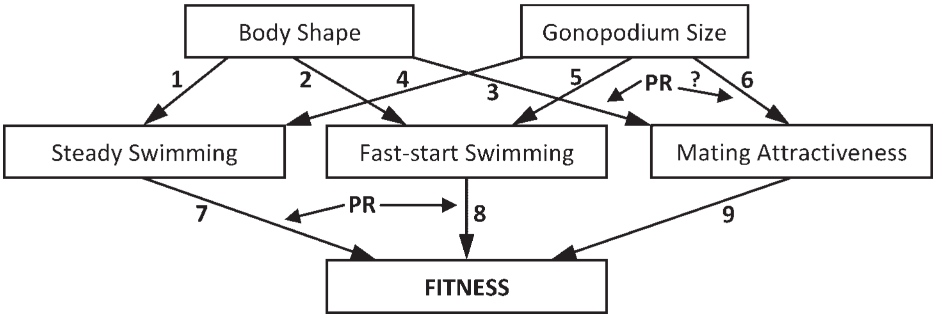

Fig. 2 Conceptual illustration of the generalized model of divergent selection examined in this study. Each path is numbered for easy reference in the text and Table 1. PR = predator regime.

Table 1 Assumptions of the generalized model of divergent selection examined here for *Gambusia* fishes (Assumptions 1–9 correspond to path numbers 1–9 in Fig. 2)

Model's assumptions	
1	Steady-swimming performance enhanced by greater streamlining of the body
2	Fast-starts enhanced by larger mid-body/caudal region, smaller anterior body/head region
3	Females prefer to mate with males having a more adaptive body shape, given their predatory environment (i.e., more streamlined body in absence of predators, larger caudal region in presence of predators)
4	Steady-swimming performance affected in unknown manner by gonopodium size
5	Fast-starts enhanced by smaller gonopodia
6	Females prefer to mate with males having a larger gonopodium, potentially dependent on predator regime
7	In the absence of predators, natural selection strongly favors steady-swimming performance, but does not do so in the presence of predators
8	In the presence of predators, natural selection strongly favors fast-start escape performance, but does not do so in the absence of predators
9	Sexual selection favors males possessing greater attractiveness to females
10	Genetic constraints do not preclude appropriate evolutionary responses to selection
11	All relevant factors are included in the model (e.g., relatively minimal importance of other traits; alternative agents of selection; gene flow)

Table 2 Predictions of the generalized model of divergent selection illustrated in Fig. 2

Trait	Model's prediction
Steady swimming	Higher under conditions of low predation
Fast-start swimming	Higher under conditions of high predation
Body shape	More streamlined under conditions of low predation; Larger mid-body/caudal region under conditions of high predation
Gonopodium size	Larger under conditions of low predation
Reproductive isolation	Greater between predator regimes than within

derived from *a priori* knowledge of the biomechanics of fish locomotion, ecological consequences of locomotor abilities in many fishes, and past empirical evidence for sexual selection. All assumptions are listed in Table 1. The model can be quantified using a combination of the Lande equation and the functional-constraints equation, as illustrated by Langerhans (2008; his Fig. 1). Components of the model were specifically selected to focus on traits and environmental factors that should capture major evolutionary patterns in this group of fish. The model predicts evolutionary divergence in locomotor performance, body shape, and gonopodium size between predator regimes, with implications for the evolution of reproductive isolation (i.e., ecological speciation; Table 2). Here, I will test this model's assumptions, evolutionary predictions, implications for speciation, and generality, using poeciliid fish in the genus *Gambusia* as the model system. I focus on intraspecific variation, rather than variation among species, in an effort to more directly assess cause-and-effect regarding the model's assumptions and predictions, since comparisons among conspecific individuals should harbor fewer potentially confounding variables (e.g., less divergence in non-focal traits) than does comparisons among species (Harvey and Pagel 1991; Rose and Lauder 1996).

The model's background

The elements of the model are based on an extensive foundation of prior theoretical and empirical work (Wu 1971; Lighthill 1975; Webb 1975, 1983, 1984, 1986a, 1986b; Blake 1983; Weihs 1989; Katzir and Camhi 1993; Videler 1993; Vogel 1994; Walker

1997; Plaut 2001; Domenici 2003; Ghalambor et al. 2003; Blake 2004; Langerhans and DeWitt 2004; Langerhans et al. 2004, 2005, 2007; Walker et al. 2005; McHenry and Lauder 2006; Fisher and Hogan 2007; Domenici et al. 2008; Tytell and Lauder 2008; Domenici 2009; Langerhans 2009a, 2009b, 2010; Langerhans and Makowicz 2009; Langerhans and Reznick 2009). These previous studies should be consulted for details underpinning the model's construction. The core of the model is the hypothesis of divergent selection on locomotor performance across predator regimes. This hypothesis derives from (1) a functional trade-off, in which fish cannot simultaneously optimize both types of swimming modes, and (2) a shift in the balance of selection on these swimming capabilities across predatory environments (see below). The model also includes one major way that sexual selection might act on traits having important consequences for locomotor abilities: females' preferences for body shape and gonopodium size of males. The inclusion of these mating preferences—and their possible dependence on context—emphasizes the varied functions that morphological traits can serve, as well as the integrated nature of adaptive evolution; it also provides a direct assessment of how evolutionary changes in phenotypes might affect sexual isolation among populations.

