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Morphological and enzymatic correlates of aerobic and burst performance in different populations of Trinidadian guppies *Poecilia reticulata*

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Summary

We examined the mechanistic basis for two whole-animal performance traits, aerobic capacity and burst speed, in six laboratory-reared Trinidadian guppy populations from different native drainages with contrasting levels of predation. Using within- and between-population variation, we tested whether variation in organs and organ systems (heart, gill and swimming motor mass) and the activities of several enzymes that support locomotion (citrate synthetase, lactate dehydrogenase and myofibrillar ATPase) are correlated with aerobic performance (maximum rates of oxygen consumption, $\dot{V}_{O_2max}$) or burst performance (maximum swim speed during escape responses). We also tested for associations between physiological traits and habitat type (different drainages and predation levels).

Organ size and enzyme activities showed substantial size-independent variation, and both performance measures were strongly correlated to body size. After accounting for size effects, neither burst nor aerobic performance was strongly correlated to any organ size or enzymatic variable, or to each other. Two principal components (PCI, PC2) in both males and females accounted for most of the variance in the organ size and enzymatic variables. In both sexes, heart and gill mass tended to covary and were negatively associated with citrate synthetase and lactate dehydrogenase activity. In males (but not females), variation in aerobic performance was weakly but significantly correlated to variation in PC1, suggesting that heart and gill mass scale positively with $\dot{V}_{O_2max}$. Neither of the component variables and no single morphological or enzymatic trait was correlated to burst speed in either sex.

Evolutionary changes in important life history traits occur rapidly in guppy populations subjected to different predation intensities (high mortality in downstream sites inhabited by large predatory fish; low mortality in upstream sites lacking large predators). We found significant differences between stream drainages in all morphological variables and most enzymatic variables, but only the mass of the swimming motor and LDH activity were significantly affected by predation regime. Overall, our data show that microevolution has occurred in the physiological foundations of locomotor performance in guppies, but evolutionary changes in physiology do not closely correspond to the predation-induced changes in life history parameters.

Key words: locomotion, swimming, predation, life history, burst speed, aerobic capacity, guppy, *Poecilia reticulata*, trade-off, lactate dehydrogenase, myofibrillar ATPase, citrate synthetase.

Introduction

Intra- and interspecific variation in locomotor performance has long been of interest to comparative physiologists. Interspecific comparative studies of trait variation are often used to provide insights into both evolutionary adaptation and the mechanistic foundations of performance. A related approach (which reduces the problem of phylogenetic non-independence among related species) is to use correlative analyses of natural intraspecific variation at different levels of integration (e.g. enzymes, organelles, cells, organs, organ systems and the intact animal) to yield insights into the functional basis of whole-animal performance (e.g. Hulbert and Else, 1981; Garland, 1984; Bennett, 1987, 1997; Bennett et al., 1989; Mangum and Hochachka, 1998). For example, several recent papers have shown correlative links between avian energy metabolism (basal metabolism and maximal aerobic capacity) and organ size and muscle enzymology (Kersten and Piersma, 1987; Daan et al., 1990; Piersma et al., 1996; Burness et al., 1998), and explore the role of central and peripheral organs in setting the limits to performance (e.g. Chappell et al., 1999; Hammond et al., 2000). These results can be used to predict what organs or organ systems are likely to be impacted by selection on whole-animal performance, or by acclimation, seasonal changes or other types of phenotypic plasticity. Of special interest in both mechanistic and

evolutionary contexts are trade-offs (antagonistic pleiotropy) between genetically based traits. Trade-offs are fundamental and explicit in life-history models and other evolutionary theory and are implicit in many aspects of comparative physiology, and a key question for both physiologists and evolutionary biologists is the extent to which selection-driven evolution of major organism-level traits affects other characters.

We examined these questions in a system that offers particularly clear insights into microevolutionary change: the cluster of guppy populations (*Poecilia reticulata* Peters) on the island of Trinidad. Life history evolution in these fish has been extensively studied by David Reznick, John Endler and their colleagues. Guppies are native to rainforest drainages throughout the mountain ranges of Trinidad, and occur even in very small tributaries (Reznick and Endler, 1982). Local habitats range from shallow, slow-moving, low-volume pool-and-riffle systems (often interspersed with barrier waterfalls) to large, deep, fast-flowing rivers. Variation in stream topography, along with other factors, results in striking interpopulation differences in predation regimes and mortality rates (large guppy predators occur in high-volume downstream habitats but are absent above barrier waterfalls). In turn, differences in predation are associated with large and genetically determined differences in life history parameters such as age at first reproduction and offspring number and size (Reznick, 1982; Reznick and Endler, 1982; Reznick et al., 1996). Experimental manipulation of predation in natural habitats has revealed surprisingly rapid evolutionary change in life history traits (Reznick and Bryga, 1987; Reznick et al., 1990, 1997).

Given the variation in stream hydrodynamics, large and genetically based differences in life history, and quick response to selection in guppy populations, a natural question is: has whole-animal physiological performance also evolved in these fish and, if so, what are the mechanistic causes of performance differences? Previous studies have reported population differences in some aspects of locomotor performance (Cullum and Bennett, 1995; Odell and Chappell, in press). In this paper we use within- and between-population variation to examine several physiological characters underlying aerobic capacity (the metabolic foundation of sustainable swimming) and burst speed (presumably crucial in escape from predators and other emergency responses). Both traits require the effective and coordinated functioning of a suite of enzymes, organelles, cells, tissues, organs and organ systems, and hence are useful integrative physiological indices. We studied burst and aerobic performance at several levels of integration, from the intact animal (size and shape) to organ systems (heart, gill and swimming motor mass) to several muscle enzymes commonly used as indicators of aerobic and anaerobic capacity (Childress and Somero, 1979; Dickson et al., 1993; Gibb and Dickson, 2002). We also examined the effects of natural predation regimes (defined as the presence or absence of large guppy predators such as the pike cichlid *Crenicichla alta*; Reznick and Endler, 1982) on morphology and enzyme activity.

We tested four specific hypotheses. (1) Variance in aerobic capacity should be positively correlated with variance in the size of the heart and gills (key organs in oxygen uptake and delivery) and with citrate synthetase activity (an indicator enzyme for oxidative metabolism and hence for the amount of slow-twitch ‘red’ muscle in the swimming motor). (2) Variance in burst performance should be positively correlated with variance in the size of the swimming motor (comprising mainly fast-twitch glycolytic fibers; Veggetti et al., 1993) and with lactate dehydrogenase (an indicator enzyme for glycolytic ATP production and fast-twitch muscle) and myofibrillar ATPase activities. (3) Due to trade-offs, there should be a negative correlation between aerobic and glycolytic traits. (4) Because of pleiotropic interactions with life history traits and direct selection by predators, organ size and enzyme activities should differ consistently in upstream (low-predation) and downstream (high-predation) populations. The numerous natural population replicates of guppies, and their amenability to laboratory culture, allowed us to use a ‘common-garden’ approach to control for environmental effects and reveal genetic differences.

