Faculty Publications

Biological Sciences

7-1-2004

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Recommended Citation

Constraints on Adaptive Evolution: The Functional Trade-Off between Reproduction and Fast-Start Swimming Performance in the Trinidadian Guppy (*Poecilia reticulata*)

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abstract: The empirical study of natural selection reveals that adaptations often involve trade-offs between competing functions. Because natural selection acts on whole organisms rather than isolated traits, adaptive evolution may be constrained by the interaction between traits that are functionally integrated. Yet, few attempts have been made to characterize how and when such constraints are manifested or whether they limit the adaptive divergence of populations. Here we examine the consequences of adaptive life-history evolution on locomotor performance in the live-bearing guppy. In response to increased predation from piscivorous fish, Trinidadian guppies evolve an increased allocation of resources toward reproduction. These populations are also under strong selection for rapid fast-start swimming performance to evade predators. Because embryo development increases a female's wet mass as she approaches parturition, an increased investment in reproductive allocation should impede fast-start performance. We find evidence for adaptive but constrained evolution of fast-start swimming performance in laboratory trials conducted on second-generation lab-reared fish. Female guppies from high-predation localities attain a faster acceleration and velocity and travel a greater distance during fast-start swimming trials. However, velocity and distance traveled decline more rapidly over the course of pregnancy in these same females, thus reducing the magnitude of divergence in swimming performance between high- and low-predation populations. This functional trade-off between reproduction and swimming performance reveals how different aspects of the phenotype are integrated and highlights the complexity of adaptation at the whole-organism level.

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Adaptation and constraint jointly shape phenotypic evolution. Adaptation, or the process of evolution by natural selection, was the cornerstone of Darwin's (1859) proposed mechanism for evolutionary change and remains the most widely accepted mechanism responsible for phenotypic evolution (Rose and Lauder 1996). Yet, it is also recognized that the response to natural selection is subject to various constraints that place limits on the course or outcome of adaptive evolution. Constraints can be genetic (e.g., Lande 1979; Arnold 1992), developmental (e.g., Maynard Smith et al. 1985), biomechanical-chemical (e.g., Gould and Lewontin 1979; Roff 1992; Stearns 1992), and functional (e.g., Arnold 1992; Reznick and Travis 1996). All such constraints can place qualitative and quantitative limits on adaptation (see Dawson et al. 1977; Gould and Lewontin 1979; Maynard Smith et al. 1985; Arnold 1992; Perrin and Travis 1992). Empirical studies of adaptation in natural populations reveal that this interplay between selection and constraint is often a common feature of evolution by natural selection. Specifically, it is often the case that adaptations involve trade-offs or compromises between competing functions (Reznick and Travis 1996).

Natural populations of Trinidadian guppies (*Poecilia reticulata*) provide a model system for studying natural selection in the wild. As such, they also provide an opportunity to study the role of constraints on adaptive evolution. Guppy populations show significant differences in color patterns, behaviors, and life histories, all of which are strongly associated with the presence or absence of

piscivorous predators (Endler 1995). In larger streams, guppies co-occur with a suite of piscivorous fish, including the pike-cichlid (*Crenicichla alta*), on south-draining slopes (e.g., Endler 1978) and predatory gobies (*Eleotris* and *Gobiomorus*) on north-draining slopes (e.g., Reznick et al. 1996). These predators are excluded from upstream tributaries by rapids and barrier waterfalls. Upstream communities have progressively fewer fish species, often resulting in small streams with just guppies and the killifish *Rivulus hartii*, which preys on juvenile guppies. This pattern of predator exclusion is repeated among drainages and results in a repeated pattern of streams with high- and low-predation communities. Field, lab, and field selection experiments have all shown guppies from high-predation communities rapidly evolve earlier maturity, increased fecundity, shorter interbrood intervals, and larger reproductive allocations compared with their low-predation counterparts (e.g., Reznick et al. 1990, 1997). The evolution of guppy life histories has thus not only served as empirical support for theoretical models of how age-specific mortality drives adaptive life-history evolution but also represents an empirical example of contemporary adaptation in a natural population (Reznick and Ghalambor 2001).

Has adaptive life-history evolution in guppies constrained the adaptive evolution of other traits? Pregnancy and reproductive burden are known to impair locomotor performance and increase the risk of predation in a wide range of vertebrate and invertebrate species (e.g., Shine 1980; Bauwens and Thoen 1981; Winfield and Townsend 1983; Seigel et al. 1987; Cooper et al. 1990; Sinervo et al. 1991; Plaut 2002). This suggests that the increased reproductive allocation (the proportion of female mass that consists of developing offspring) in guppies from high-predation streams imposes an increased cost to locomotor performance. There are several reasons why adaptive differences in guppy reproductive allocation may result in reduced swimming performance, particularly with respect to the fast-start evasion response, which is a rapid burst of acceleration that allows fish to escape the path of a striking predator. First, given that mass is the property of matter that resists acceleration, any increase in nonlocomotor muscle mass should decrease accelerations. Second, the increased volume necessary to accommodate larger broods may increase flexural stiffness and limit axial bending during burst swimming (Beamish 1978; James and Johnston 1998). Third, the increased surface and cross-sectional area that result from the increased volume may increase drag and mass (Beamish 1978; Plaut 2002). Finally, excess allocation of energy to eggs and developing embryos may negatively influence the contractile properties of muscle and decrease power output (James and Johnston 1998). Paradoxically, then, the adaptive life-

history response to increased predation (increased reproductive allocation) may result in increased vulnerability to predation. Fast-start performance may itself be a direct target of selection. Predators have been shown to select for traits that increase the probability of avoiding predation (e.g., Seghers 1974; Feder 1983; Taylor and McPhail 1985; Magurran et al. 1992; McPeek et al. 1996; Watkins 1996; O'Steen et al. 2002; Domenici 2003). Predator-mediated selection on swimming performance leads to the expectation that guppies from high-predation environments should have improved escape performance (O'Steen et al. 2002; Chappel and Odell 2004). Predators on guppies may therefore impose opposing patterns of selection on fast-start swimming performance by directly favoring increased performance while indirectly favoring reduced performance via the evolution of a greater reproductive allocation. The functional integration of pregnancy with locomotion thus provides a framework for testing whether adaptive life-history evolution and fast-start evolution are mutually constrained by a functional trade-off (Ghalambor et al. 2003).