Here, I provide a brief description of the hypothesis of divergent selection included in the model. Natural selection is expected to favor steady-swimming performance in low-predation environments thereby increasing competitive abilities, but favor fast-start swimming performance in high-predation environments thereby increasing survival during predatory encounters. Steady swimming (cruising) describes constant-speed locomotion in a straight line, and is commonly employed in nature during competition for limited resources, such as searching for food (which is often patchily distributed), obtaining mates (sometimes involving long courtship chases), and seeking favorable abiotic conditions (e.g., maintaining preferred environmental temperature). Because steady-swimming activities are often of critical importance, natural selection will often favor various means of reducing the energetic cost of movement. Fast-start swimming refers to a more complicated locomotor pattern involving rapid acceleration, and is commonly employed in the wild in predator–prey interactions (i.e., capturing evasive prey and evading predatory strikes) and social interactions (e.g., courtship, antagonistic interactions). Here, I focus on an escape response initiated by Mauthner-cells. It is present in most fish and is called a C-start. During this fast-start, the fish's body rapidly bends into a ''C'' shape and then produces a propulsive stroke of the caudal region in the opposite direction, resulting in a sudden, high-energy burst of swimming. Because these two swimming modes are optimized with different propulsor arrangements, and because many fish employ a mechanically coupled locomotor system—e.g., propulsion by the body and caudal fin both for steady-swimming and for fast-start swimming—fish generally cannot exhibit high levels of performance in both steady-swimming and fast-start swimming. Based on the functional morphology of fish locomotion, steady-swimming performance should often be enhanced by more streamlined bodies (fusiform shape; deep/wide anterior body, tapering to a shallow/narrow caudal peduncle), while fast-start performance should often be enhanced by posteriorly large bodies (small anterior body/head region, large mid-body/caudal region); these morphological features are expected to increase thrust and stability while minimizing drag and recoil energy losses during different swimming activities. Additionally, since gonopodia unlikely contribute useful thrust, but instead contribute to drag, smaller gonopodia are generally expected to enhance both steady swimming and fast-start swimming. However, because longer gonopodia might actually delay the separation of the boundary layer during steady swimming, it is possible that gonopodium size could positively influence steady-swimming. Thus, it is currently unclear how gonopodium size might affect steady-swimming performance.

In the next sections, I evaluate the empirical evidence from *Gambusia* for the assumptions of the model described in Table 1 (with the exceptions of Assumptions 9–11). These latter assumptions are simply assumed to be true for the sake of generality. First, the assumption that males with greater mating attractiveness to females actually experience higher fitness (e.g., greater success in mating and achieving fertilization; production of more offspring) has not yet been directly examined in *Gambusia*. Considering these, fish exhibit high frequencies of sneak matings, the relationship between attractiveness and fitness may not be a simple one, although a positive association is probably a safe assumption. Second, there is no reason to believe that genetic constraints (e.g., additive genetic variances and covariances of traits) would be of a form sufficient to preclude significant evolutionary responses in the directions predicted by the hypothesized selection. Finally, Assumption 11 is unlikely to be satisfied in this case or in any other natural system—most populations are open, complex systems (e.g., gene flow may occur; multiple selective agents may act on numerous traits)—however, it does serve as a simplifying assumption of *ceteris paribus*. For the model to serve its purpose well and to provide evolutionary predictions with accuracy and wide applicability, it should prove robust to such violations.

Assumptions about biomechanics: morphology → locomotion

I begin testing the model's assumptions by examining assumptions linking body shape and gonopodium size to locomotor abilities (Assumptions/Paths 1, 2, 4 and 5 in Table 1, Fig. 2). For Assumption 1, recent work demonstrated that fish possessing more streamlined bodies exhibit greater steady-swimming performance (e.g., use less hydromechanical power for the same speed of swimming) and increased endurance in Western mosquitofish (*G. affinis*) (Langerhans 2006, 2009b; Langerhans and Reznick 2009; Fig. 3A). For Assumption 2, experimental studies have revealed that within two *Gambusia* species, fish with larger mid-body/caudal regions and smaller anterior body/head regions exhibit greater fast-start performance (higher burst velocity, greater maximum acceleration, and greater rotational velocity during Stage 1 of the C-start) (Langerhans 2009a; Langerhans et al. 2004; Fig. 3B). Further, recent work using digital-particle image velocimetry (DPIV) has elucidated the primary body region responsible for producing thrust during a C-start in the Bahamas mosquitofish (*G. hubbsi*): the mid-body/caudal region of the fish (Fig. 3C). Thus, both assumptions regarding the influence of body

