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SYMPOSIUM

Predator-Induced Phenotypic Plasticity in Metabolism and Rate of Growth: Rapid Adaptation to a Novel Environment

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Synopsis Novel environments often impose directional selection for a new phenotypic optimum. Novel environments, however, can also change the distribution of phenotypes exposed to selection by inducing phenotypic plasticity. Plasticity can produce phenotypes that either align with or oppose the direction of selection. When plasticity and selection are parallel, plasticity is considered adaptive because it provides a better pairing between the phenotype and the environment. If the plastic response is incomplete and falls short of producing the optimum phenotype, synergistic selection can lead to genetic divergence and bring the phenotype closer to the optimum. In contrast, non-adaptive plasticity should increase the strength of selection, because phenotypes will be further from the local optimum, requiring antagonistic selection to overcome the phenotype–environment mismatch and facilitate adaptive divergence. We test these ideas by documenting predator-induced plasticity for resting metabolic rate and growth rate in populations of the Trinidadian guppy (*Poecilia reticulata*) adapted to high and low predation. We find reduced metabolic rates and growth rates when cues from a predator are present during development, a pattern suggestive of adaptive and non-adaptive plasticity, respectively. When we compared populations recently transplanted from a high-predation environment into four streams lacking predators, we found evidence for rapid adaptive evolution both in metabolism and growth rate. We discuss the implications for predicting how traits will respond to selection, depending on the type of plasticity they exhibit.

Introduction

During range expansions or colonizations of novel environments, populations may experience directional selection pressures that result in contemporary adaptation (Reznick and Ghalambor 2001). Traditional evolutionary models predict that directional selection on standing genetic variation will move the mean phenotype toward a new adaptive peak (Lande 1979; Lande and Arnold 1983; Barrett and Schluter 2008). However, many traits are differentially expressed across environments, such that novel environments can also induce phenotypic plasticity. Given that phenotypic plasticity results in predictable phenotypic responses across environments, and populations can harbor genetic variation in the form of genotype–environment interactions (G × E) (Falconer 1981), selection can act on plastic traits and produce adaptive responses (Schmalhausen 1949; Bradshaw 1965). Thus, novel environments can simultaneously impose directional selection and induce plasticity, yet traditional models and empirical studies of adaptive evolution tend to ignore an explicit role for plasticity.

How might selection and plasticity jointly shape adaptive evolution? Quantifying plastic responses within the native or historic range of environments of a population can provide insight into the ancestral patterns of plasticity (Schlichting and Pigliucci 1998;

Conover et al. 2009), and be compared with derived patterns of plasticity when a population moves into a new environment (Ghalambor et al. 2007; Conover et al. 2009). When the local optimum in the new environment is known, insight can be gained into whether the pattern of plasticity in the ancestral population exhibits adaptive or non-adaptive plasticity. For example, threespine sticklebacks (*Gasterosteus aculeatus*) have colonized freshwater lakes and evolved different trophic morphologies in limnetic and benthic habitats that in part reflect adaptation to different diets (Schluter 1993). However, trophic morphology is also plastic, such that when sticklebacks are forced to feed on the preferred food of a limnetic or benthic ecotype, they develop a trophic morphology that makes them phenotypically more similar to these respective ecotypes (e.g., Day et al. 1994; Day and McPhail 1996; Wund et al. 2008). Thus, the plastic response to feeding on a particular diet appears adaptive as it is in the same direction of evolutionary change. When the environment induces plastic changes that are in the same direction favored by selection, it is termed synergistic (Falconer 1990) and can result in cogradient variation in which there is a positive covariance between genetic and environmental influences on the phenotype (Conover et al. 2009). In contrast, many non-adaptive plastic responses that often are induced by stress, or poor-quality environments that result in physiological limitations (e.g., temperature, limited nutrients, and predators) result in antagonistic selection (Falconer 1990) because the environment induces a plastic response that is opposite the direction of selection. Such antagonistic selection in turn can lead to the evolution of countergradient variation, where there is a negative covariance between genetic and environmental influences on the phenotype (Levins 1968; Conover et al. 2009). For instance, lower temperatures and low availability of food often favor the evolution of faster growth in many ectotherms, but the plastic response to these environments results in slower growth (Berven 1982a, 1982b; Conover and Present 1990; Conover and Schultz 1995; Finstad et al. 2004a; Conover et al. 2009). In such cases of countergradient variation, selection must overcome the plastic response, which often leads to cryptic adaptive evolution (Grether 2005; Conover et al. 2009). The implications of such adaptive and non-adaptive plasticity for adaptive evolution remain a largely unexplored area, but are likely to be important during the early stages of adaptive divergence when directional selection is strong.

Plasticity and evolution of energy budgets in novel environments

Constraints and trade-offs from allocating finite energy budgets form central tenets of life-history theory (Stearns 1992). For instance, organisms allocate energy stores between reproduction and somatic growth only after meeting the energy requirements of self-maintenance, or resting metabolism (Myrick 2011). Thus, the evolution of a relatively high or low cost of self-maintenance may reflect variation in the availability of resources and the energy costs associated with their acquisition, and we would expect individuals to exhibit plasticity in response to environmental conditions that alter either the availability or the acquisition of resources (e.g., risk of predation, availability of food, and temperature).