Materials and methods

Animals

We used second and third generation laboratory-born guppies *Poecilia reticulata* Peters to minimize the possible influences of maternal effects (which could be present in F_1 offspring) and differential acclimatization to local environments. Ancestral fish were collected from six isolated populations (20–25 gravid females for each locale) from three major river drainages (Caroni, Oropouche and Yarra). Two drainages contained naturally occurring population pairs in upstream and downstream habitats: Oropouche (Quare and Oropouche sites) and Yarra (Yarra tributary, Yarra River sites). The Caroni drainage contained a downstream control site (Aripo River) and an experimental population of guppies introduced from the control site to an upstream tributary (Aripo introduction site) where guppies were previously absent (Reznick and Bryga, 1987).

All F_2 and F_3 offspring were reared under standardized conditions (water temperature 24.5±1°C and an *ad libitum* diet of commercially available granular food and liver paste; Reznick and Bryga, 1987). For each generation, two fry were collected daily from stock tanks (to provide roughly equal representation of offspring from each wild-caught ancestor). Fry were reared together in population-specific 18 liter tanks until mature, and then individually housed in 7.25 liter tanks during the measurement period. Females were not gravid during experiments. All aspects of the care and use of animals in this study were approved by the institutional animal care and use committee of the University of California, Riverside, USA.

Because of logistical constraints, we simultaneously reared upstream and downstream populations from the same drainage, while populations from different drainages were reared sequentially. That design allows robust comparisons between

upstream and downstream (low- and high-predation) habitats, but the temporal separation in rearing fish from different drainages introduces the possibility of uncorrected 'block' effects in comparisons among drainages.

Whole-animal performance

Aerobic performance and burst speed were measured for each animal (Odell, 2002; Odell and Chappell, in press). Briefly, we measured maximal oxygen uptake rates ($\dot{V}_{O_2max}$) during forced exercise using closed-system respirometry. The cylindrical respirometry chamber contained an adjustable magnetic stirbar, which generated a variable current that the fish swam against. Current speed was increased in a stepwise fashion every 2 min until fish began to swim unsteadily and fatigued (i.e. failed to maintain position). Output from an oxygen microelectrode (Strathkelvin Instruments, Glasgow, Scotland) was sampled by a computer and converted to oxygen content. Oxygen saturation remained above 84% in these tests and validation studies revealed no decrease in exercise performance at O_2 saturations as low as 60–70% (Odell and Chappell, in press). Oxygen consumption rate ($\dot{V}_{O_2}$) was calculated as the time derivative of oxygen content, and we computed $\dot{V}_{O_2max}$ as the highest continuous average $\dot{V}_{O_2}$ over a 1 min period during forced swimming, using custom software ('LabAnalyst'; http://warthog.ucr.edu). The $\dot{V}_{O_2max}$ measured in this way is repeatable over periods of 7 and 90 days (Odell and Chappell, in press).

Burst performance during the escape response (C-start) was measured as maximum speed over a short interval (we used maximum speed because it is less prone to measurement error than other burst indices such as acceleration or rates of body bending; Walker, 1998). Guppies were filmed at 500 frames s^{-1} with a MotionScope camera (RedLake Instruments, San Diego, CA USA); C-starts were initiated by tapping on the side of the tank with a small metal object. Video recordings were captured to a Macintosh computer and digitized over 50–100 sequential frames (100–200 ms). Within that interval, we determined the maximal forward velocity of the bending axis (approximately the center of mass) over 20 ms, using custom software ('Motion Analysis'; http://warthog.ucr.edu). This measurement is highly repeatable, at least over short inter-measurement intervals (several minutes; Odell and Chappell, in press).

Morphology

After performance measurements were completed, guppies were euthanized with a lethal dose of MS-222. Wet mass *M* and standard length *SL* were determined and fish were bisected transversely immediately posterior to the anus. Tail portions were weighed (to ±0.1 mg) and immediately frozen in liquid nitrogen for use in enzyme analyses. Anterior portions were preserved in 5% formalin. Hearts (ventricle tissue only) and gills were dissected from preserved fish under a 20× microscope, placed individually in the wells of plastic microtiter plates, dried overnight at 55°C, and weighed to the nearest μg using an electrobalance (Cahn C-41; Cahn Instruments, Madison, WI, USA). We were particularly careful to harvest hearts and gills in a standardized manner to insure equivalent sampling of these small organs in all fish. The head and viscera were removed from the anterior portion of the carcass and the remainder was weighed to ±0.1 mg. The mass of the 'swimming motor' (primarily muscle, but also containing bone and skin) was calculated as the sum of the masses of the eviscerated headless anterior carcass and the tail portion.

For an index of shape differences, we used the relationship between wet mass and standard length, based on the principle that the mass of geometrically similar bodies should vary as the cube of linear dimensions. Thus, if guppies from different populations have similar shapes, they should exhibit a constant ratio of $M^{1/3}/SL$, and we computed that ratio for each individual.

Enzyme activities

We assayed the swimming motor for an aerobic marker enzyme (citrate synthetase, CS), an anaerobic marker enzyme (lactate dehydrogenase, LDH), and myofibrillar ATPase, an enzyme with activity proportional to the speed of fiber contraction (Barany, 1967; Johnston and Walesby, 1977). Because we had small amounts of tissue (especially in males), we assayed the entire post-anal tail assembly.

Tail assembly tissue was homogenized in ground-glass tissue grinders in 20 volumes (females) or 40 volumes (males) of extraction buffer containing 10 mmol l^{-1} Tris-HCl, pH 7.2 at 20°C, 1% Triton X-100 (w/v), 5 mmol l^{-1} ETDA, 100 mmol l^{-1} KCl. The crude homogenate was centrifuged for 10 min at 7500 ***g***, and the supernatant was assayed for CS and LDH activity. To obtain myofibrils (Watkins, 2000), the pellet was washed and resuspended in 2 ml of extraction buffer and centrifuged again for 10 min at 7500 ***g***. This procedure was repeated three times, each time discarding the supernatant. After the third centrifugation, the pellet was resuspended in 500 μl of rinse buffer (30 mmol l^{-1} Tris-HCl, 150 mmol l^{-1} KCl, pH 7.2 at 0°C) and assayed for myofibrillar ATPase activity.