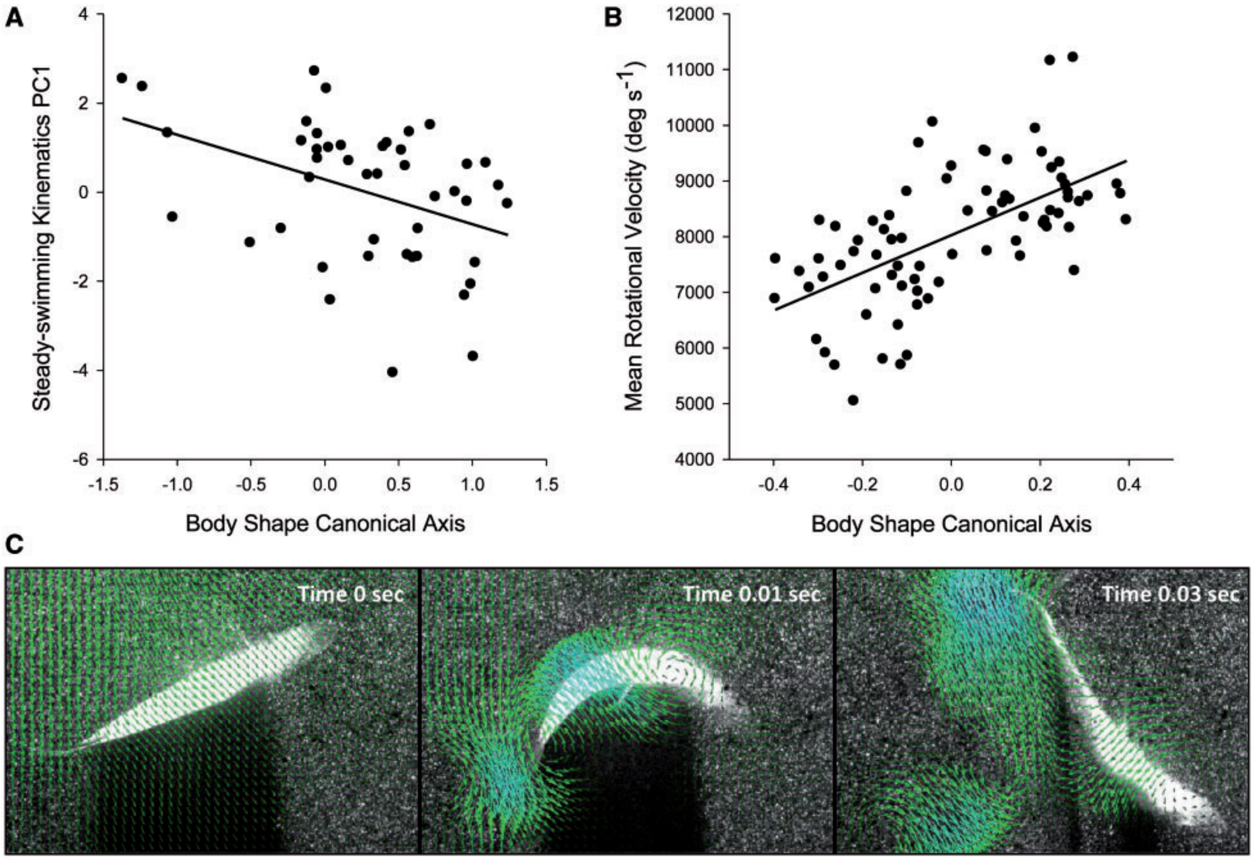

Fig. 3 Relationship between body shape and locomotor performance in *Gambusia*. (A) In Western mosquitofish (*G. affinis*), fish with larger caudal regions and smaller heads (positive end of the *x*-axis) exhibit reduced steady-swimming performance as evidenced by lower propulsive wave speeds and greater rostral and tail-beat amplitudes (negative end of *y*-axis) while swimming at the same speed (data from Langerhans 2009b; $n = 45$, $P = 0.006$). (B) In Bahamas mosquitofish (*G. hubbsi*), fish with larger caudal regions and smaller heads (positive end of the *x*-axis) exhibit greater escape performance as measured by rotational velocity of the body during Stage 1 of the C-start (data from Langerhans 2009a; $n = 77$, $P < 0.0001$). (C) Three video frames (1000 Hz) of the ventral view of a Bahamas mosquitofish (*G. hubbsi*) performing a C-start, with vectors of water velocity depicted using DPIV (data from R.B. Langerhans, G.V. Lauder, unpublished data). Note the large jet of water moving upward in Frames 2–3, which propels the fish toward the bottom-right region of the frame. This jet is largely produced by the mid-body/caudal region of the fish.

shape on locomotor performance possess significant empirical support.

For the effects of gonopodium size on locomotor performance, only Assumption 5 comprised a directional hypothesis. For Assumption 4, *a priori* predictions for how gonopodium size might influence steady-swimming are uncertain—knowledge of the biomechanics of fish locomotion suggests that both positive and negative effects are possible (Anderson et al. 2001; Fish and Lauder 2006). Specifically, while a greater surface area of the gonopodium might increase drag, a larger gonopodium could reduce energetic costs of steady swimming by delaying the separation of the boundary layer, especially if males depress the gonopodium to the ventral surface of the body. Unfortunately, no prior study has directly examined the link between gonopodium size and steady-swimming performance, although Basolo and Alcaraz (2003) showed that larger swords—elongate extensions on the ventral part of the caudal fin, which are superficially similar to gonopodia—do incur energetic costs. However, recent results from a swim-tunnel experiment with *G. affinis* suggests that males with relatively larger gonopodia actually tend to exhibit higher endurance (time before fatigue) during steady swimming (R.B. Langerhans, unpublished data). Clearly, the relationship between gonopodium size and steady-swimming performance needs further investigation. For Assumption 5, Langerhans et al. (2005) found that populations of *G. affinis* possessing larger gonopodia (relative to body size) exhibit lower burst-swimming speeds. Moreover, in high-speed videos ($\geq$500 Hz) of C-starts in *Gambusia*, the gonopodium can often be seen flailing about, likely contributing to elevated drag (R.B. Langerhans, personal observation). Recent work has also found that in both *G. affinis* and *G. hubbsi*, males with longer gonopodia (relative to body length) experience lower survivorship in the presence of a predatory fish (Langerhans 2010; A.M. Makowicz and R.B. Langerhans, unpublished data), suggesting that the gonopodium's effects on escape performance may have reduced survival. Although some empirical support exists for Assumption 5, further research is needed to gain a better understanding of the relationship between gonopodium size and locomotor abilities.