Resting metabolic rate (RMR), the minimum energy expenditure achieved in a post-absorptive (i.e., digestive) state (reviewed by Burton et al. 2011), represents the energetic cost of self-maintenance. RMR is considered to be central to evolutionary and ecological physiology because it dictates the residual resources available for competing physiological functions (e.g., activity, growth, and reproduction) (Garland and Carter 1994; McNab 2002; Ricklefs and Wikelski 2002). However, there are competing predictions on how evolution shapes the cost of self-maintenance (reviewed by Burton et al. 2011). The ''compensation hypothesis'' views RMR as a direct cost of maintenance and, therefore, predicts that organisms with a low relative RMR can increase their allocation of energy to growth and reproduction and have higher relative fitness (Gadgil and Bossert 1970; Nilsson 2002; Boratyński and Koteja 2010). In contrast, the ''increased intake hypothesis'' predicts that individuals with a high relative RMR will have higher relative fitness due to their propensity for greater intake of energy and thus turnover (Nilsson 2002; Boratyński and Koteja 2010). In the latter case, higher RMR is due to increases in organ mass (Daan et al. 1990; Konarzewski and Diamond 1995; Speakman and McQueenie 1996; reviewed by Blackmer et al. 2005) and facilitates greater maximum metabolic rates that can sustain higher levels of processing and absorption of energy (McNab 1980; Thompson 1992). Although both hypotheses have some empirical support, many studies have produced conflicting or ambiguous results (Blackmer et al. 2005; Boratyński and Koteja 2010; summarized in table 1 of Burton et al. 2011).

In an effort to address how environmental variation influences the relationship between RMR and

Table 1 Sample sizes for statistical analyses of oxygen consumption and rate of somatic growth

Population	Reared without cues from a predator	Reared with cues from a predator
Oxygen consumption		
HP	24	24
Intro1	20	20
Intro2	24	24
Intro3	9	9
Intro4	13	11
LP	7	7
Somatic growth rate		
HP	51	57
Intro1	14	15
Intro2	16	16
Intro3	18	18
Intro4	20	20
LP	11	11

fitness, and reconcile the two aforementioned hypotheses, Burton et al. (2011) proposed the "context-dependent hypothesis." The context-dependent hypothesis proposes different environmental conditions that would favor either high or low RMR. For example, higher RMRs may positively correlate with fitness under favorable environmental conditions, but prove disadvantageous in unstable or poor environments in which the cost of self-maintenance may become cumbersome (Burton et al. 2011). Thus, plastic or evolutionary responses that lower RMR could serve to buffer or safeguard individuals in variable environments.

The cost of self-maintenance should also be correlated with other traits related to fitness, such as survival, growth, and reproduction (Stearns 1992; Ricklefs and Wikelski 2002). Despite some ambiguity in the relationship between RMR and fitness, growth rates can be susceptible to variation in RMR and can in turn affect fitness (Arendt 1997; Metcalfe and Monaghan 2003; Auer et al. 2010). Growth rate may also be decoupled from RMR and evolve independently in response to different environmental conditions. For example, faster growth and early maturity should be favored in environments with high extrinsic mortality despite any costs that may be incurred (Arendt 1997; Metcalfe and Monaghan 2003; Auer et al. 2010). Conversely, when extrinsic mortality is low, lower growth rates may be favorable, allowing individuals to mature in better condition than can be achieved with faster growth rates. Collectively, these hypotheses and perspectives argue for a potentially important link between fitness and metabolism, as well as the potential for plasticity in metabolic rates. Nevertheless, few studies have simultaneously evaluated plasticity and evolution in metabolic rates along with potentially correlated traits such as growth rate. We take such an approach here.

Trinidadian guppies as a model system

In natural populations of Trinidadian guppies (*Poecilia reticulata*), suites of life-history, behavioral, and morphological traits exhibit differences across populations that are consistent with adaptive responses to differences in extrinsic mortality among environments (Endler 1995; Reznick et al. 1996; Magurran 2005). We tested for evolutionary divergence and phenotypic plasticity in RMR and growth rate in populations of Trinidadian guppies (hereafter, guppies). Specifically, we used a combination of field-transplant experiments and laboratory common gardens to measure plastic and evolved responses. Guppies are particularly suited to study the evolution and maintenance of RMR due to the contrasting environments in which natural populations occur. Guppies in high-predation locales co-occur with a suite of piscivorous predators and have high extrinsic mortality. In contrast, low-predation habitats are associated with lower extrinsic mortality, but the quality of resources can be poor (Reznick and Endler 1982; Reznick et al. 2001; Zandonà et al. 2011; El-Sabaawi et al. 2012). Under laboratory conditions, we reared male guppies in the presence or absence of chemical cues from a predator to mimic these contrasting environments and examined whether populations showed evidence for evolutionary divergence and developmental plasticity in rates of metabolism and growth. We measured these traits in a natural populations subjected to high predation and to low predation and four experimentally low-predation populations that were established with individuals from the high-predation population.