Here we test whether high- and low-predation guppies reared in a common environment for two generations differ in fast-start swimming performance, whether pregnancy results in a locomotor cost of reproduction, and whether this cost is greater in high-predation guppies. In the absence of any constraints, if there is directional selection on fast-start swimming speed in high-predation localities, then these guppies should attain higher fast-start performance than their low-predation counterparts (fig. 1*A*). If the cost of pregnancy was similar in both groups, then females should show an equal decline in swimming performance with advancing pregnancy (fig. 1*B*). However, if guppies from high-predation localities have a higher locomotor cost of reproduction because of their increased reproductive allocation, then their swimming performance should decline more rapidly as pregnancy advances despite selection for faster-swimming performance (fig. 1*C*). In the first two cases (fig. 1*A*, 1*B*), the results would be interpreted as showing evidence for adaptive evolution without constraint, whereas in the third scenario (fig. 1*C*), we would conclude that adaptive evolution in swimming performance is mutually constrained by a functional trade-off with reproductive allocation.

Methods

Pattern of Wet Mass Change in Developing Embryos

Because guppies give birth to live young, pregnancy is expected to reduce locomotor performance in females as developing embryos grow and presumably increase in volume and wet mass. However, we know of no study that

Burst Swimming Performance (y-axis)

Stage of Pregnancy (x-axis)

Legend:
— low predation
· · · high predation
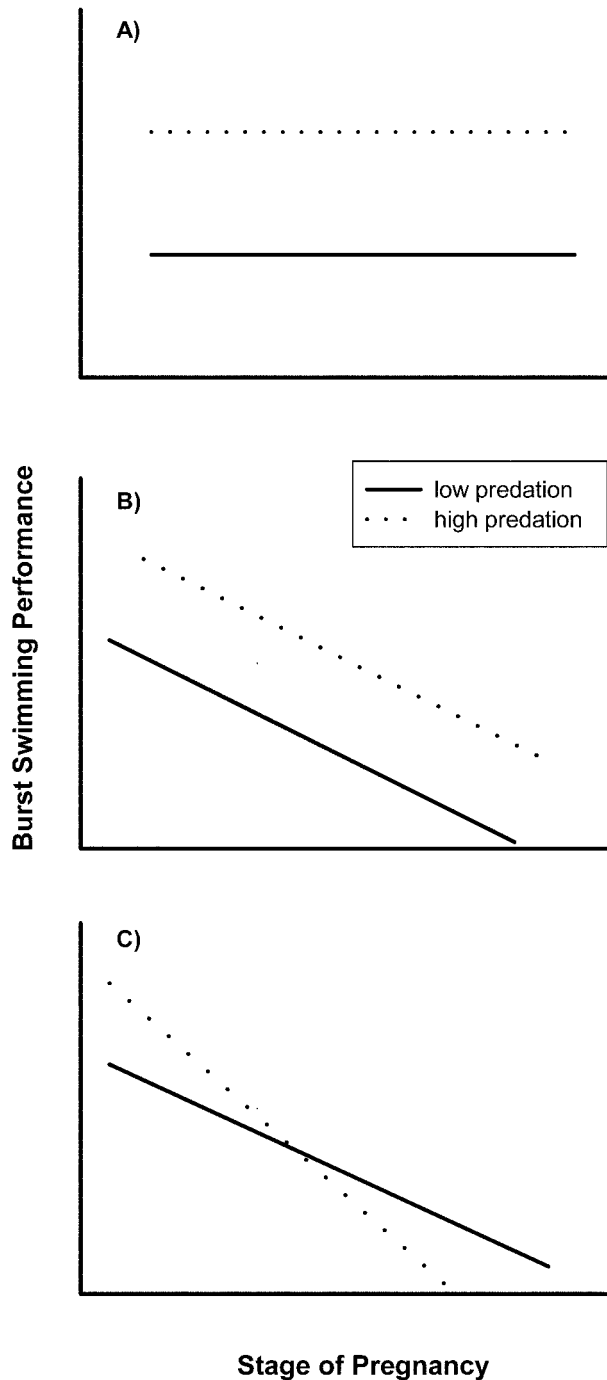
Figure 1: Some possible outcomes for the independent and interactive effects of predation risk and pregnancy on the swimming performance of Trinidadian guppies. *A*, Predators select for increased performance, but there is no cost of pregnancy. *B*, Predators select for increased performance, and there is a similar cost of pregnancy. *C*, Predators select for increased performance, and there is a higher cost of pregnancy in high-predation populations that have larger reproductive allocations.

has quantified how wet mass of developing embryos changes over the course of pregnancy in guppies. We tested this assumption by dissecting 26 wild-caught females from the El Cedro River in Trinidad and measuring the wet mass of embryos at different stages of development. The El Cedro is an experimental low-predation locality containing guppies derived from a high-predation locality. These females were collected in 1995 and preserved in formalin. Formalin preservation does not change the wet mass (D. N. Reznick, personal observation). We removed all developing embryos from each female, measured female standard length, and weighed the embryos. Embryos were classified by stage of development following Haynes (1995). Total embryo wet mass was divided by the number of embryos to obtain an average wet mass for an individual embryo within a litter. To visualize the change in wet mass of a developing embryo, we converted Haynes's (1995) developmental stages into a numeric scale that ranged from 0 (egg with yolking ova; close to conception) to 90 (fully developed eyed embryo; close to parturition) and plotted this number versus embryo wet mass.