Assumptions about ecology: locomotion → fitness

Two of the most critical assumptions of the model describe a shift in the balance of natural selection across predator regimes (assumptions/paths 7 and 8 in Table 1, Fig. 2). The assumptions are relatively straightforward, but yet have proven difficult to test. Ideally, large-scale studies would measure the strength of natural selection on both steady-swimming performance and fast-start swimming performance for *Gambusia* in the wild in both low- and high-predation environments. However, the measurement of locomotor abilities is a demanding feat in the laboratory, much less in the field. Moreover, how one might measure fitness in low-predation environments for such a study is less than obvious due to the low levels of mortality (e.g., may require genetic tracking of parentage). Because of these logistical difficulties, such studies in the wild have not yet occurred. Yet, to properly assess these assumptions, rigorous tests in the field must overcome these obstacles. To date, these assumptions have been tested experimentally only in the laboratory or in large mesocosms.

For Assumption 7, experimental work has shown that *G. affinis* with greater steady-swimming performance (i.e., those generating more efficient thrust as quantified by a suite of swimming kinematics measured with high-speed video) have greater endurance (Langerhans 2009b). That is, fish exhibiting lower consumption of hydromechanical power during steady swimming can swim at a given speed for a longer period of time before fatiguing. Because endurance should provide a useful surrogate for fitness in the high-competition scenarios characteristic of low-predation environments (fish continually cruise through the water in these high-density environments in search of food, mates, favorable abiotic conditions), this finding suggests that selection in the absence of predators favors increased steady-swimming performance. For Assumption 8, a mesocosm experiment involving *G. hubbsi* found strong selection for increased fast-start performance (mean rotational velocity, maximum acceleration) in the presence of a predatory fish (Langerhans 2009a). In this experiment, fitness was measured as survival in the presence of a piscivorous fish, which likely provides a useful surrogate for selection in high-predation environments. Thus, this finding suggests that selection probably favors enhanced fast-start abilities in the presence of predators. Further, indirect evidence both from *G. hubbsi* and *G. affinis* also supports this conclusion, as selection was found to favor fish with larger mid-body/caudal regions and smaller anterior body/head regions in mesocosms with predatory fish (Langerhans 2009a; A.M. Makowicz and R.B. Langerhans, unpublished data; see Fig. 4). Put together, empirical evidence

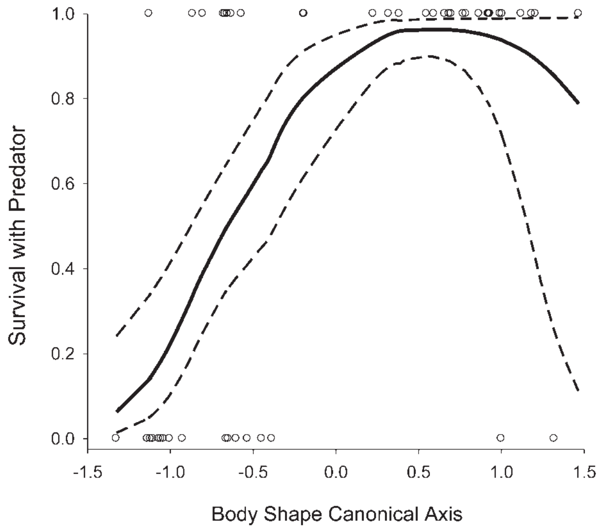

Fig. 4 Natural selection favors fish with larger mid-body/caudal regions and smaller anterior body/head regions (positive end of axis) in *Gambusia hubbsi* in the presence of a predatory fish (*Gobiomorus dormitor*). Fitness function estimated using the non-parametric cubic-spline regression technique. The solid line represents mean survival probability and the dashed lines indicate ± 1 SE of predicted values from 1000 bootstrap replicates of the fitness function. Data from Langerhans (2009a).

does support these assumptions; however, additional studies are desirable.

Assumptions about sexual selection: morphology → attractiveness to mates

Assumptions regarding putative effects of morphology on attractiveness to potential mates (hereafter abbreviated as "mating attractiveness") derive from the existence of mating preferences for larger fin size and for larger body size in some poeciliid fishes (Rosen and Tucker 1961; Bischoff et al. 1985; McPeek 1992; Basolo 1995; Endler and Houde 1995; Ptacek 1998; Gould et al. 1999; MacLaren et al. 2004) and from the hypothesis that sexual selection via mating preferences might reinforce natural selection (Assumptions/paths 3 and 6 in Table 1, Fig. 2). Assumption 3 is based on little *a priori* theoretical knowledge other than the fact that honest indicators of fitness are sometimes utilized in choosing mates (Kodric-Brown and Brown 1984; Grafen 1990; Johnstone 1995; Kokko et al. 2006). Yet, experimental work has so far provided support for the assumption. Langerhans et al. (2007) used video-playbacks to demonstrate that female *G. hubbsi* prefer males from their own populations over those from foreign populations, and that this preference appears at least partially based on differences in males' body shapes between populations. Recently, a mate-choice experiment was conducted using within-population variation in males' body shapes, and found that female *G. hubbsi* prefer males having a more "adaptive" body shape in terms of their particular predator regime (A.M. Makowicz and R.B. Langerhans, unpublished data). That is, using graphic animations (to control for all traits other than body shape), it was found that females within three low-predation populations preferred males having a smaller mid-body/caudal region and a larger anterior body / head region, while females from two high-predation populations exhibited the opposite preference. For Assumption 6, it seems plausible that females might exhibit mating preferences based partially on gonopodium size since males sometimes extend or abduct their gonopodium during mating displays, gonopodia sometimes exhibit distinctive coloration (e.g., black speckling or blue iridescence; orange pigmentation on rays 6–10), and some poeciliids are known to exhibit mating preferences for larger fins (Rosen and Gordon 1953; Rosen and Tucker 1961; Bischoff et al. 1985; Hughes 1985; Basolo 1995; Langerhans et al. 2005; Langerhans, 2010); however, whether such a preference might depend on the predatory environment, similar to preference for swords in green swordtails (*Xiphophorus helleri*) (Johnson and Basolo 2003), is unknown. To date, experimental studies have demonstrated in two *Gambusia* species that females prefer males with larger gonopodia (Langerhans et al. 2005; Kahn et al. 2010). In one study, Langerhans et al. (2005) found that female *G. affinis* from both low- and high-predation populations exhibited similar preferences, indicating that mating preferences for gonopodium size does not appear to differ between predator regimes. Thus, experimental results to date support the model's assumptions about sexual selection and suggest that females prefer mates having a more "adaptive" body shape and a larger gonopodium.