Methods

Sampling of natural and experimental populations of guppies

We sampled six populations of guppies within the Guanapo River drainage in the Northern Range Mountains of Trinidad, West Indies. The first population, hereafter referred to as HP, is a native population subject to high predation in the Guanapo River drainage that contains a variety of predator

species, including the common predator on guppies, the pike cichlid *Crenicichla frenata* (Gilliam et al. 1993; Torres-Dowdall et al. 2012b). The second population, hereafter referred to as LP, represented a native low-predation population from the same drainage and was sampled from the Tumbason tributary of the Guanapo River where guppies co-exist with only one other species, a killifish (*Rivulus hartii*). *Rivulus hartii* are gape-limited omnivores that prey primarily on juvenile guppies (Mattingly and Butler 1994). Thirty adult females and 30 adult males were sampled from the HP population in March of 2008 and from the LP population in March of 2012. Fifty juveniles (likely between 4 and 6 weeks old given their size class) were also sampled from the HP population in March 2010. The remaining four populations were descendants of high-predation individuals from the HP population that had been experimentally introduced into four low-predation streams (within the Guanapo River drainage) that previously lacked guppies. The four introduced populations were established in 100–180 m reaches of small, first-order tributaries that contained only *R. hartii*. Waterfalls bound the upper and lower limits of each reach and were artificially enhanced (if necessary) to prevent emigration and the establishment of populations above the streams receiving introductions and immigration from downstream populations, respectively. Upstream barrier waterfalls were enhanced in two reaches (Intro2 and Intro3) and a downstream barrier was enhanced in Intro4 (see below).

Paired introductions were conducted across two consecutive years. In March 2008, HP guppies were introduced into the Lower La Laja and Upper La Laja tributaries of the Guanapo drainage (hereafter Intro1 and Intro2, respectively). Each stream was stocked with 38 gravid females and 38 mature males. To minimize the potential for founder effects and equalize genetic diversity in each stream, males and females from each random cross were introduced into alternate streams with the consequence that the introduced females carried sperm stored from the 38 males that they were mated with. Then, the females were paired in the introduction site with 38 new males. In March 2009, this protocol was replicated in the Caigual and Taylor tributaries (hereafter Intro3 and Intro4, respectively), but 45 males and females were introduced into each site. The riparian forest canopy was experimentally thinned (opened) in one stream of each pair, 6 months prior to the introductions (Kohler et al. 2012). Canopy thinning increased light levels relative to the undisturbed (closed) canopies of the paired reach (as part of a separate experiment) (Kohler et al. 2012). Collectively, the four introduced populations are called ''introduced populations'' hereafter. We did not find any significant effects of canopy thinning (data not shown). Forty juveniles were collected from each introduced population 2 years (six to eight generations) after their establishment: in March 2010 from the Intro1 and Intro2 populations, and in March 2011 from the Intro3 and Intro4 populations. These juveniles were reared to adulthood in the laboratory, and then mated to produce laboratory lines.

To minimize maternal and other environmental effects, we reared all wild-caught guppies for two generations under common garden laboratory conditions (modified from Reznick 1982) in 1.5-l tanks (Aquatic Habitats, Apopka, FL) connected to a custom-made recirculating system and maintained on a 12-h light cycle at $27 \pm 1^{\circ}C$. Fish were reared on standardized food levels adjusted weekly for age and number of individuals per tank (a.m.—Tetramin® tropical fish flakes, Spectrum Brands, Inc., Cincinnati, OH; p.m.—brine shrimp nauplii *Artemia* spp.). The quantity of food offered daily approximated *ad libitum* and was comparable to the high level of food administered by Reznick (1982).

To establish a G1 generation, when wild adults were sampled (see above), gravid females were housed individually until parturition. Each G1 brood was housed separately. Females that did not give birth within about 30–35 days of capture were randomly crossed with a wild-caught male. No two females were crossed with the same male. When juveniles were sampled from the wild (see above), guppies were anesthetized in buffered MS-222 ($0.85\ mg\ ml^{-1}$; ethyl 3-aminobenzoate methane sulfonic acid salt) (Sigma-Aldrich, St Louis, MO) and separated by sex. Juvenile females (28–56 days) can be identified by the presence of melanophores in a triangular patch that appears on their ventral abdomens, which is absent in males (Reznick 1982). After reaching maturity, each wild-caught female was crossed with a single male to produce the G1 generation. Males are considered to be sexually mature when the apical hood grows even with the tip of their gonopodium; females usually mature within ±1–2 days of males (Reznick 1990; Auer et al. 2010). The protocol was replicated on the G1 generation to produce the second (G2) generation.