Enzyme activities were determined under saturating substrate conditions in a microplate spectrophotometer (Molecular Devices, Inc., Sunnyvale, CA, USA). In all assays, the reaction volume was 160 μl, and included 10 μl of either supernatant or myofibril suspension. Assays were performed in duplicate and with a simultaneous negative control (no substrate). In addition, a blank reaction (no enzyme) was run on each plate. Reaction mixtures were as follows. Citrate synthetase: 80 mmol l^{-1} Tris, pH 8.0 at 20°C, 2 mmol l^{-1} 5,5′-dithio-bis(2-nitrobenzoic acid), 400 mmol l^{-1} $MgCl_2$, 0.1 mmol l^{-1} acetyl coenzyme-A, 0.5 mmol l^{-1} oxaloacetate (omitted in control), absorbance read at 412 nm at 25°C. Lactate dehydrogenase: 3.2 mmol l^{-1} NADH, 500 mmol l^{-1} KCl, 50 mmol l^{-1} imidazole buffer pH 7.0 at 20°C, 1.0 mmol l^{-1} pyruvate (omitted in control), absorbance read at 340 nm at 25°C. Myofibrillar ATPase: a commercially available kit (EnzChek® phosphate assay kit E-6646; Molecular Probes, Inc., Eugene, OR, USA) was used to

Table 1. *Correlations between size, performance, organ mass, and enzyme activity for Trinidadian guppies*

		Mass						Enzyme activity		
	Length	Body	Heart	Gill	Swim motor	$\dot{V}_{O_2max}$	Burst speed	CS	LDH	ATPase
Standard length		**0.941***	**0.612***	**0.702***	**0.903***	**0.841***	**0.420***	0.222*	0.197*	0.052
Body mass	**0.919***		**0.643***	**0.794***	**0.965***	**0.826***	**0.408***	0.222*	**0.318***	0.070
Heart mass	**0.475***	**0.458***		**0.609***	**0.621***	**0.565***	**0.294***	0.133	0.199*	0.084
Gill mass	**0.546***	**0.550***	**0.558***		**0.797***	**0.632***	**0.293***	0.241*	0.227*	0.076
Swimming motor mass	**0.892***	**0.972***	**0.423***	**0.522***		**0.828***	**0.382***	0.180*	**0.339***	0.110
$\dot{V}_{O_2max}$	**0.721***	**0.764***	**0.470***	**0.517***	**0.742***		**0.415***	0.133	0.208*	0.059
Burst speed	0.175*	0.180*	0.012	0.003	0.192*	0.135		0.152	0.173*	–0.063
CS	–0.223*	–0.213*	–0.027	–0.065	–0.181*	–0.162*	–0.030		**0.479***	0.016
LDH	**0.348***	**0.539***	0.061	0.199*	**0.557***	**0.332***	0.172*	–0.118		0.222*
ATPase	–0.006	–0.050	–0.021	–0.158	–0.049	0.041	0.020	–0.026	–0.134	

Results for females are in the upper right and results for males are in the lower left.

N=133–147 for females (*N*=99 for ATPase) and 160–168 for males.

Asterisks indicate significance at the *P*=0.05 level; numbers in boldface remained significant after Bonferroni correction for multiple simultaneous tests (Rice, 1989; adjusted α=0.00172).

$\dot{V}_{O_2max}$, maximum oxygen consumption; CS, citrate synthetase activity; LDH, lactate dehydrogenase activity; ATPase, myofibrillar ATPase activity.

Heart mass and gill mass are dry mass; swimming motor mass is wet mass.

measure the presence of phosphate released by the hydrolysis of ATP. After pre-incubating the samples at 25°C for 2 min to remove endogenous phosphate, the reaction was initiated with 1 mmol l^{-1} ATP and the rate of change in absorbance was measured at 360 nm.

Total protein concentration (mg ml^{-1}) was determined for both supernatants and resuspended myofibril pellets by Bradford assay (BioRad, Hercules, CA, USA), using a gamma globulin standard. We expressed enzyme activities as i.u. mg^{-1} protein in the sample (1 i.u. = μmol substrate converted to product per minute). Protein content variation in both phases was low and we did not reject data from any individual because of unusually high or low total protein (i.e. there were no noticeable outliers). However, a problem with protein precipitation during the purification stages compromised myofibrillar ATPase results for Yarra drainage females, and these data had to be discarded.

Statistical analyses

Performance, organ size and some enzyme activities were strongly related to body size (Table 1), so for most population comparisons we used analysis of covariance (ANCOVA, with size as a covariate) or residuals of log–log mass regressions (using wet mass). Because of minimal size overlap, the sexes were analyzed separately in most tests.

We looked for patterns in combinations of organ mass and enzymatic variables using principal components analysis (PCA) of a Pearson product–moment correlation table. PCA creates composite variables that are relatively independent of one another (Tabachnick and Fidell, 1996). Because composite variables are orthogonal, they reduce the problem of collinear data when analyzed by multiple regression. Preliminary analysis revealed that the overall relationships did not differ among drainages or locales, so data for all individuals within a sex were pooled. Parallel analysis (Franklin et al., 1995) was used to determine significant eigenvalues for component retention. The contribution of each raw variable to the composite variables was considered significant if its factor loading was ≥0.316, meaning that it accounted for >10% of the variation in that component. Relationships between whole-animal performance and retained PCA axes were examined with multiple regression. Composite variables were also analyzed by ANOVA with drainage and predation regime as the main effects.

Analyses were performed using JMP 4.0.4 (SAS Institute, 2000), Statistica/Mac 4.1 (1994 Statsoft Inc.), and SAS/Mac 6.12 (1996 SAS Institute). The significance level (α) was 0.05; for multiple simultaneous tests (e.g. correlation matrices) we adjusted α using a sequential Bonferroni correction (Rice, 1989) to avoid Type I errors.

Results

Size, shape and trait variation

Guppies from the six populations exhibited a 3.6-fold mass range in males (0.053–0.193 g, *N*=168), a 6.7-fold mass range in females (0.082–0.553 g; *N*=147), and a 10.4-fold mass range overall (*N*=315). In general, guppies did not match the geometrical expectation of body mass varying with the cube of standard length (Fig. 1). On average within the 12 groups analyzed (6 populations, 2 sexes), mass increased slightly more rapidly with length than predicted (exponents relating length to mass ranged from 2.59 to 3.62 and averaged 3.12), indicating that small individuals had a ‘slimmer’ shape than larger fish (t=2.64, N=12, P=0.0248 that the exponent=3.0). Both locale and drainage affected shape ($F_{5,308}$=60.2, P<0.000001; $F_{2,308}$=130, P<0.000001, respectively).

However, there were no shape differences between the sexes (P=0.80), no interaction between sex and either locale (P=0.77) or drainage (P=0.64), and no effect of predation regime on shape (P=0.58). Shape did not affect burst speed after correcting for body size (expressed as either *SL* or *M*; P>0.22) but was significantly correlated to $\dot{V}_{O_2max}$ (P=0.0224, ANCOVA with *SL* as the other covariate, P=0.0000398, ANCOVA with *M* as the other covariate).