Testing the model's predictions

Now that each assumption of the model has been sufficiently considered, and most found to posses strong empirical support, the utility of the GMDS in predicting major evolutionary patterns will be evaluated. Does evolution proceed in the manner predicted by the GMDS (see Table 2)?

First, divergence in steady-swimming performance between predator regimes has so far been directly tested for one species, *G. affinis*. In this species, fish from different predator regimes produce thrust during steady swimming in distinctly different manners: fish from low-predation regimes exhibit lower rostral and tail-beat amplitudes, and higher propulsive wavelengths and wave speeds than do fish from high-predation fish while swimming at a common speed (Langerhans 2009b). These differences in steady-swimming kinematics result in more efficient generation of thrust (lower power consumption) and greater endurance (swimming a longer time prior to fatigue) for fish from low-predation environments. These differences were also shown to have a genetic basis, as laboratory-born fish raised in a common environment were used in experimentation. Thus, the first prediction of the GMDS has been confirmed for one species.

Second, fast-start escape performance has diverged between predator regimes in the manner predicted for the two *Gambusia* species in which it has been tested (Langerhans 2009a; Langerhans et al. 2004). Fish from high-predation environments exhibit ~20% higher burst speeds in *G. affinis* and ~42% higher maximum acceleration and ~18% higher average rotational velocity during a C-start in *G. hubbsi* compared to conspecific fish from low-predation localities. In both species, the morphological traits partially responsible for fast-start divergence have a genetic basis, but the genetic basis of fast-start performance *per se* has not yet been directly examined.

Third, divergence in body shape between predator regimes has now been tested in a number of *Gambusia* species, representing the most thoroughly tested prediction of the GMDS (Langerhans and DeWitt 2004; Langerhans et al. 2004, 2007; Langerhans 2009a, 2009b; Langerhans and Makowicz 2009; R.B. Langerhans and C.A. Layman, unpublished data). In each case, fish from high-predation environments exhibit larger caudal regions and smaller heads than do fish from low-predation populations. In two species (*G. affinis*, *G. hubbsi*), a genetic basis for the morphological differences have been confirmed (Langerhans et al. 2004; Langerhans 2009a, 2009b). Considering these species inhabit different geographic regions (e.g., Texas, Bahamas, Cayman Islands) and habitat types (e.g., freshwater and hyper-saline ponds, blue holes, tidal creeks), and experience different species of predatory fish (e.g., largemouth bass, bigmouth sleeper, great barracuda), the GMDS has yielded some accurate and robust evolutionary predictions for the evolution of body shape. Interestingly, however, not all species and sexes generated the predicted patterns through identical mechanisms. For instance, larger caudal regions in high-predation localities were sometimes achieved primarily through elongation (e.g., *G. affinis*; Langerhans et al. 2004; Langerhans 2009b) and other times mainly through deepening (e.g., *G. hubbsi*; Langerhans et al. 2007; Langerhans 2009a). While both sexes typically exhibited similar morphological trends across predator regimes, males and females of *G. caymanensis* responded to predation pressure in unique ways: (1) smaller heads in high-predation localities were achieved by a decrease in depth of the head in males, but a shorter head length in females, and (2) larger caudal regions in high-predation sites were realized by a lengthening and deepening of the caudal peduncle in males, but only a deepening in females (Langerhans and Makowicz 2009). Overall, these findings provide strong empirical support for the evolutionary predictions of divergence of body shape between fish from different predatory environments, and also suggest that multiple routes to similar adaptive peaks exist—functional convergence through multiple anatomical pathways.

Fourth, gonopodium size has been shown to exhibit the predicted pattern of divergence in *G. affinis* and *G. hubbsi*; fish in low-predation populations exhibit larger gonopodia than fish at high-predation sites (Langerhans et al. 2005). Moreover, a genetic basis for divergence in gonopodium size has been demonstrated in *G. affinis* (Langerhans et al. 2005). However, no consistent differences in gonopodium size between predator regimes were observed in *G. caymanensis* (R.B. Langerhans and A.M. Makowicz, unpublished data). Of all the predictions derived from the GMDS, this one is the most tenuous due to our incomplete understanding of how gonopodium size influences steady-swimming performance. So far, results match predictions for two species, but exhibit no clear pattern in a third species—this prediction requires further investigation.