Within 24 h of birth, G2 full-sibling broods were randomly assigned to two 1.5-l tanks (2–10 full siblings per tank) that differed in exposure to chemical cues from a predator (reared with or without cues

from a predator) using a split-brood design. Siblings reared with cues from predators were reared in recirculating units that housed a pike cichlid within the sump that supplied water to the tanks (Torres-Dowdall et al. 2012a; Ruell et al. 2013). Cues from predators included both kairomones and alarm pheromones, or chemical signals released from pike cichlids consuming two guppies daily. Guppies reared without cues from predators were housed in identical recirculating units without predators in the water supply. G2 juveniles were anesthetized and sexed at 29 days (see above), and one male per family per rearing treatment was randomly selected and reared individually under the same conditions thereafter.

Rates of somatic growth

Somatic growth must be measured prior to maturity because male guppies shift resources away from growth and toward reproduction (male guppies exhibit asymptotic growth that ceases shortly after maturity). After being separated by sex, G2 males were carefully dried of all free surface water and weighed for wet mass (g) at 29 days. Males were weighed again at 43 days. This time period reflects juvenile growth prior to the onset of metamorphosis of the anal fin (investing in the intromittent organ). The initiation of metamorphosis of the anal fin was observed to commence at 50 days in our fastest growing HP population (Reznick 1990; Reznick and Bryga 1996; E. Ruell, personal observation). Thus, males' somatic growth rates were measured over a 14-day period when they were housed and fed individually, and prior to the metamorphosis of their anal fins. Growth rate was calculated as the change in mass per unit time (g day^{-1}) as follows:

$$\frac{\text{mass}_{\text{final}} - \text{mass}_{\text{initial}}}{\text{time}},$$

where $\text{mass}_{\text{final}}$ is wet mass at 43 days, $\text{mass}_{\text{initial}}$ is wet mass at 29 days, and time is the 14-day growth period.

Resting metabolic rate

Male guppies were fasted for 24 h prior to measurement of their RMR. Fish reared with predator cues were transferred to tanks without predator cues 24 h prior to any respiratory measurements. Each fish was then placed in a jar-type static respirometer (fig. 10.1 of Cech 1990) using water without predator cues, and acclimated for a minimum of 60 min (range = 60–70 min). Water current was permitted to flow through each respirometer during acclimation. Respirometers were on shelves that were covered by an opaque blind throughout the experiment to minimize additional stress to the fish that could elevate O_2 consumption. A blank respirometer was measured along with each group of fish to correct for microbial respiration.

Oxygen concentrations of water samples were measured with a SI130 Microcathode Oxygen Electrode housed in a MC100 Microcell using a Strathkelvin 928 6-channel O_2 Interface connected to a PC running Strathkelvin 928 Oxygen System software (Strathkelvin Instruments Ltd, Glasgow, UK). Prior to all measurements, the O_2 electrode was calibrated with saturated water sampled from the aerated water supply (100% saturation) and anoxic water (achieved by dissolving a small amount of anhydrous sodium sulfite in the same water; 0% saturation) and adjusted for temperature and barometric pressure. Following the acclimation period, an initial measurement of O_2 concentration (mg O_2 l^{-1}) was taken. After approximately 90 min (range = 90–100 min), a second water sample was measured for O_2 concentration. $\dot{M}O_2$ (mg O_2 h^{-1}) was calculated for each individual in as follows:

$$\frac{\dot{M}O_2 = (cO_{2\text{initial}} - cO_{2\text{final}})V}{T},$$

where $\dot{M}O_2$ is measured O_2 consumption, $cO_{2\text{initial}}$ is the initial O_2 concentration, $cO_{2\text{final}}$ is the ending O_2 concentration, V is the respirometer volume (l), and T represents the time duration between initial and final O_2 sampling. Wet mass (g) of the fish was measured immediately following the measurement of cO_2 final. Mass-independent $\dot{M}O_2$ was calculated by dividing the raw data by wet mass raised to the mass exponent. The mass exponent was determined from the slope of a linear regression of log-transformed $\dot{M}O_2$ on log-transformed wet mass (Innes and Wells 1985; Cech 1990). The mass exponent was 0.508 (SE = 0.1045, $P < 0.001$). All further analyses of $\dot{M}O_2$ were performed on the mass-independent data.