Source of Laboratory Stocks and Experimental Design

All guppies used in escape performance trials were second generation (F2) descendants of wild-caught females collected in 1998 from four localities in Trinidad. Localities of origin included two high-predation streams and two low-predation tributaries paired within the Yarra and Oropuche drainages. These two drainages were chosen because they represent independently derived high- and low-predation lineages from the northern and southern slopes, respectively, of Trinidad's Northern Range Mountains (Carvalho et al. 1991; Fajen and Breden 1992). Twenty-five adult females were collected from each of these localities and individually housed in aquaria. Because female guppies store sperm, each female produced one or more broods of young (the F1 generation). The experimental design used to generate the F2 generation followed the same protocol used in previous studies (for details, see Reznick 1982; Reznick and Bryga 1987). Each wild-caught female generated a distinct F1 pedigree; F1 lineages were then crossed with others from the same locale in a way that equalized the contribution of each to the F2 generation. All such crosses were unique and among different lineages to avoid inbreeding. Such a design retains the genetic diversity of the original collection of wild-caught females, prevents adaptation to the laboratory environment, and greatly reduces any environmental or maternal influences so that remaining differences among populations can be attributed to genetic differences (Reznick 1982; Reznick and Bryga 1987). All fish received measured quantities of flake paste (a mixture of finely milled flake

food and water) in the morning and live brine shrimp nauplii in the afternoon. Water temperature was kept at 25°C for all tanks. An F2 pair from each population was grouped together in a randomized block.

Females were mated once per week until they began to reproduce. They were then mated for a day after the birth of each litter because females are especially receptive to mating at this time (Crow and Liley 1979). Such repeated matings ensured that females always had a supply of viable sperm and maintained a regular interbrood interval. Finally, to reduce the potential for cannibalism, a net wall was installed at the back of each aquarium made of mesh large enough for the young to swim through but too small for the mother.

Confirmation of Life-History Differences

To confirm that the four populations exhibited typical life-history differences between high- and low-predation populations (i.e., high-predation females have larger reproductive allotments), we measured the total wet mass at birth for 36 randomly chosen litters from all four populations. Elsewhere (Reznick et al. 2001), we show that these same high- and low-predation populations differ in a fashion that is consistent with all of our earlier studies of their life histories. Specifically, the high-predation guppies began to reproduce at an earlier age and smaller size, had shorter interbrood intervals, and produced more and smaller young than did the low-predation guppies. All of these differences were statistically significant (Reznick et al. 2001).

Fast-Start Swimming Trials in Pregnant Fish

A total of 80 females (20 from each population) were used in this study. We examined escape response by measuring the fast-start performance of female guppies in 1999. Guppies, like all teleost fish, present a stereotyped startle response called the "fast" or "C" start (Weihs 1973; Domenici and Blake 1997), which is used to initially evade a predation strike. We evaluated fast-start performance by temporarily moving females to a glass tank with a 1-cm² reference grid on the bottom. This tank was subdivided using aquarium dividers that prevented the fish from seeing any approaching stimuli and resulted in a 12 × 12-cm swimming area. Relative to body size, this arena is approximately five body lengths in distance and is in the same range used in similar studies of escape performance (e.g., Law and Blake 1996; Andraso 1997; James and Johnston 1998). This focal tank was in turn placed within a larger glass tank that served as a water bath and helped regulate water temperature. Both tanks were placed on a white background to maximize the contrast between the fish and

the background. Four halogen lights were focused on the swimming arena to create even illumination. Water depth within the focal tank was approximately 3 cm to limit vertical swimming. Because water temperature can affect swimming performance, water temperature was monitored and maintained close to 25°C (range 23.8°–26.6°C) for all experiments. Females were acclimated to the new surroundings for 15 min. Water temperature was recorded when females were placed in the water, at the end of the 15-min acclimation period, and a third time at the end of the experiment. The fast starts were filmed at 500 frames/s (Redlake Motionscope camera) and recorded to (VHS) videocassette tape. The fast-start response was elicited by dropping a golf ball from a height of 25 cm outside the swimming area into the focal tank. This approach proved very effective in repeatedly eliciting a fast start, and the ripples caused by the dropping of the ball did not influence subsequent digitizing because fast starts were initiated long before any ripples reached the location of the fish.

Film Analysis

Only a single fast-start response for each female was used in the analyses. When multiple responses were available, we selected the fastest response for inclusion in our analyses. We did not include events where fish exhibited only minimal curvature of the body. Recorded sequences were converted from analog (VHS) to digital format and saved as AVI files. The dorsal midlines of the first three tail beat cycles of the fast-starting fish were digitized using a modification of the public domain National Institutes of Health Image program (developed at NIH and available on the Internet at http://rsb.info.nih.gov/nih-image/) for the Apple Macintosh (the modification is available from the author on request). We fit a cubic spline function to each digitized midline within a sequence and used this function to find the coordinates of an unmeasured landmark at 0.35 total length, which we used as a proxy for the center of mass.