Finally, the model predicts that divergence of traits across predator regimes should lead to ecological speciation. Ecological speciation describes the process whereby barriers to gene flow evolve between populations due to ecologically-based divergent selection (Rundle and Nosil 2005). For *Gambusia*, multiple lines of evidence provide support for this prediction. First, divergent phenotypes across predator regimes are predicted to result in reduced fitness of immigrants across predatory habitats, effectively reducing gene flow between areas with different predator regimes (but not affecting gene flow within a regime). That is, empirical studies indicate that migrants between predator regimes would suffer reduced endurance in low-predation environments and reduced survival in high-predation localities (Langerhans 2009a, 2009b). Thus, local adaptation *per se* should reduce gene flow among populations from divergent habitats if migration is non-zero—this has not yet been directly tested. Second, mate-choice experiments have revealed that prezygotic sexual isolation appears stronger between predator regimes than within predator regimes. When given the choice between mates from different populations (within *G. hubbsi*) or from different species (four species of *Gambusia*), sexual isolation is consistently stronger between populations/species from different predator regimes than between populations/species from the same predator regime (Langerhans et al. 2007). These findings suggest that traits under divergent selection are involved in mating preferences, a contention supported by recent work on within-population mating preferences for body shape (A.M. Makowicz and R.B. Langerhans, unpublished data). In contrast, mating preferences for gonopodium size may not facilitate ecological speciation: while immigrants from high-predation sites should suffer reduced mating attractiveness in low-predation environments due to smaller gonopodia, immigrants from low-predation populations to high-predation localities might actually exhibit greater attractiveness to potential mates than would native males due to the larger gonopodia of the former. However, preferences for body shape might overwhelm preferences for gonopodium size, or natural selection against a large gonopodium due to its negative consequences for fast-start performance could negate its putatively positive effects on mating attractiveness. Mate-choice tests evaluating the independent effects of gonopodium size and body shape have not yet been performed. In summary, there is significant evidence that predictable evolutionary changes have played important roles in the process of ecological speciation in *Gambusia*.

Generality of the model

The GMDS examined here has proven quite useful in predicting major patterns of phenotypic evolution in *Gambusia*, and appears to encapsulate some of the causes of speciation in this group of fish—but how general are these patterns? Can this GMDS help us understand the causes and consequences of the evolution of traits in other groups of fish? To capture major agents of evolutionary diversification, and identify common forms of divergent selection, the GMDS would ideally be highly robust to violations of assumptions and yield accurate predictions in a range of organisms. That is, an ideal GMDS would be explicitly constructed in such a general manner that the model might be usefully applied to diverse organismal groups not originally considered in the creation of the model. Because the present model included a trait not present in most fish other than livebearers (gonopodium), which component of the model cannot exhibit a great degree of generality. However, all other aspects of the model might apply to many fishes across the planet. The model examined here was only recently described, and thus the number of relevant tests outside the original system is not exceedingly high. Fortunately, there is one exception—a reasonable number of studies have examined intraspecific variation in body shape across predator regimes, spanning a diverse set of fishes. To date, however, no study other than those of *Gambusia* has investigated the direct links between the model's phenotypic predictions and the evolution of reproductive isolation. Therefore, an assessment of the generality of the model's implications for ecological speciation cannot yet be made. I will assess the existing evidence for each of the model's phenotypic predictions in non-*Gambusia* fishes, with a particular emphasis on variation in body shape.

First, tests of locomotor differences in fish inhabiting divergent predator regimes are exceedingly scarce. Other than *Gambusia*, I know of only four species that have been examined. In Trinidadian guppies (*Poecilia reticulata*), Hart's rivulus (*Rivulus hartii*), crucian carp (*Carassius carassius*), and three-spine stickleback (*Gasterosteus aculeatus*), populations in high-predation environments have been shown to exhibit higher fast-start escape performance (e.g., maximum velocity, maximum acceleration, rotational velocity) than conspecifics in low-predation environments (Huntingford et al. 1994; Ghalambor et al. 2004; Domenici et al. 2008; C.E. Oufiero, M.R. Walsh, D.N. Reznick, T. Garland, unpublished data). However, Chappell and Odell (2004) found that burst speed did not differ among predator regimes in *P. reticulata*. In *R. hartii*, fish from high-predation regimes are additionally known to suffer reduced steady-swimming abilities (critical swimming speed) (C.E. Oufiero, M.R. Walsh, D.N. Reznick, T. Garland, unpublished data). Thus, five of six tests of locomotor differences in non-*Gambusia* fishes provide support for the predictions of the GMDS. Given the paucity of studies to date, more work is clearly needed.

For gonopodium size, some poeciliids other than *Gambusia* are known to exhibit differences between predator regimes. However, regarding the predicted nature of differences results so far are mixed, as some researchers have found larger gonopodia in high-predation environments (*P. reticulata*: Cheng 2004), while others have found the opposite pattern (*P. reticulata*: Kelly et al. 2000; *Brachyrhaphis episcopi*: Jennions and Kelly 2002). More tests are necessary to better understand the factors driving the evolution of gonopodium size.

Differences in body shape between predator regimes have been investigated in many fishes, and similar patterns of differentiation of body shape have now been uncovered in a number of distantly related groups (Table 3 and Fig. 5). A review of the literature reveals that existing empirical data provide strong support for the GMDS prediction of divergence in body shape divergence between animals from different predator regimes. In the vast majority of cases, there is evidence that fish inhabiting environments with greater risk of predation from piscivorous fish tend to have larger mid-body/caudal regions (sometimes because of larger median fins) and smaller anterior body/head regions than their conspecific counterparts in environments with lower risk of predation (Table 3). This evidence comes from disparate groups of fish, geographic regions, habitat types, and predatory fish species. Such replicated patterns of morphological divergence suggest that variation in intensity of predation by piscivorous fish can drive relatively predictable phenotypic trends in many taxa. Of course, patterns are certainly not identical in each species, with some patterns not matching the predictions, and there is variation among taxa in the particular anatomical routes taken to achieve similar outcomes (Table 3).