Statistical analyses

We used linear mixed effects (lme) models to analyze the effects of genetic background and cues from a predator on RMR and growth rate. Variation among families in intercept was modeled as a random effect. RMR and growth rate were used as response variables and modeled with Gaussian error. Residual plots were used to visually determine whether model assumptions of normality and homoscedasticity were met. A log transformation was applied to the RMR data to adjust for deviation from homoscedasticity. All other data conformed to model

Table 2 Statistical results for the full factorial and reduced linear mixed models (fit by REML) on RMR

Fixed effect	Estimate (SE)	HPD 95% interval	pMCMC
Full factorial model			
HP (intercept)	−1.11 (0.03)	−1.16, 1.06	<0.001***
Intro1	0.01 (0.04)	−0.07, 0.08	0.89
Intro2	−0.01 (0.04)	−0.08, 0.07	0.85
Intro3	−0.02 (0.05)	−0.13, 0.07	0.64
Intro4	0.08 (0.05)	−0.01, 0.17	0.08
LP	0.08 (0.06)	−0.03, 0.18	0.17
Reared without predator's cues	−0.01 (0.04)	−0.08, 0.07	0.84
Intro1 × without predator's cues	0.08 (0.06)	−0.02, 0.20	0.13
Intro2 × without predator's cues	0.04 (0.05)	−0.06, 0.14	0.47
Intro3 × without predator's cues	0.16 (0.07)	0.01, 0.29	0.02*
Intro4 × without predator's cues	−0.002 (0.07)	−0.13, 0.12	0.97
LP × without predator's cues	0.11 (0.08)	−0.04, 0.27	0.16
Reduced model			
HP (intercept)	−1.14 (0.02)	−1.18, 1.10	<0.001***
Intro1	0.05 (0.03)	−0.01, 0.11	0.09
Intro2	0.01 (0.03)	−0.04, 0.06	0.64
Intro3	0.06 (0.04)	−0.02, 0.13	0.11
Intro4	0.08 (0.03)	0.02, 0.15	0.01*
LP	0.13 (0.04)	0.06, 0.21	0.001**
Reared without predator's cues	0.04 (0.02)	0.01, 0.08	0.03*

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

assumptions. All lme models were performed in R (R Core Team 2012) with the lme4 package (Bates et al. 2012). We tested for significance of fixed effects with Markov chain Monte Carlo sampling of the posterior distribution of the parameters using the languageR package (Baayen 2011).

The full RMR model was not significantly different between populations or treatments (Table 2). However, there was a significant interaction between the Intro3 population and experimental treatment ($P = 0.024$; Table 2). This pattern was driven by the tendency for RMR values to converge in all populations when reared with the predator cue and the Intro3 population exhibiting a greater response to the rearing environment than we observed in the other populations. Additionally, given the combination of the number of parameters estimated by the model and our sample sizes (see Table 1 for sample sizes), there was insufficient power to detect population and treatment effects. Because we were primarily interested in the direction of plasticity and evolved differences between genetic backgrounds, we also fit a reduced lme that excluded the population by treatment interaction term (Table 2). We focus our interpretation on the reduced model while acknowledging the significant interaction present in the full model.

We also examined the effect of RMR on growth rate with an lme. RMR and treatment were modeled as fixed effects and family intercept was modeled as a random effect.

Results

Resting metabolic rate

RMR was higher in the derived LP population than in the ancestral HP population (Fig. 1; Table 2 reduced model). Thus, our expectation was that introduced populations should evolve a higher RMR relative to the HP population from which they were derived. Indeed, three of the four experimentally introduced populations exhibited higher RMR (Fig. 1). However, there was also plasticity in RMR, as most of the populations tended to increase RMR when reared without the cues from a predator (Fig. 1). This pattern of plasticity also influenced the patterns of divergence between populations, as there was greater divergence between the LP and

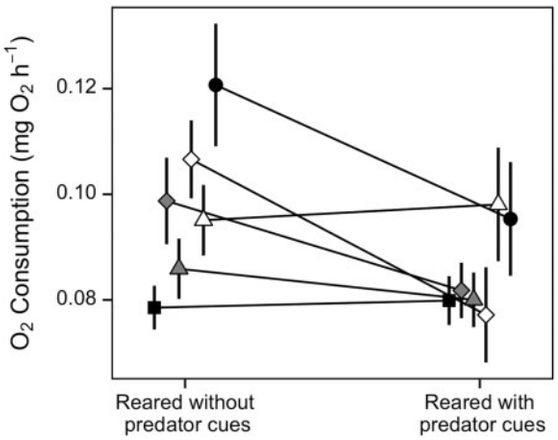

Fig. 1 Phenotypic plasticity and evolutionary divergence in mean RMR (±SE) for the ancestral high-predation population (closed squares), derived low-predation population (closed circles), and the introduced populations (Intro1 = gray diamonds, Intro2 = gray triangles, Intro3 = white diamonds, and Intro4 = white triangles) reared with and without cues from a predator.