Estimates of velocity and acceleration are very sensitive to the method of numerical differentiation (Walker 1998*a*). To minimize error variance that would reduce the power of detecting predator and pregnancy effects, we used an MSE quintic spline algorithm to smooth the displacement versus time data and to estimate instantaneous velocities and accelerations throughout a digitized sequence (Walker 1998*b*). From the fitted functions, we computed cumulative displacement over the first 22 ms, average velocity and acceleration within the first 22 ms, maximum velocities and accelerations occurring within the first 22 ms, total turning angle of the anterior 10% of the body though the end of stage 1 (the initial C bend), and average rotational velocity through the end of stage 1 (Walker 1997*b*).

This time frame covers the first two kinematic stages of the fast start and approximates the duration of a guppy predator-prey interaction (J. A. Walker, unpublished data).

Statistics and Analysis

Differences in reproductive allocation between populations were initially tested using a general linear model that included female wet mass as a covariate, population as a fixed main effect, and the total wet mass of the brood as the dependent variable. Because populations within a predation regime did not differ significantly ($P > .20$), we repeated the same analysis but substituted predation regime (high vs. low) as the main effect. For our analysis of fast-start swimming performance, we first used multiple regression as an exploratory tool to identify significant correlates of escape performance. We separately examined the relationship between our measures of performance and female age, body mass, number of embryos inside the female at the time of the experiment (determined by the number of offspring born following the experiment), the total number of litters produced before the evaluation of burst performance, water temperature at the time of the experiment, date, time of day the experiment was conducted, and the number of days preparturition at the time of the swimming trial. As in previous studies (e.g., Reznick 1982), we found the length of the gestation period to be significantly shorter in females from high-predation populations (25.1 vs. 28.9 d; $F = 45.57$, df $= 1, 72$, $P < .0001$), thus reducing the number of comparable observations early in the reproductive cycle. To control for differences in interbrood interval, we transformed the number of days preparturition at the time of the swimming trial to the percentage of time remaining until birth (percent pregnancy) by dividing the number of days postbirth at the time of the trial by the total number of days in the cycle. Body mass was correlated with all measures of burst swimming performance except maximum acceleration. The percentage of time pregnant was a significant and independent predictor of maximum velocity and cumulative distance traveled. The number of embryos at the time of the experiment, although not statistically significant in the multiple regression, did explain some residual variance in velocity and was therefore included along with body mass and time until parturition as covariates in subsequent analyses of velocity. We then separately tested whether our measures of swimming performance differed within a predation regime (high vs. high and low vs. low) in order to determine whether we could group populations across drainages. We first tested each measure of performance with a general linear model that included predation as a fixed main effect, drainage as a random effect, and predation regime within drainage as a nested term. Appropriate covariates were included when necessary. In all cases, we found no significant difference within a predation regime ($P > .21$) and therefore combined the populations within the high- and low-predation treatments.

Our model of adaptive evolution of the fast-start and reproductive systems yields three directional hypotheses: H_1 guppies from high-predation populations have elevated fast-start performance, H_2 fast-start performance declines with increased pregnancy in guppies from both high- and low-predation sites, and H_3 guppies from high-predation sites exhibit a steeper decline in fast-start performance as pregnancy progresses. We used general linear models similar to those described above with predation regime (low vs. high) as a main effect, percent pregnancy as the primary covariate, and body mass and number of offspring as secondary covariates. Interactions between secondary covariates and predation were dropped from the analysis if nonsignificant. The main effect (predation) is a test of H_1, the primary covariate (percent pregnancy) is a test of H_2, and the interaction between these is a test of H_3. For all three tests, we used the directional probability, $P_{dir} = P_{1\text{-tailed}}[1 + (\delta/\gamma)]$, where $P_{1\text{-tailed}}$ is the one-tailed probability $P_{2\text{-tailed}}/2$; δ is the portion of the error, α, asymmetrically assigned to the less likely direction; and γ is the portion of the error as asymmetrically assigned to the more likely direction (Rice and Gaines 1994). Because a difference in performance in the direction opposite to that expected is possible, just unlikely given what we know about the mechanics of the fast start, we follow Rice and Gaines's (1994) suggestion and assign $\gamma = 0.04$ and $\delta = 0.01$. Our rationale for using one-tailed hypotheses is derived from our prior knowledge of the system. Specifically, we have repeatedly demonstrated that high-predation guppies have larger reproductive allocations (Reznick and Endler 1982; Reznick et al. 1996) and that there is a genetic basis to these differences (Reznick and Bryga 1996). On this basis, we argue that if there is a locomotor cost of reproduction, then this cost should be greater in high-predation guppies.
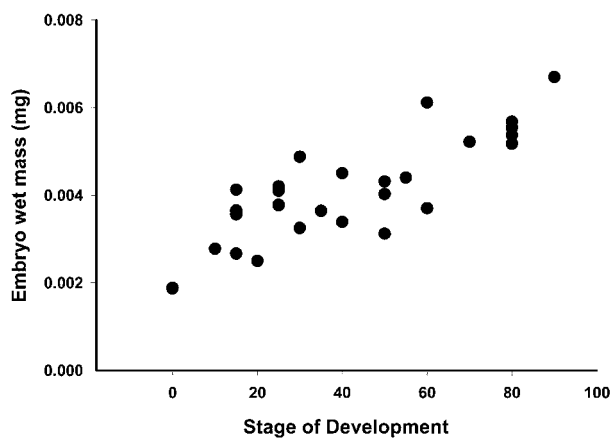
If the interaction between predation and percent pregnancy is significant, then the test of H_1 is relevant only at the beginning of gestation, whereas the test of H_3 is relevant only at the end of gestation. We used a nonparametric permutation test to further test these hypotheses. For this test, separate least squares regressions of swimming performance on percent pregnancy were fit to the high-predation and low-predation data subsets. The test statistic is the observed difference in expected performance at 0% and 100% gestation between the two groups. The group assignment (high or low predation) was randomly permuted, and the regressions for each pseudogroup and associated pseudodifference were recomputed 9,999 times. We then compared the test statistic to the distribution of the 10,000 pseudodifferences (one of which was the ob-

served difference). If the test statistic lay outside the 95% confidence intervals of the distribution, we considered the test statistic significant. We applied a directional one-tailed test as above to test the a priori prediction that high-predation populations would have higher performance at the beginning of gestation but not at the end. We visualized the effects of pregnancy by plotting raw and residual values of swimming performance versus the time until birth for high- and low-predation populations.