Several points should be mentioned regarding the trends in body shape elucidated so far. First, some prior studies were not designed as tests of the predictions examined here, and did not directly measure (or present results from) traits necessary to properly assess its correspondence to the model's predictions. To thoroughly evaluate the utility of this GMDS in improving our understanding of morphological evolution in fishes, future work should explicitly test this GMDS, directly examining traits of particular interest and evaluating key assumptions when possible. When excluding studies that did not explicitly measure the relevant body parts, the existing literature indicates strong support for the GMDS predictions for both the mid-body/caudal region (patterns from 16 of 17 species unambiguously matched predictions) and the anterior body/head region (patterns from 15 of 17 species unambiguously matched predictions) (both $P < 0.0025$, binomial test). Second, observed morphological patterns arose from some combination of genetic differentiation and phenotypic plasticity—some of the observed morphological patterns are known to have a genetic basis, some to have a large environmental component, and others to comprise both sources of variation. The particular underlying genetic/developmental basis is irrelevant for this GMDS, as its predictions of divergent body shapes might be achieved via genetic divergence, the evolution of adaptive phenotypic plasticity, or a combination of the two—such details depend on intricacies of particular systems, which were not included in the GMDS. Finally, researchers must be careful of obtaining the "right" results for the "wrong" reasons. That is, we know *a priori* that the GMDS cannot comprise a thoroughly accurate representation of the system at hand (by definition, it is supposed to be a generalized model, with numerous simplifying assumptions); this means that alternative mechanisms might additionally be capable of producing outcomes predicted by the model. Thus, studies should assess the feasibility of the GMDS in their given system, and consider whether confounding factors or alternative mechanisms should be included in the model (or statistically or experimentally controlled). For instance, the effects of environmental gradients other than predator regime (e.g., water flow, foraging regime) may require attention in some systems, especially if covariation between predator regime and these other factors exist. Such consideration may often be necessary to directly test the model's predictions, but GMDS that truly capture fundamental and primary mechanisms of diversification should be robust to many violations of the model. Multi-functionality of traits also needs to be considered, as body shape affects more performance variables than steady swimming abilities and fast-start swimming capabilities, or attractiveness to mates. For instance, a deeper mid-body may enhance a fish's ability to avoid predation by both exceeding the gape of the predator as well as improving fast-start performance (see Domenici et al. 2008). Thus, subtle revisions of the model by incorporating additional terms may be necessary in some systems; although maintaining broad generality is obviously desired.

Conclusions

Evolutionary biologists wish to understand the predictability of evolutionary change (Robinson and Wilson 1994; Travisano et al. 1995; Reznick et al. 1997; Losos et al. 1998; Huey et al. 2000; Gould 2002; Grant and Grant 2002; Langerhans and DeWitt 2004; Langerhans et al. 2006; Bull and Molineux 2008; Langerhans 2008, 2009a; Stern and Orgogozo 2008; Langerhans and Makowicz 2009; Langerhans and Reznick 2009; Stern and Orgogozo 2009). GMDS has been offered as a means of addressing the predictability of evolution and of improving our understanding of the causes and

Table 3 Differences in body shape between fishes from different predator regimes

Species	Region	Primary predators	Shape characteristic of high-predation localities/treatment	References
Family Centrarchidae				
Lepomis gibbosus	Ontario, Canada	*Sander vitreus*	Deeper body	Januszkiewicz and Robinson 2007
Family Cichlidae				
Astatotilapia velifer	Uganda	*Lates niloticus*	Longer, deeper caudal region; shallower, shorter anterior region	L.J. Chapman et al., unpublished data
Haplochromis annectidens	Uganda	*Lates niloticus*	Longer, deeper caudal region; shallower, shorter anterior region	L.J. Chapman et al., unpublished data
Paralabidochromis beadlei	Uganda	*Lates niloticus*	Longer, deeper caudal region; shallower, shorter anterior region	L.J. Chapman et al., unpublished data
Family Cyprinidae				
Carassius auratus	Domestic (Asia)	*Esox lucius*	Deeper body	Chivers et al. 2008
Carassius carassius	Scandinavia	*Esox lucius*	Deeper body, shorter anterior region	Domenici et al. 2008; Brönmark and Miner 1992; Andersson et al. 2006; Johansson and Andersson 2009; Poleo et al. 1995
Rastrineobola argentea	Uganda	*Lates niloticus*	Deeper caudal region; shallower, shorter anterior region	R.B. Langerhans et al., unpublished data
Rutilus rutilus	Sweden	*Esox lucius*	Longer caudal region; shallower anterior region	Eklöv and Jonsson 2007
Family Gasterosteidae				
Culaea inconstans	Michigan	*Salvelinus fontinalis, Esox lucius*	Deeper caudal region; shallower anterior region	Zimmerman 2007
Gasterosteus aculeatus	Alaska; Canada	*Oncorhynchus clarki*	Longer, deeper caudal region; shallower, shorter anterior region	Walker 1997; Walker and Bell 2000; Spoljaric and Reimchen 2007
Family Percidae				
Perca fluviatilis	Sweden; Finland	*Esox lucius, Sander lucioperca*	Longer, deeper caudal region; deeper, shorter anterior region[a]	Eklöv and Jonsson 2007, Kekalainen et al. 2010
Family Poeciliidae				
Brachyrhaphis rhabdophora	Costa Rica	*Parachromis dovii, Rhamdia guatemalensis*	Longer, deeper caudal region; shallower anterior region	Langerhans and DeWitt 2004
Gambusia affinis	Texas, USA	*Micropterus salmoides, Lepomis cyanellus*	Longer, deeper caudal region; shallower, shorter anterior region	Langerhans and DeWitt 2004; Langerhans et al. 2004; Langerhans 2009b
Gambusia caymanensis	Cayman Islands	*Sphyraena barracuda, Megalops atlanticus*	Longer, deeper caudal region; shallower, shorter anterior region[a]	Langerhans and Makowicz 2009
Gambusia hubbsi	Bahamas	*Gobiomorus dormitor*	Deeper caudal region; shallower, shorter anterior region	Langerhans et al. 2007; Langerhans 2009a; Langerhans and Gifford 2009
Gambusia manni	Bahamas	*Sphyraena barracuda, Strongylura* spp.	Deeper caudal region; shallower, shorter anterior region	R.B. Langerhans and C.A. Layman, unpublished data
Poecilia mexicana	Mexico	*Cichlasoma salvini, Rhamdia guatemalensis*	Deeper caudal region; shorter anterior region	Tobler et al. 2008
Poecilia reticulata	Trinidad	*Crenicichla alta*	Longer, deeper caudal region; shallower, shorter anterior region[a]	Langerhans and DeWitt 2004, Hendry et al. 2006; Burns et al. 2009
Poecilia vivipara	Brazil	*Hoplias malabaricus*	Shallower, longer caudal region; shallower anterior region	Gomes and Monteiro 2008