All organ size measurements (heart, gill and swimming motor mass) and both performance traits were positively correlated with body size in both sexes (Table 1), but most variables scaled allometrically with mass (Table 2). Among enzymatic variables, LDH activity scaled positively with mass in both sexes, while CS activity scaled negatively with mass in males. There were no significant effects of size on CS activity in females or on myofibrillar ATPase activity in either sex.

The range of variation in mass-adjusted organ size and enzyme activity indices was 5–32% (Figs 2, 3), with the lowest variation in swimming motor mass. After removing the effects of mass (Table 3), neither $\dot{V}_{O_2max}$ nor burst speed was significantly correlated to any single morphological or biochemical variable in either sex. In addition, $\dot{V}_{O_2max}$ and burst speed were not significantly correlated to each other (i.e. there was no evidence of a trade-off between burst and aerobic performance). Gill and heart mass, and CS and LDH activities, were positively correlated in both sexes but no other mass-adjusted variables were consistently related in both males and females.

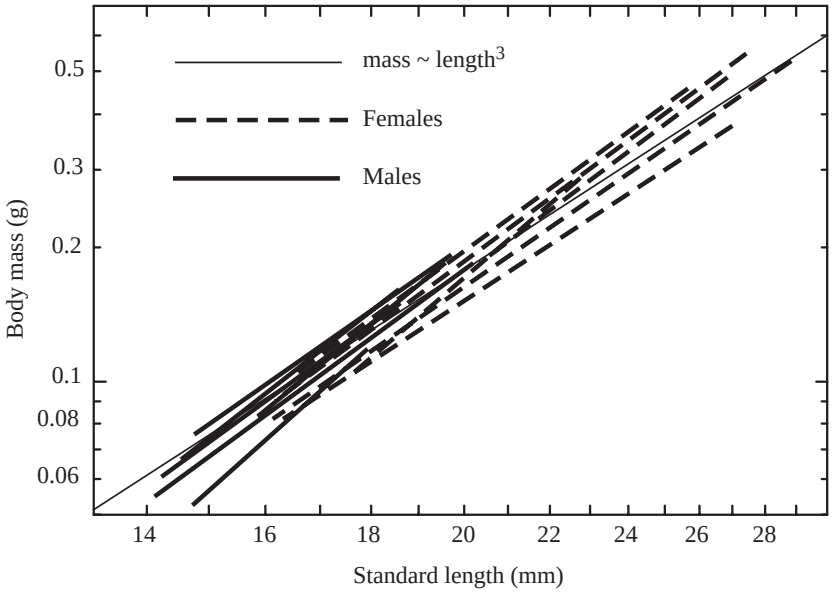

Fig. 1. Relationship between length and body mass in guppies from six populations. The expected scaling for geometrically similar shapes (mass varies as length^3) is shown by the thin diagonal line. The following populations had length exponents significantly different from 3.0: Quare males (3.17, P<0.05); Yarra Tributary males (3.54, P=0.01); Oropouche females (3.62, P<0.05); Quare females (3.26, P<0.05); Yarra Tributary females (3.31, P<0.01).

Table 2. *Mass scaling exponents for organ size and enzymatic variables in Trinidadian guppies (slope ± S.E.M.)*

	Females	Males
$\dot{V}_{O_2max}$	0.753±0.040*	0.782±0.054*
Burst speed	0.216±0.035*	0.149±0.043*
Swimming motor mass	0.998±0.014	1.11±0.013*
Heart mass	0.871±0.056*	0.722±0.096*
Gill mass	0.824±0.044*	0.872±0.085*
CS activity	0.097±0.049	–0.116±0.046*
LDH activity	0.278±0.069*	0.693±0.086*
ATPase activity	0.081±0.056	0.014±0.083

Abbreviations as in Table 1.

Asterisks indicate scaling factors that differ significantly from isometry (i.e. scaling to body mass^{1,0} for organ size, or to body mass^0 for enzyme activities, which were corrected for the mass of protein in tissue samples).

Note that CS and ATPase activities in females and ATPase activity in males do not change significantly with body mass.

N=133–147 for females (N=99 for ATPase) and 160–168 for males.

Relationships among groups of variables

Principal components analysis indicated that interactions among groups of morphological and biochemical variables were complex, inconsistent between males and females, and largely unrelated to burst or aerobic performance. PCA yielded two significant component axes for each sex (Table 4). The fraction of variation in the data set accounted for by retained components was 54.6% in males and 60.5% in females.

In males, the first PC axis (PC1) was described by heart, gill, swimming motor, CS and LDH residuals. Heart and gill residuals were negatively related to swimming motor mass, CS and LDH residuals. The second PC axis (PC2) was characterized by residual ATPase activity at one end, and gill, CS and LDH residuals at the other.

In females, ATPase was not included because of flawed data from Yarra fish. In the remaining 99 females, PC1 was described by a negative relationship between heart and gill residuals and the enzyme residuals (LDH and CS). The second axis described a negative relationship between swimming motor residuals and heart and CS residuals.

Multiple regression of performance on composite PC variables explained no more than 5% of performance variation (Table 5) and was significant for only one analysis: in males, there was a slight but significant negative relationship between residual $\dot{V}_{O_2max}$ and PC1, suggesting that aerobic performance in males increases with heart and gill mass and decreases with