Results

Reproductive and Life-History Patterns

Guppy embryos increase in wet mass by nearly fourfold between conception and birth (fig. 2). However, populations differ significantly in the total amount of resources allocated to reproduction (fig. 3). High-predation populations have higher reproductive allocations (i.e., total wet mass of brood) compared with low-predation populations (fig. 3), as seen in earlier studies (Reznick and Endler 1982; Reznick et al. 1996). Indeed, high-predation females carry a reproductive mass that is 40% higher than that of their low-predation counterparts ($F = 10.88$, df $= 1, 33$, $P = .002$). The burden of pregnancy thus increases as the offspring progress through development (fig. 2), and the size of the burden is larger in guppies from high-predation environments (fig. 3).
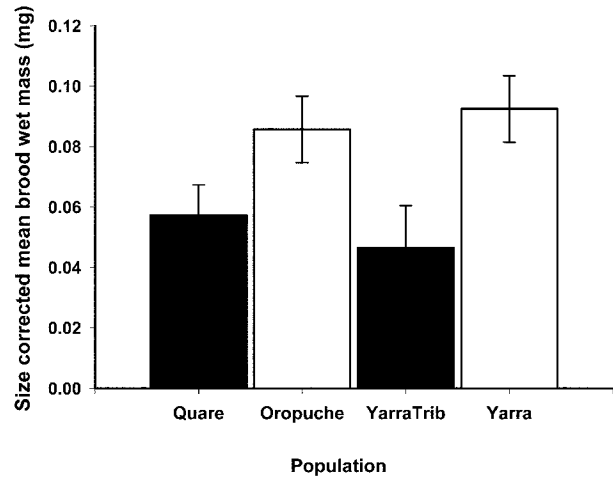
Figure 3: Reproductive allocation (mean total wet mass of brood in mg ± SE) corrected for female mass in four populations of guppies (*solid bars* = low predation, *open bars* = high predation). Populations significantly differ in reproductive allocation (ANCOVA, $F = 3.67$, df $= 3, 31$, $P = .02$).

Fast-Start Swimming Performance

Six of the 80 females in the study exhibited irregular reproductive cycles (i.e., did not give birth in the month prior to or following the swimming trials). We excluded these females from all analyses because we could not evaluate the stage of pregnancy at the time of the experiment. Maximum and average acceleration were not correlated with any of the covariates tested. In ANCOVAs for the remaining dependent variables, body mass was positively correlated with maximum velocity (table 1), cumulative distance traveled (table 2), total turning angle ($F = 3.92$, df $= 1, 69$, $P = .052$), and average rotational velocity ($F = 7.73$, df $= 1, 69$, $P = .007$). The stage of pregnancy was highly negatively correlated with both velocity (table 1) and cumulative distance traveled (table 2). There was no significant effect of predation on total turning angle ($F = 0.563$, df $= 1, 69$, $P = .456$) or average rotational velocity ($F = 0.835$, df $= 1, 69$, $P = .364$; table 3). In contrast, both maximum and average acceleration were significantly faster in females from high-predation populations (table 3; one-way ANOVA, $F = 6.22$, df $= 1, 70$, $P_{dir} = .009$, $F = 4.09$, df $= 1, 68$, $P_{dir} = .03$, respectively). Velocity and distance traveled were also significantly higher in high-predation females compared with low-predation females (tables 1–3). In both cases, there were also significant interactions between predation regime and the stage of pregnancy (tables 1, 2) for velocity and distance traveled. Such interactions suggest a differential locomotor cost of pregnancy in high- and low-predation populations.

Figure 2: Relationship between stage of development and wet mass of a typical guppy embryo. Embryo wet mass increases rapidly ($r = 0.83$, $n = 30$, $P < .001$) from the earliest stages of development (0 = fertilized egg with yolking ova) to the final stages close to birth (90 = fully developed eyed embryo).

Table 1: ANCOVA for maximum and average velocity (m/s) of high- and low-predation guppies, correcting for female mass, stage of pregnancy, and the number of embryos inside the female at the time of swimming

Source	df	MS	F	P
Maximum velocity:				
Mass	1	.238	10.71	>.001
Pregnancy	1	.699	31.47	**.001**
Embryos	1	.042	1.88	.176
Predation	1	.119	5.34	**.015**
Predation × pregnancy	1	.070	3.16	**.050**
Error	66	.022		
Average velocity:				
Mass	1	.112	14.61	>.001
Pregnancy	1	.134	17.50	>.001
Predation	1	.036	4.70	**.021**
Predation × pregnancy	1	.029	3.74	**.036**
Error	67	.008		

Note: *P* values in bold are modified to reflect directed hypotheses (Rice and Gaines 1994).