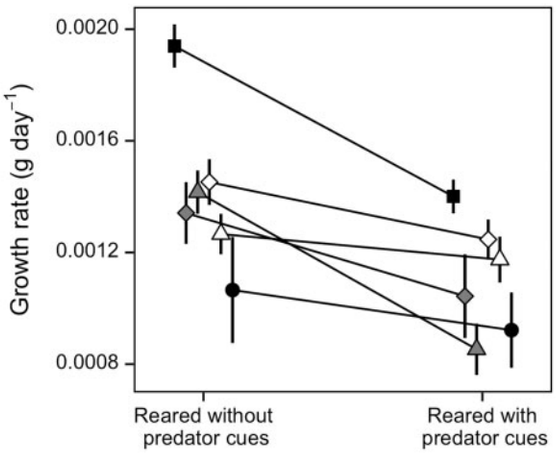

Fig. 2 Phenotypic plasticity and evolutionary divergence in mean rate (±SE) of somatic growth for the ancestral high-predation population (closed squares), derived low-predation population (closed circles), and the introduced populations (Intro1 = gray diamonds, Intro2 = gray triangles, Intro3 = white diamonds, and Intro4 = white triangles) reared with and without cues from a predator.

introduced populations and the ancestral HP population when guppies were reared without cues from a predator (Fig. 1 and Table 2). Casual observations revealed that spontaneous activity was minimal inside the respirometers and did not appear to differ between fish reared with and without cues of a predator or among populations (C. A. Handelsman, personal observation).

Rate of somatic growth

The LP population had the lowest growth rate and the HP population had the highest growth rate (Fig. 2 and Table 3), again providing an expectation that growth rate should evolve to become slower in the introduced populations. Indeed, the Intro1 and Intro2 populations exhibited slower growth than did the HP population and the Intro4 showed the same trend (Fig. 2 and Table 3). Again, as with RMR, the pattern of divergence between the experimental populations and their HP source is greater in the absence of cues from a predator (Fig. 2). This pattern arises because growth rate was plastic in response to the predator's cue, increasing when fish were reared without the cues. Variation among populations in this plasticity led to significant interaction terms in the model for the Intro3 and Intro4 populations (Table 3). These populations exhibited less plasticity in response to the rearing environment (Fig. 2). Thus, they are diverging toward the native LP phenotype but are less sensitive to the rearing environment than are the other four populations. Because the HP population had a faster growth rate than the LP population and the plastic response to predators' cues was to slow growth rate, genetic divergence in growth rate showed countergradient variation.

Relationship between rate of somatic growth and RMR

RMR was not correlated with growth rate across all individuals when controlling for the rearing environment (slope = 0.0025, $n = 7$–24 per population, $P = 0.9$). Nonetheless, the HP population had the highest growth rate and the lowest RMR, whereas the reciprocal pattern was found in the LP population. The lack of a relationship between RMR and growth rate suggests that RMR does not constrain growth rate and that these traits can evolve independently.

Discussion

Newly established populations are likely to experience the dual effects of directional selection and plasticity in response to new environmental conditions. Physiological and life-history traits may be particularly sensitive to changes in risk of predation and in availability of food, as these features of the environment are known to induce plasticity and act as sources of selection (Lima and Dill 1990; Werner and Anholt 1993; Beckerman et al. 2007; Steiner and Van Buskirk 2009). Here, we find that RMR and growth rate of guppies show a general pattern of being plastic in response to cues from predators. A

Table 3 Statistical results for the full factorial linear mixed model (fit by REML) on somatic growth rate

Fixed effect	Estimate (SE)	HPD 95% interval	pMCMC
HP (intercept)	1.4×10^{-3} (6.0×10^{-5})	1.3×10^{-3}, 1.5×10^{-3}	<0.001***
Intro1	-3.5×10^{-4} (1.3×10^{-4})	-6.0×10^{-4}, -1.0×10^{-4}	0.01**
Intro2	-5.5×10^{-4} (1.3×10^{-4})	-8.0×10^{-4}, -3.0×10^{-4}	<0.001***
Intro3	-1.4×10^{-4} (1.2×10^{-4})	-4.0×10^{-4}, 1.0×10^{-4}	0.21
Intro4	-2.3×10^{-4} (1.2×10^{-4})	-5.0×10^{-4}, 0.00	0.06
LP	-4.5×10^{-4} (1.5×10^{-4})	-8.0×10^{-4}, -2.0×10^{-4}	0.002**
Reared without predator's cues	5.5×10^{-4} (7.7×10^{-5})	4.0×10^{-4}, 7.0×10^{-4}	<0.001***
Intro1 × without predator's cues	-2.6×10^{-4} (1.7×10^{-4})	-6.0×10^{-4}, 1.0×10^{-4}	0.19
Intro2 × without predator's cues	1.5×10^{-5} (1.6×10^{-4})	-3.0×10^{-4}, 4.0×10^{-4}	0.89
Intro3 × without predator's cues	-3.5×10^{-4} (1.6×10^{-4})	-7.0×10^{-4}, 0.00	0.05*
Intro4 × without predator's cues	-4.5×10^{-4} (1.5×10^{-4})	-8.0×10^{-4}, -1.0×10^{-4}	0.01**
LP × without predator's cues	-4.1×10^{-4} (1.9×10^{-4})	-8.0×10^{-4}, 0.00	0.07