[a] Results varied among sexes or studies.

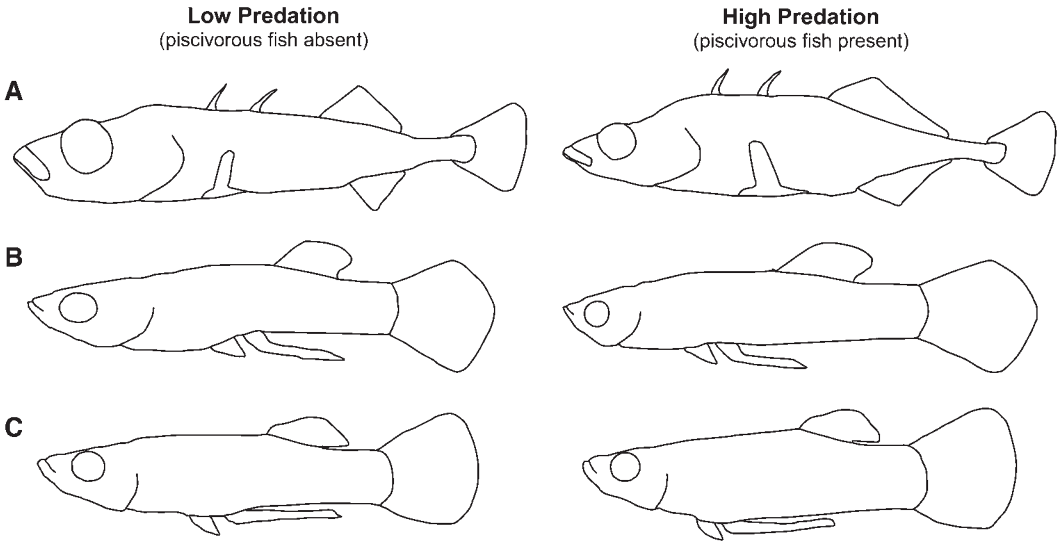

Fig. 5 Illustrated examples of morphological differences between populations inhabiting low- and high-predation environments within three fishes. Note the smaller anterior body/head region and larger mid-body caudal region in populations coexisting with predatory fish. Figures represent results of thin-plate spline deformations from the grand mean in each species, and are magnified 2 × to aid visualization. (A) Threespine stickleback, *Gasterosteus aculeatus*, from 40 lakes in Alaska, USA (data from Walker and Bell 2000). (B) Western mosquitofish, *Gambusia affinis*, from six ponds in Texas, USA (data from Langerhans et al. 2004). (C) Bahamas mosquitofish, *Gambusia hubbsi*, from 12 blue holes on Andros Island, Bahamas (data from Langerhans et al. 2007).

consequences of phenotypic and genetic change. The GMDS examined here is relatively simple, founded on a clear set of assumptions, and is integrative in nature (combining biomechanics, ecology, sexual selection, and speciation). The model performed well for *Gambusia* fishes, as empirical evidence largely supported all of its predictions. Changes in traits were quite predictable, and these changes were shown to be capable of driving the process of ecological speciation. Thus, the GMDS provided insight into the predictability of phenotypic changes and its consequences for proliferation of lineages in *Gambusia* fishes.

A key utility of the GMDS approach lies in the assessment of the generality of a simple model, helping pinpoint factors responsible for broadly predictable evolutionary patterns. When testing the model's predictions in the present case, there was strong correspondence between observations and predictions for the predicted phenotypic shifts with available data from other fishes. Results suggest that variation in intensity of predation by piscivorous fish may be a major driver of predictable morphological variation for many, distantly related fishes. By explicitly testing predictions of a GMDS, for which the mechanistic assumptions have strong empirical and experimental support, we can confidently conclude that divergent natural selection on locomotor performance between populations from different predator regimes (favoring steady swimming where predation is low and fast-starts where predation is high) may represent a widespread catalyst of predictable divergence of body shape in many fishes. Thus, the GMDS approach has proven useful in strengthening our understanding of the origin of phenotypic diversity in fish, the predictability of that diversity, and its consequences for speciation. Future assessments of the GMDS approach are warranted—the method may prove invaluable in elucidating the general predictability and peculiarity of evolutionary change, and in discovering particular forms of divergent selection responsible for producing evolutionary patterns that are repeatable and predictable across disparate groups of organisms.

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