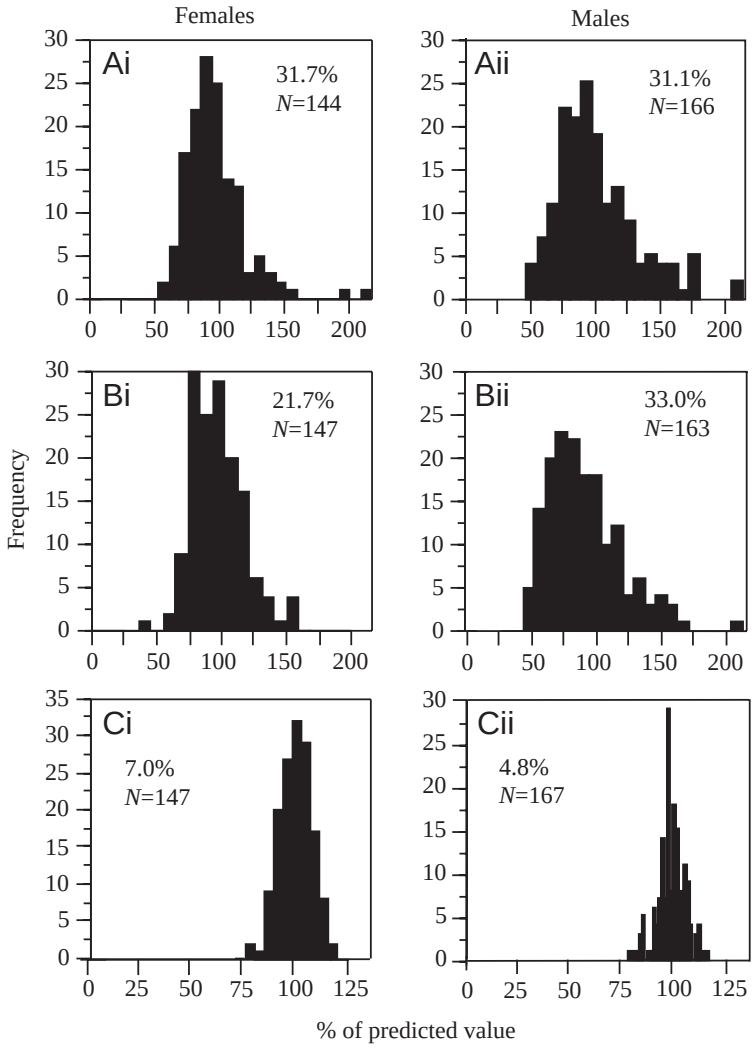

Fig. 2. Organ mass variation of (A) heart, (B) gill and (C) swimming motor in male and female guppies. Data are expressed as percentage of predicted values from a mass regression (a value of 100% indicates that the observed value equals the predicted value). Average percentage differences from predicted values are shown for each variable.

increasing swimming motor mass and muscle enzyme activities.

Population and drainage comparisons

All organ mass variables and most enzymatic variables (except myofibrillar ATPase activity) differed significantly among drainages, and most variables in both categories were affected by sex (Tables 6, 7). The range of variation in drainage means (minimum to maximum) was 8% for swimming motor mass, 30% for heart mass, 34% for gill mass, 16% for CS activity and 29% for LDH activity (Table 8). There were numerous significant interaction terms that included drainage effects.

In contrast to the widespread effects of sex and drainage, only the size of the swimming motor and (with marginal significance) LDH differed between low- and high-predation populations. On average, fish from low-predation environments had slightly but significantly larger swimming motors (64.8% of body mass) than did fish from high-predation environments (63.1% of body mass), and this effect was apparent in all three drainages. Males had significantly larger swimming motors than females (67.2% *versus* 60.6% of body mass, respectively) but the relative magnitude of the difference between predation regimes was about the same for both sexes. LDH activity averaged about 6% higher in low-predation populations.

There was substantial variation in PC variables among populations, but little consistency with predation regime (Fig. 4). Male PC1 scores differed in naturally occurring high–low predation pairs (Oropouche and Yarra drainages). However, this pattern was not observed in the Caroni drainage, which was field-manipulated within the last 25 years (Reznick and Bryga, 1987). Males from the Oropouche drainage had the highest overall PC1 scores, consistent with greater swimming motor mass and LDH and CS

Table 3. *Size-adjusted correlations (based on residuals from body mass regressions) for performance, organ mass and enzyme activity*

		Mass				Enzyme activity		
	$\dot{V}_{O_2max}$	Burst speed	Heart	Gill	Swim motor	CS	LDH	ATPase
$\dot{V}_{O_2max}$		0.147	–0.071	–0.127	0.004	–0.085	–0.113	–0.067
Burst speed	0.027		0.038	–0.142	–0.073	0.210*	0.045	–0.229*
Heart mass	0.161*	–0.116		**0.282***	–0.116	0.129	–0.054	0.041
Gill mass	0.177*	–0.083	**0.365***		0.133	0.086	–0.029	0.207*
Swim motor mass	–0.075	0.123	–0.107	**–0.237***		–0.152	0.143	0.127
CS	–0.014	0.058	0.095	0.052	–0.005		**0.340***	–0.137
LDH	–0.133	0.161*	–0.195*	–0.210*	**0.362***	**0.245***		0.164
ATPase	0.104	–0.073	–0.037	–0.163*	–0.227*	–0.015	–0.191*	

Results for females are in the upper right and results for males are in the lower left.
Sample sizes, abbreviations and symbols as in Table 1; the adjusted α (Rice, 1989) was 0.00278.

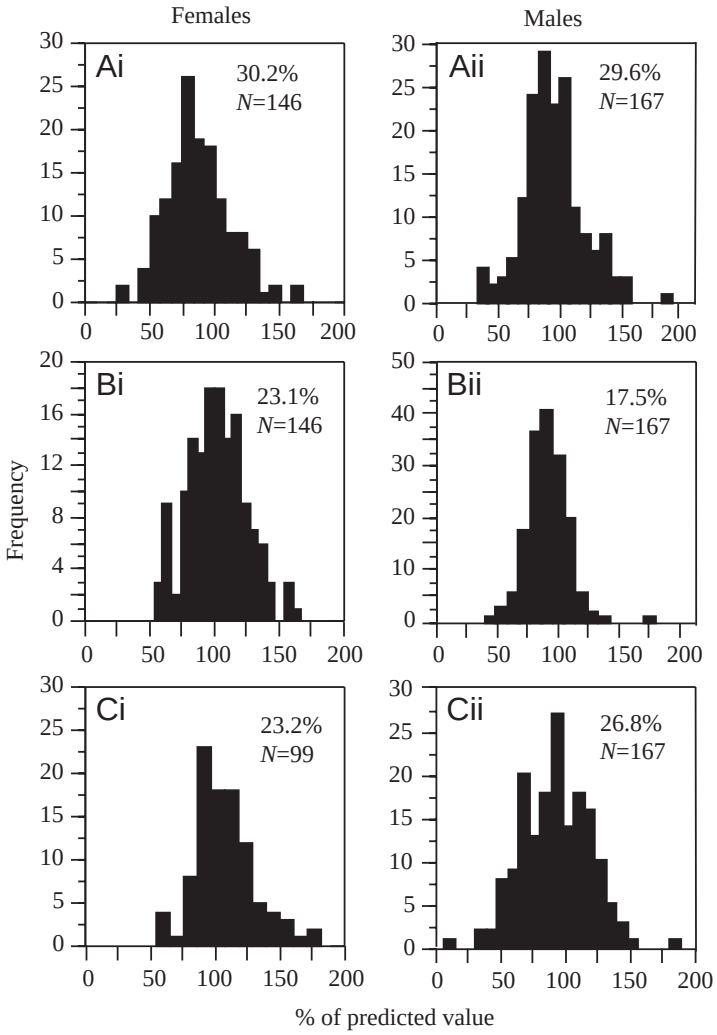

Fig. 3. Variation in the activities of (A) lactate dehydrogenase (LDH), (B) citrate synthetase (CS) and (C) myofibrillar ATPase (ATPase) in male and female guppies. Data are shown as percentage of predicted values from a mass regression (LDH, male CS) or as percentage of mean value (ATPase; female CS). The average percentage difference from predicted values is shown for each variable.

activities. PC2 varied with predation regime but not between drainages. In the naturally occurring stream pairs (Oropouche and Yarra), low-predation guppies had higher PC2 scores, suggesting that they have increased gill mass and CS and LDH activities. Guppies in high-predation locales had higher myofibrillar ATPase activities.