We tested this hypothesis with a one-tailed permutation test to test for predation effects on performance at the beginning of gestation (0%), when the burden of pregnancy should be minimal, and at the end of gestation (100%), when the burden should be greatest. As expected, both maximum velocity and distance traveled were significantly greater for high-predation females at the start of the gestation period ($P_{dir} = .025$ and .012, respectively) and lower at the end of the gestation period, although this difference was not significant ($P_{dir} = .10$ and .075, respectively). The difference at the beginning but not end of gestation suggests a greater cost of pregnancy in the females from high-predation streams, as predicted by their increased reproductive allocation. This increased cost of pregnancy on distance traveled and velocity is conspicuous when plotted graphically (fig. 4*B*, 4*C*). In contrast, maximum acceleration is largely invariant with respect to the stage of pregnancy in both high- and low-predation populations (fig. 4*A*).

Discussion

The stereotyped fast-start swimming response of fish is the primary means by which they are able to escape the path of a striking predator (e.g., Weihs 1973; Domenici and Blake 1997). This response is thus thought to be under strong directional selection from predators (e.g., Domenici 2003; Ghalambor et al. 2003). Female guppies from high-predation populations achieve significantly faster accelerations (table 3; fig. 4*A*), reach higher velocities (table 1; fig. 4*B*), and travel a greater distance (table 2; fig. 4*C*) in comparison to females from low-predation populations.

Because these trials were conducted on second-generation lab-born fish from independently derived high- and low-predation populations, the results suggest a genetic basis to these differences in swimming performance (Reznick 1982; Garland and Adolph 1991). These results also provide a mechanistic explanation for recent work by O'Steen et al. (2002), who showed that high-predation guppies consistently survive better than low-predation guppies in staged encounters between the predatory *Crenicichla* and either wild-caught or second-generation lab-reared guppies. Collectively, our results and those of previous researchers (e.g., Seghers 1974; Magurran et al. 1992; O'Steen et al. 2002) provide strong evidence that predators have selected for genetically based adaptive changes in guppy escape ability. However, predators have also selected on guppy life-history strategies (e.g., Reznick 1982; Reznick and Bryga 1987; Reznick et al. 1990), resulting in an apparent functional trade-off between reproduction and swimming performance. Like other live-bearing fish, guppies experience an increasing reproductive burden because embryos expand in mass and volume as development progresses (fig. 2; see also Plaut 2002). Furthermore, guppies from high-predation environments carry a significantly larger reproductive burden compared with that of their low-predation counterparts (fig. 3). Yet, the degree to which pregnancy and differences in reproductive allocation differentially reduce burst swimming performance between high- and low-predation populations depends on which components of the response are examined. Rotational velocity and turning angle of the body do not differ among high- and low-predation populations (table 3) and are not correlated with the stage of pregnancy. The maximum and average accelerations differ significantly between high- and low-predation populations and are also not significantly correlated with the stage of pregnancy (fig. 4*A*). Maximum and average velocity and the cumulative distance traveled during a fast start decline as pregnancy progresses, but the rate of this decline is higher in guppies from high-predation environments (fig. 4*B*, 4*C*).

Table 2: ANCOVA for cumulative distance traveled (cm) of high- and low-predation guppies, correcting for female mass and stage of pregnancy at the time of swimming

Source	df	MS	F	P
Mass	1	.502	12.69	.001
Pregnancy	1	.584	14.77	>.001
Predation	1	.207	5.24	**.016**
Predation × pregnancy	1	.147	3.72	**.036**
Error	67	.040		

Note: *P* values in bold are modified to reflect directed hypotheses (Rice and Gaines 1994).

Table 3: Descriptive statistics from female guppy burst swimming trials

	Predation	
Trait	Low	High
Body mass (mg)	447 (32)	491 (29)
Maximum acceleration (m/s/s)	53.25 (4.37)	67.35 (3.68)
Average acceleration (m/s/s)	33.37 (3.15)	41.54 (2.59)
Maximum velocity (m/s)	.854 (.03)	.913 (.03)
Average velocity (m/s)	.594 (.02)	.615 (.01)
Cumulative distance traveled (cm)	1.32 (.04)	1.37 (.03)
Total turning angle (deg/s)	11,000 (558)	11,527 (520)
Average rotational velocity (deg)	101 (6.5)	103 (6.1)

Note: Shown are least square means ($\pm$SE) from general linear models, except for maximum acceleration and body mass.

Thus, high-predation female guppies attain faster velocities and move greater distances early in gestation but cannot maintain this advantage throughout the entire reproductive cycle and are either similar to or perhaps slightly slower than low-predation females at the end of the gestation period (fig. 4*B*, 4*C*).

Arnold (1992) recognized four key properties of constraints: source, strength, consequence, and persistence (see also Maynard Smith et al. 1985). If we apply these key properties to swimming performance in guppies, then we see that the source or proximate cause of the constraint appears to be the increased friction drag associated with larger reproductive burdens in high-predation guppies (fig. 3). The difference in slope describing how performance changes over the reproductive cycle between high- and low-predation populations (fig. 4) quantifies the strength of reproduction as a constraint on burst swimming performance (Arnold 1992). The consequence or outcome of the constraint is that high-predation guppies must compromise escape ability for increased fecundity, presumably because of a large selection gradient for higher reproductive investment. Alternatively, the consequence could be that high-predation guppies sacrifice reproductive investment to maintain escape ability. More generally, the consequence is that these two components of fitness interact with one another as they evolve. The persistence or stability of this constraint over evolutionary time remains unknown, but it is difficult to imagine how any live-bearing fish would be able to completely overcome the locomotor costs associated with increasing fecundity. The fact that this constraint has evolved in very similar ways in two drainages that are genetically quite distinct from each other (Carvalho et al. 1991; Fajen and Breden 1992) argues that the interaction is a persistent one.