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

native LP population from an upstream tributary of the Guanapo drainage exhibited a higher mean RMR and lower mean growth rate than did the downstream ancestral HP population under common garden conditions (Figs. 1 and 2). Faster growth rates and a lower RMR in the HP population are thought to be adaptive in the face of high mortality from predators (e.g., McPeek et al. 2001; McPeek 2004). Indeed, we also found that the four experimental introductions of HP guppies into low-predation tributaries showed similar patterns of divergence within only six to eight generations (Figs. 1 and 2). These patterns of divergence and rapid evolution complement a suite of other life-history (Reznick and Endler 1982), behavioral (Seghers 1974; Endler 1995; Godin and Briggs 1996; Templeton and Shriner 2004; Torres-Dowdall et al. 2012a), and morphological traits (Layman et al. 2003; Langerhans and Dewitt 2004; Alexander et al. 2006; Hendry et al. 2006; Burns et al. 2009, C. K. Ghalambor et al., in review) that have been shown between contrasting habitats with high and low predation. Furthermore, although RMR in the HP source population was not plastic in response to cues from predators, the rapid evolution of RMR in the introduced populations also resulted in the rapid evolution of plasticity (i.e., an increase in the slope of the reaction norm; Fig. 1). In contrast, the HP source population exhibited rapid evolution of a lower growth rate without a change in plasticity (Fig. 2). However, the direction of this evolutionary change was opposite to the plastic response to being reared without the predator's cues (Fig. 2), suggesting a non-adaptive plastic response and antagonistic selection on growth. Below, we elaborate on how evolutionary divergence and plasticity in RMR and growth rate corresponds to different selection pressures.

Resting metabolic rate

Animals living under the risk of predation often face a trade-off between foraging to fulfill their energy needs and foregoing foraging to avoid predators (Lima and Dill 1990; Ball and Baker 1996; McPeek et al. 2001; Fraser et al. 2004). As a result, prey species can respond to the risk of predation by reducing their energy demands via lowering their metabolic rates (Ball and Baker 1996; Beckerman et al. 2007). Consistent with these patterns, we found that, within this drainage, a native LP population and the introduced populations generally exhibited higher RMRs than did the HP population from which they were derived (Fig. 1). The environmental effect of rearing guppies with and without predators' chemical cues on RMR generally paralleled the pattern between the ancestral HP population and the derived LP population, with RMR increasing in the absence of the predators' cues (Fig. 1). This represents adaptive plasticity as it is in the same direction as evolution. However, the ancestral HP population was not plastic in response to the cues (Fig. 1), and the evolution of plasticity for increased RMR in the absence of predators' cues followed the colonization of an LP stream by a HP population. This divergence was much stronger in the derived environment (without cues from predators), given that most populations exhibited the ancestral phenotype when reared with the cues (Fig. 1), suggesting that the evolution of plasticity is driven by selection acting on RMR in

the low-predation environment (see also Torres-Dowdall et al. 2012a).

A general assumption in predator–prey interactions is that predators constrain the activity of the prey, which in turn reduces the prey's ability to forage (Lima and Dill 1990; Brown et al. 2006). Thus, predators could serve to lower metabolism because they either induce plastic changes in activity, or act as a selection pressure that reduces activity and associated energy demands (Werner et al. 1983; Ball and Baker 1996; McPeek et al. 2001; Brown et al. 2006; Beckerman et al. 2007). Although there was certainly some spontaneous activity during our measurements of metabolic rate, we did not observe any qualitative differences between the two treatments, suggesting that guppies are plastically changing energy demands. Nevertheless, guppies are prey for larger fish throughout their range (Reznick and Endler 1982) and thus exhibit various plastic behavioral strategies in nature that reduce activity and exposure to predators. For example, guppies preferentially utilize the peripheral banks of streams, where they take shelter under vegetation and in shallow water (Seghers 1973; Reznick et al. 2001; C. K. Ghalambor et al., in review). Moreover, guppies will cease feeding at night in the presence of predators (Fraser et al. 2004). Collectively, while these behaviors may help guppies avoid predators, they also likely reduce access to food, which in turn favors lower metabolism, such that some combination of changes in metabolism and activity likely occur in nature. A prediction that arises from these results is that those individuals with higher RMRs should be more likely to engage in risk-taking behavior in order to meet their energy demands relative to those individuals with lower RMRs. In support of this, risk-taking behavior was positively correlated with RMR in the common carp (*Cyprinus carpio*) (Huntingford et al. 2010). Similarly, deprivation of food increased risk-taking behavior in European sea bass (*Dicentrarchus labrax*) in general, but individuals with the highest RMRs increased risk-taking the most (Killen et al. 2011).