Significant drainage effects occurred in both component scores in females. Females from high-predation regimes had significantly higher PC1 scores on two of the three drainages. Interestingly, this difference was strongest in the experimentally manipulated Caroni drainage. In general, females from high-predation locales had higher residual activities of CS and LDH and smaller gill and heart masses. PC2 scores differed in only the Yarra drainage, with higher PC2 scores in upstream (low-predation) fish. This suggests that low-predation Yarra females had larger hearts and higher CS activities than high-predation Yarra females. Oropouche females had the lowest overall PC2 scores, indicating that they had the highest residual swimming motor mass.

Table 4. *Results of principal components analysis for organ mass and enzyme activity: factor loadings for retained components (PC1, PC2)*

	Females		Males	
	PC1	PC2	PC1	PC2
Aerobic				
Heart	**–0.394**	**0.688**	**–0.684**	0.313
Gill	**–0.698**	0.172	**–0.604**	**0.520**
CS	**0.764**	**0.344**	**0.343**	**0.643**
Anaerobic				
Swimming motor	–0.226	**–0.738**	**0.691**	–0.139
LDH	**0.762**	–0.051	**0.706**	**0.478**
ATPase	–	–	–0.120	**–0.553**
Explained variance	0.371	0.234	0.324	0.222

Traits are sorted into aerobic (heart, gill, CS) and anaerobic (swimming motor, LDH and ATPase) sectors; the initial hypothesis was that factor loadings would correspond to these sectors. ATPase is absent from female PC axes because of unreliable data from one drainage. The combined components explained 60.5% of total variance in females and 54.6% of total variance in males.

Symbols and abbreviations as in Table 1.

Discussion

Microevolution of life histories in guppy populations in the river systems of Trinidad is a textbook case of Darwinian adaptation in response to natural selection (e.g. Campbell and Reece, 2001). Impressively large shifts in resource allocation between somatic and reproductive functions have evolved along trajectories predicted by life

Table 5. *Multiple regression of factor scores from principal components (PC1, PC2) on residual locomotor performance in guppies*

	$\dot{V}_{O_2max}$			Burst speed		
	r^2	F	P	r^2	F	P
Males						
Overall	0.051	4.23	0.0163	0.019	1.48	0.23
PC1		8.17	0.0048		2.38	0.125
PC2		0.29	0.59		0.56	0.46
Females						
Overall	0.003	0.20	0.82	0.005	0.32	0.73
PC1		0.40	0.53		0.08	0.77
PC2		0.01	0.96		0.56	0.46

$\dot{V}_{O_2max}$, maximum oxygen consumption.
F and P values are shown for each component axis.
N=159 for males and 142 for females.

Table 6. *Guppy organ mass as a function of predation regime, drainage and sex (ANCOVAs with body mass as covariate)*

		Swimming motor mass		Heart mass		Gill mass	
Effect	d.f.	*F*	*P*	*F*	*P*	*F*	*P*
Predation	1,302	25.3	<0.000001	0.60	0.44	0.05	0.83
Drainage	2,302	32.3	<0.000001	12.1	0.000009	56.6	<0.000001
Sex	1,302	44.2	<0.000001	0.54	0.46	14.8	0.00015
Predation × drainage	2,302	1.77	0.17	0.30	0.74	1.52	0.22
Predation × sex	1,302	4.96	0.027	0.009	0.92	0.24	0.62
Drainage × sex	2,302	3.24	0.041	0.66	0.52	13.1	0.0000036
Predation × drainage × sex	2,302	10.6	0.000036	1.15	0.32	8.55	0.00024

N=315 for all variables.

history models, and field experiments show that rates of evolution can be surprisingly rapid (Reznick and Bryga, 1987; Reznick et al., 1997). Much evolutionary theory (especially life history theory; Stearns, 1992; Roff, 1992) and many concepts in comparative physiology are based on the idea of trade-offs, so it is reasonable to expect that guppy populations with different levels of reproductive effort, offspring size, maturation rate, etc. could experience pleiotropic changes in traits based on somatic investment, such as locomotor and aerobic performance. Also, selection from the different predation regimes in upstream and downstream habitats might be expected to affect burst performance directly (O'Steen et al., 2002) and affect aerobic capacity indirectly (through trade-offs between fast-twitch anaerobic muscle fibers used in burst swimming *versus* slow-twitch oxidative 'red' fibers powering aerobic performance). In partial support of that idea, our previous work (Odell, 2002; Odell and Chappell, in press) revealed significant variation in aerobic capacity among guppy populations, although we did not find differences in burst speed. Similarly, Cullum and Bennett (1995) found that wild-caught guppies from high-predation populations were faster than fish from low-predation populations, but the difference did not persist in 'common-garden' laboratory-reared fish (A. J. Cullum, personal communication). In terms of functional causality, considerable work with a range of species suggests that variance in organismal-level performance traits can often be explained by correlated variance in organ size or enzyme function (e.g. Hulbert and Else, 1981; Garland and Else, 1987; Kersten and Piersma, 1987; Daan et al., 1990; Weibel et al., 1991; Piersma et al., 1996; Bennett, 1997; Chappell et al., 1999; Hammond et al., 2000).