Why velocity and distance traveled are more sensitive to differences in the stage of pregnancy compared with acceleration is unclear. We expected that the increased mass, surface area, and body stiffness associated with pregnancy would primarily reduce acceleration and subsequently reduce velocity and distance traveled. However, despite maximum velocity being highly correlated with mean and maximum acceleration ($P < .0001$), there is no significant pregnancy effect on acceleration (fig. 4*A*). The lack of a significant pregnancy effect on acceleration despite its significant effect on velocity and distance suggests either that an augmented error variance inherent in acceleration estimates reduced the power to detect trends (Walker 1998*a*) or that acceleration is somehow buffered against the changes associated with pregnancy. Given the relatively few studies that have examined the consequences of reproduction on swimming performance, there is clearly a need for further studies examining the physiological, morphological, and kinematic consequences of pregnancy on different components of swimming performance.

Chappell and Odell (2004) also evaluated swimming performance in guppies and concluded that there were no differences in maximum velocity among guppies from high- and low-predation localities. However, their methods significantly differed from ours because they evaluated maximum velocity at a time interval that was 10 times longer than that considered here and without any reference to when the maximum velocity was achieved. While their approach provides insight into the anaerobic capacity of a guppy to outswim a potential chasing predator, it does not evaluate the fast-start or C-start response more commonly used to evade a striking predator. Chappell and Odell (2004) also did not control for age, the effects of pregnancy, or the genetic background. Any combination of these factors could account for the differences between their results and the results of other research (Seghers 1974; Magurran et al. 1992; O'Steen et al. 2002; this study) concluding strong directional selection by predators on components of escape ability.

Because natural selection acts on the whole organism, traits that are functionally integrated can either facilitate or constrain the response to selection acting on multiple
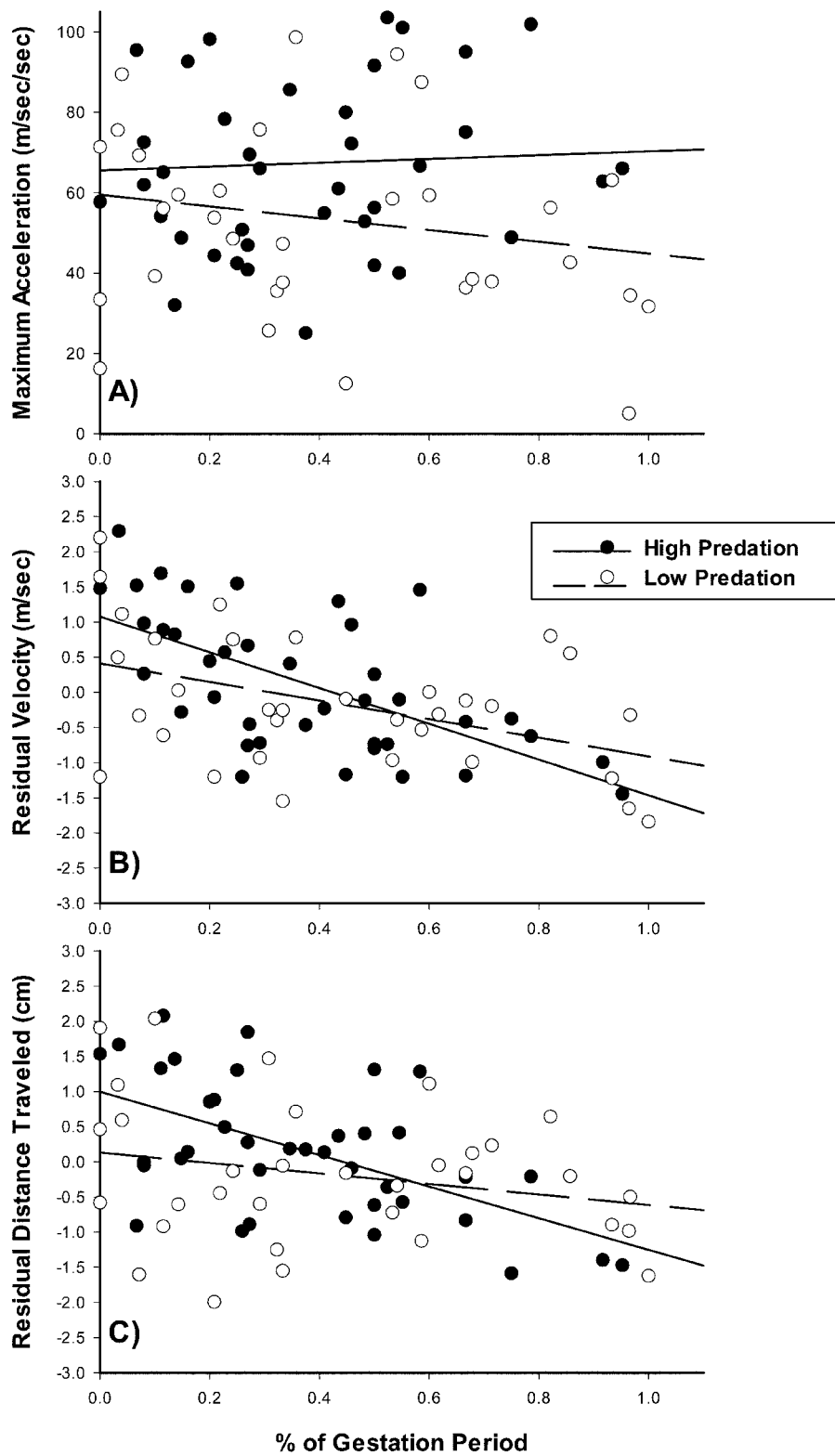
Figure 4: Scatterplots showing the relationship between the stage of pregnancy (expressed as a percentage of the gestation period) and different components of the burst swimming response. *A*, Raw values for maximum acceleration. *B*, Residual values for maximum velocity, controlling for body mass and number of embryos at the time of the experiment. *C*, Residual values for cumulative distance traveled during the fast-start response, controlling for body mass.

components of the phenotype (e.g., Ghalambor et al. 2003). Such integration and trade-offs are expected to result in different evolutionary outcomes depending on the nature of the local selection pressures (Ghalambor et al. 2003). However, these patterns often become evident only when studies are conducted in a comparative context. For example, work by Billerbeck et al. (2001) and Lankford et al. (2001) show that strong selection for increased growth rate in northern latitude populations of Atlantic silversides (*Menidia menidia*) comes at the expense of both sustained and burst swimming performance, which in turn increases the risk of predation. This trade-off can be seen only when they are compared with lower-latitude populations, which appear to be under weaker selection for rapid growth but stronger selection for superior swimming performance and predator avoidance (Billerbeck et al. 2001; Lankford et al. 2001). Likewise, in comparisons of brook stickleback (*Culaea inconstans*) with variable amounts of defensive armor, all aspects of burst swimming performance decline in morphs with more fully developed pelvic girdles and spines. The armor protects against predators but interferes with the ability to bend during a fast-start response (Andraso 1997). Finally, in results that are analogous to those reported here, Sinervo et al. (1991) show that sprint speed declines in gravid western fence lizards (*Sceloporus occidentalis*) and that populations with increased reproductive investment exhibit a greater decline in performance. Thus, as in guppies, selection for increased fecundity comes at the expense of locomotor performance. Other similar studies exist, yet most studies of life-history evolution do not explicitly evaluate how traits such as reproduction and growth rate interact with locomotor performance and how such interactions shape the joint evolution of these different components of fitness (Ghalambor et al. 2003).

A challenge to comparative studies of whole-organism performance that invoke adaptive evolutionary changes is identifying the mechanistic changes that underlie observed differences between populations or species (e.g., Garland and Carter 1994). We see two nonmutually exclusive explanations for how predators may have selected for faster burst swimming speeds in high-predation guppies. First, predators may have selected for body shapes and fin dimensions that result in improved burst performance (Webb 1978, 1986; Walker 1997*a*). Second, predators may have selected for properties of the neuromuscular components that mediate escape performance (e.g., Hale et al. 2002). We have no data on the neural or muscular physiology of these populations, but, given that rotational velocity and turning angle did not differ significantly (table 3), it appears that faster speeds are not being achieved through greater contraction and bending of the body. Preliminary data on wild-caught females indicate that high-predation populations do have more fusiform body shapes,

longer and more shallow caudal peduncles, and larger caudal fins (C. Ghalambor et al., unpublished data). Such differences in body shape may improve performance in evading predators (Walker 1997*a*) and could explain faster speeds by high-predation fish.

The ability of a fish to evade a predatory strike is determined by the coordination of numerous biochemical, neurological, physiological, morphological, and behavioral parameters (e.g., Domenici and Blake 2000). In this study, we have considered only one aspect of the ability to escape, the fast-start burst swimming response. However, as we show, burst swimming can be decomposed into different components (e.g., acceleration, velocity), and each of these components may or may not vary over the course of pregnancy (fig. 4). Thus, while we do not know how much the decline in performance in high-predation females with advancing pregnancy negatively impacts the likelihood of escaping an attack, we assume that these changes increase predation risk. In addition, we do not know the degree to which females might compensate for the cost of pregnancy by altering their behavior to reduce their potentially increased vulnerability to predatory attacks (e.g., Brodie 1989). For example, in three-spine stickleback, Rodewald and Foster (1998) show that gravid females alter habitat use and escape behavior compared with nongravid females. Such changes in behavior are thought to increase the probability of survival during the gravid period when females may be more vulnerable to predators (Rodewald and Foster 1998). It would be interesting to know whether female guppies employ similar behavioral shifts as they approach parturition.

In conclusion, we know that locomotor performance is impaired by pregnancy in a wide range of invertebrate and vertebrate taxa in both aquatic and terrestrial environments (e.g., Shine 1980; Bauwens and Thoen 1981; Winfield and Townsend 1983; Sinervo et al. 1991; Plaut 2002). We also know that predation can select for improved locomotor performance (e.g., Taylor and McPhail 1985; McPeek et al. 1996; Watkins 1996; Domenici 2003), increased reproductive allocation (e.g., Reznick 1982; Reznick et al. 1990, 1997), changes in morphology (e.g., Bell et al. 1985; Domenici 2003), and behavior (Seghers 1974; Feder 1983; Magurran et al. 1992; O'Steen et al. 2002) in some organisms. We know far less about how such seemingly unrelated traits are functionally integrated in their response to selection. Yet, an individual's fitness may be determined by how certain trait combinations act to compensate for the costs associated with functions that contribute to fitness (e.g., Feder 1983; Brodie 1989; Cooper et al. 1990; Foster and Baker 1995; Rodewald and Foster 1998). Such integration between a wide range of traits and their varied functions could ultimately either constrain or facilitate the evolution of different aspects of the phenotype

or performance in response to different selection pressures (Ghalambor et al. 2003). What our current results show is that despite a general frustration associated with studying constraints on adaptive evolution (e.g., Antonovics and van Tienderen 1991), such interactions are real and can be empirically quantified. A challenge to future studies of adaptation is to more explicitly consider the functional relationships between traits and to examine adaptation within the context of the integrated whole organism.

Acknowledgments

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