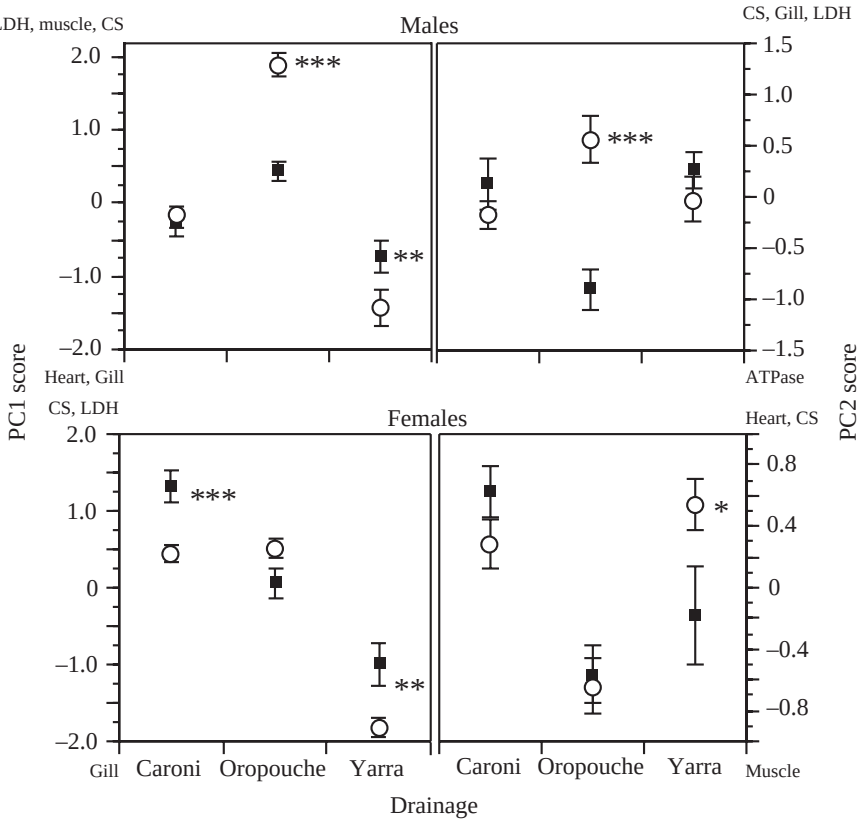

Fig. 4. Principal components scores by population for male and female guppies. Asterisks show significant differences from planned comparisons (**P*<0.05; ***P*<0.01; ****P*<0.001). High predation locales are represented by solid squares; low predation locales are indicated by open circles; values are means ±1 S.E.M. Variables with significant factor loadings are indicated on each axis. Differences between drainages were significant in both analyses, but cannot be distinguished unequivocally from block effects.

Accordingly, we began our study of guppy locomotor physiology expecting to find positive correlations between aerobic performance and aerobic organs and enzymes (heart and gill mass; CS activity), positive correlations between burst performance and glycolytic organs and enzymes (swimming motor mass; LDH and myofibrillar ATPase activities), negative correlations (trade-offs) between

Table 7. *Enzymatic activities in guppy swimming muscle as a function of predation regime, drainage and sex*

		CS activity		LDH activity		ATPase activity	
Effect	d.f.	*F*	*P*	*F*	*P*	*F*	*P*
Predation	1,302	0.36	0.83	4.13	0.0431	2.20	0.139
Drainage	2,302	18.7	<0.000001	23.0	<0.000001	0.001	0.98
Sex	1,302	34.0	<0.000001	3.33	0.069	36	<0.000001
Predation × drainage	2,302	13.0	0.0000037	20.5	<0.000001	14.9	0.00015
Predation × sex	1,302	0.70	0.40	0.11	0.74	5.06	0.025
Drainage × sex	2,302	19.0	<0.000001	5.44	0.0048	0.65	0.42
Predation × drainage × sex	2,302	3.19	0.0423	10.6	0.000035	34.5	<0.000001

Abbreviations as in Table 1.

For CS and ATPase, results are from ANOVA; LDH was tested with ANCOVA (with body mass as the covariate), as this enzyme varies with body mass (Table 1).

N=217 for ATPase and 315 for CS and LDH; ATPase results exclude the Yarra drainage because of flawed data from Yarra females.

burst and aerobic traits, and consistent differences between morphology and enzyme activities between upstream (low-predation) and downstream (high-predation) populations. Somewhat to our surprise, the results gave little support to any of these predictions.

Size effects on performance

Both of the measured locomotor performance indices were strongly correlated to body size (and consequently organ size; Tables 1, 2): as expected, large guppies have higher absolute aerobic capacities and can swim faster than small guppies. However, after correcting for body size, there were few significant correlations between either $\dot{V}_{O_2max}$ or burst speed and any organ size or enzymatic variable (Table 3), and Bonferroni correction for multiple simultaneous tests suggests that the small number of significant individual correlations may be statistical artifacts (Type I errors).

Aerobic and anaerobic trait associations

We found several significant associations among mass-adjusted morphological and enzymatic traits (Table 3), but only the positive relationships between heart and gill mass, and between LDH and CS activities, occurred in both sexes. The correlation between heart and gill – two organs closely associated with oxygen uptake and transport – is not surprising, although neither organ significantly predicted locomotor performance.

We did not anticipate the positive correlation between LDH and CS, since these enzymes (indicative of glycolytic and oxidative muscle fibers, respectively) were expected to exhibit a trade-off. Lack of such a trade-off could have several causes. At the whole-animal level, amounts of oxidative and glycolytic fibers may be positively correlated because of individual differences in total muscle mass. However, CS and LDH remained significantly correlated (P<0.006 in both sexes) after inclusion of swimming motor mass in the regression (which should largely account for differences in muscle mass). Alternatively, since glycolytic fibers are the predominant fiber type in the guppy swimming motor (Veggetti et al., 1993), these results may reflect a positive correlation between LDH and CS activity within glycolytic fibers, as has been shown in interspecific comparisons of fish muscle (Dickson et al., 1993;

Table 8. *Differences between sexes and drainages in organ mass and enzyme activities*

		Mass				Enzyme activity		
Drainage	Sex	Body	Swimming motor	Heart	Gill	CS	LDH	ATPase
Caroni	M	87.5 (23.8)	57.6 (17.7)	0.0450 (0.0148)	0.327 (0.145)	17.6 (3.35)	208 (60.9)	0.121 (0.036)
	F	280 (112)	162 (63.4)	0.0924 (0.0359)	0.835 (0.295)	15.8 (2.20)	336 (115)	0.0927 (0.0162)
Oropouche	M	115 (34.2)	80.8 (27.4)	0.041 (0.017)	0.315 (0.139)	16.4 (2.66)	333 (92.6)	0.119 (0.035)
	F	246 (96.6)	155 (65.6)	0.170 (0.033)	1.39 (0.286)	12.5 (3.33)	368 (110)	0.0968 (0.0265)
Yarra	M	120 (27.2)	80.5 (19.1)	0.0645 (0.030)	0.510 (0.164)	15.6 (2.58)	274 (70.8)	0.116 (0.022)
	F	290 (96.9)	176 (53.8)	0.103 (0.053)	1.17 (0.395)	16.3 (3.31)	377 (130)	

Values are means ± S.D. (in parentheses).

Abbreviations as in Table 1; units are mg wet mass (body and swimming motor), mg dry mass (heart and gill), and i.u. mg^{-1} protein (CS, LDH, ATPase).

After correction for mass effects, differences between drainages were significant for all variables except ATPase, and differences between sexes were significant for body mass, swimming motor mass, CS and ATPase (excluding Yarra drainage fish).

Dickson, 1995). Small animal size and use of whole-muscle homogenates did not allow enzyme activities to be assayed directly and independently from each fiber type.

Other unexpected findings were a negative correlation between LDH and myofibrillar ATPase activities in males (both enzymes should have maximal activities in glycolytic fibers and hence should covary; Leonard, 1999; Watkins, 2000), and lack of correlation between ATPase activity and swimming motor size.

We hypothesized that organ sizes and enzyme activities should covary according to function and therefore expected that principal components analysis (PCA) would separate clusters of distinctly anaerobic and aerobic variables, either at opposite ends of a component axis, or on different axes. The results (Table 4) provide little support for this idea. In females, there was a distinct gradient between two aerobic variables (heart and CS) and residual swimming motor mass (an 'anaerobic' trait due to the high fraction of glycolytic fibers) on the second PC axis, but a very different pattern occurred along PC1 (the extremes – gill and CS – are both aerobic traits). In males, two aerobic traits (heart and gill) were closely associated at one end of the PC1 axis, with two anaerobic traits (LDH and swimming motor mass) clustered at the other end, as expected. However, CS (an aerobic trait) and ATPase (an anaerobic trait, given its association with rapid contraction velocity and high activity in fast-twitch glycolytic fibers) were both intermediate. On PC2, there was a gradient from two aerobic traits (CS and gill) to two anaerobic traits (swimming motor mass and ATPase), but heart and LDH were not in their predicted positions. In no case did either PC axis cleanly separate all variables into the expected groupings.

In general – for both simple correlations and PCA – we did not find evidence for functional competition between aerobic and anaerobic morphological and enzymatic traits in guppies. In terms of whole-animal locomotor physiology this is not surprising, since guppies apparently are not constrained to 'trade off' aerobic and anaerobic swimming performance (as indicated by lack of negative correlation between burst speed and $\dot{V}_{O_2max}$ after mass correction; Table 3; Odell, 2002; Odell and Chappell, in press). In other words, these fish may show individual variation in combined exercise capacity (both burst and sustainable swimming), instead of being constrained to specialize in either burst speed or aerobic performance but not both. Similar results were reported for Atlantic cod *Gadus morhua* by Reidy et al. (2000): individuals with high sprint speeds during escape responses also had high maximum sustainable swimming speeds.

Mechanistic foundations of performance

Both singly and in PCA combinations, the measured organ mass and enzymatic traits accounted for only a small fraction of the variation in mass-adjusted aerobic performance in males (and essentially none in females), and no single or combined variables were significantly correlated to burst speed (Tables 3, 5). This is somewhat surprising, as similar studies in a number of species have shown substantial size-independent performance correlates with organ size or enzyme function. For example, aerobic performance (measured as $\dot{V}_{O_2max}$) is positively correlated to heart mass in house sparrows (Chappell et al., 1999), male red junglefowl (Hammond et al., 2000), and garter snakes (Garland and Bennett, 1990). Aerobic performance also correlated positively with muscle mass in house sparrows and male junglefowl, and with muscle CS activity in the latter (Hammond et al., 2000). Watkins (2000) reported a relationship between burst speed and myofibrillar ATPase in tadpoles. However, some studies found little or no correlation between whole-animal performance traits and morphology (Garland and Huey, 1987; Garland and Else, 1987; Bennett et al., 1989) or enzyme function (Gibb and Dickson, 2002). The absence of consistent patterns suggests that mechanistic factors that determine performance capacity in vertebrates may be taxon-specific or very complex.

Population differences and predation effects

In previous work we found no effect of predation regime on either burst or aerobic performance in Trinidadian guppies, but there were small differences in aerobic capacity between drainages (Odell, 2002; Odell and Chappell, in press). Other studies of guppy burst speed (Cullum and Bennett, 1995) and ability to escape capture by large predatory fish (O'Steen et al., 2002) did find differences between animals from high- *versus* low-predation environments. The contrast between those results and ours may stem from differences in measurement techniques (the superior escape ability of high-predation guppies may be due to aspects of burst performance we did not quantify, such as response time or acceleration during the early phases of the escape response) or acclimation (Cullum and Bennett used wild-caught fish instead of laboratory-reared guppies). Whatever the reason for these apparently contradictory results for whole-animal locomotor traits, morphological and enzymatic comparisons of fish from different drainages and predation regimes are of considerable interest.

We found strong effects of drainage on all three organ size indices and all but one of the three measures of enzyme activity (Tables 6–8). Use of F_2 and F_3 fish reared under identical 'common-garden' conditions makes it likely that the inter-drainage differences had a genetic basis. They may be due to genetic drift (founder effects) or to selection arising from abiotic (hydrodynamics, water chemistry, etc.) or biotic (productivity, food availability, food quality, etc.) differences between drainages. However, caution is appropriate when postulating a genetic basis for inter-drainage differences because our experimental design, although robust for comparisons of contrasting predation regimes within drainages, did not completely control for 'block' effects in comparisons between drainages.

In contrast to the numerous differences between drainages, only two variables, swimming motor mass and LDH activity, were significantly affected by predation regime, and the direction of change was counterintuitive for both: fish from high-predation populations, which presumably need additional muscle power for rapid escape from attacks, had smaller

swimming motors and lower LDH activities than fish from low-predation populations. Patterns in data for PCA axes (Fig. 4) suggest some differentiation among high- and low-predation guppies. In males, high-predation fish from both of the naturally occurring high/low predation stream pairs in our sample (Oropouche and Yarra drainages) had lower PC2 scores than their low-predation upstream counterparts, suggesting that high-predation fish had increased myofibrillar ATPase activity. However, this difference was not evident in the Caroni stream pair containing an experimentally transplanted low-predation population dating from the early 1980s (Reznick et al., 1990). In females, individuals from two high-predation sites – including the Caroni drainage – tended to have higher PC1 scores, an indication that they possessed higher overall muscle enzyme activities than low-predation animals from the same drainage. The low-predation experimental Caroni population was established recently, so differences between these fish and their ancestors from high-predation downstream Caroni habitats have evolved relatively quickly.

In summary, our results show that large differences in guppy life history traits – including those that impact the allocation of resources to reproductive or somatic functions – can evolve with little or no concomitant effects on locomotor and aerobic performance and its underlying physiological mechanisms. We found only two clear contrasts between guppies from low- and high-predation populations: small differences in the relative size of the swimming motor and in LDH activity. Those differences – a larger swimming motor and higher LDH activity in low-predation populations – are opposite to what would be expected from selection on escape ability (e.g., O'Steen et al., 2002), but fit one prediction of life history theory (reduced somatic investment in populations with high mortality rates).

In that context it is appropriate to emphasize the crucial role of body size in locomotor performance as well as life history. In our study, both measures of performance were strongly correlated with animal mass: larger guppies swam faster and had higher aerobic capacity than smaller individuals. Given the ecology of guppies in Trinidad, size, locomotor performance and life history interact in complex and sometimes contradictory ways. Guppies grow more rapidly in high-predation downstream habitats than in low-predation upstream sites (apparently because of differences in food availability, not genetic factors; Rodd and Reznick, 1997; Reznick et al., 2001). However, because of higher mortality rates (Reznick et al., 1996), guppies in downstream populations are, on average, younger and hence smaller than guppies from upstream populations (Rodd and Reznick, 1997). Our data on the effects of size on swimming performance indicate that, despite strong predator-mediated selection on escape ability (O'Steen et al., 2002), guppies from high-predation downstream habitats are probably, on average, slower in absolute burst speed than their larger counterparts from upstream low-predation sites.

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