Although a low metabolic rate could help offset the costs of reduced foraging in high-predation environments, other aspects of the environment may also contribute to differences in metabolism. In addition to increased predation, guppy populations in downstream environments also experience faster average velocities of water (Reznick and Endler 1982; C. K. Ghalambor et al., in review), which likely increase the energetic costs of sustained swimming. For example, Atlantic salmon parr with low RMRs outgrew conspecifics with higher RMRs when forced to forage in swiftly flowing water (Armstrong et al. 2011). Thus, selection may favor individuals with lower RMRs in high-predation environments because both they are better equipped to endure periods of food-deprivation and because they can cope with the energetic demands of higher velocities of water (Burton et al. 2011). Alternatively, competitive ability may also be positively correlated with RMRs given that RMR is positively correlated with aggression and dominance in many taxa (reviewed by Biro and Stamps 2010). Guppies in low-predation environments are thought to experience high intraspecific competition for food (Reznick et al. 2001), which could favor a higher RMR. Indeed, dominance in Atlantic salmon was found to increase proportionally with the relative difference in RMR between conspecifics (Metcalfe et al. 1995). Although we found that guppies have a higher RMR when reared without cues from a predator, this cue may also be associated with increased competition. However, given that feeding levels in this study approximated *ad libitum*, it is not clear whether the perception of ample resources triggered the observed response by the guppies reared without cues from a predator, rather than the lack of risk of predation. If so, our RMR measurements for guppies reared without cues from a predator may represent the mean RMR of each population, and the rearing environment with cues could have suppressed RMR.

Rate of somatic growth

A behavioral reduction in feeding and growth is commonly observed in animals that are under the risk of predation (e.g., Brown et al. 2006). However, a plastic reduction in growth rate could either reflect a behavioral reduction in foraging, or a physiological change that reduces growth (e.g., Beckerman et al. 2007). In contrast, life-history models and empirical evidence all predict the evolution of faster growth rates when predation and mortality are high (Stearns and Koella 1986; Fraser and Gilliam 1987; Liebold and Tessier 1991; Spitze 1991; Stearns 1992). We find evidence for both a plastic reduction in growth rate in response to cues from a predator, and the evolution of slower growth rates when the risk of predation is reduced (Fig. 2 and Table 3). The ancestral HP population had a higher growth rate than did the naturally derived LP population in the common gardens. Additionally, three of the four introduced populations showed an evolutionary response, in which growth rate became slower within six to eight

generations (Fig. 2 and Table 3). Growth rate was also plastic and increased in the absence of cues from a predator (Fig. 2). Because these fish were all kept on controlled food levels, plastic changes in growth rate occurred either because of physiological changes or because of differences in rates of food consumption (Beckerman et al. 2007).

There is precedent for the adaptive evolution and optimization of growth rate and conversion efficiency. For example, work on Atlantic silversides *Menidia menidia* by David Conover and colleagues provides an informative contrast to the growth rate results presented above. Northern populations of Atlantic silversides grow faster than southern populations by converting food to biomass with greater efficiency (Conover and Present 1990; Schultz et al. 1998). This ability to undergo rapid growth increases survival in northern populations where the growing season is short (Conover and Present 1990) but comes at the cost of predator-escape performance (Billerbeck et al. 2001; Lankford et al. 2001). Similarly, preliminary evidence suggests that guppies in high-risk environments (reared with cues of a predator) retain more of the nitrogen they consume compared with individuals reared without cues of a predator (C. Dalton, unpublished data). Populations of Trinidadian guppies with high extrinsic mortality are also under selection for faster growth, earlier maturity, and elevated predator-escape abilities compared with populations with low extrinsic mortality (Reznick and Endler 1982; Ghalambor et al. 2003; Walker et al. 2005). Yet, unlike the silversides no trade-off between growth rate and swimming performance has been found in guppies, given that they exhibit faster growth and superior predator-escape performance under high-predation compared with conspecifics that experience low predation (O'Steen et al. 2002; Ghalambor et al. 2003; Walker et al. 2005). Given the absence of cues from a predator caused an increase in metabolic rate, the concomitant increase in growth rates is likely due to increased feeding and activity. Although we did not quantify feeding or daily activity in this study, other work suggests that cues from a predator result in guppies restricting their movements to the surface of the water (Torres-Dowdall et al. 2012a), which likely reduces movement and their opportunities for foraging.

What is the consequence of this plasticity for the evolution of growth rate? Because growth rates increased when fish were reared without cues from a predator, but evolved to be lower when risk of predation was reduced, these patterns reflect nonadaptive plasticity and antagonistic selection, in which the plastic response is in the opposite direction of evolutionary divergence (Falconer 1990; Grether 2005; Conover et al. 2009). The presence of cues in the rearing environment suppressed growth rate in all but the Intro1 population. We also found significant $G \times E$ in the Intro1 and Intro2 populations (Table 3), which reflects their lack of plasticity in growth rate relative to the other populations (Fig. 2). Nonetheless, the HP population exhibited the fastest growth rate whereas the LP population had the slowest growth rate under both rearing environments, and the growth rates of the introduced populations were intermediate or closer to the rates of the LP population. Such results suggest that there must be relatively strong directional selection for slower growth in the absence of predators, despite the plastic response to increase growth when cues from a predator are absent (Perrin and Rubin 1990; Metcalfe and Monaghan 2003; Biro et al. 2006).

Relationship between RMR and rate of somatic growth

The cost of self-maintenance should be tightly correlated with fitness due to its influence on survival, growth, and reproduction (Stearns 1992; Ricklefs and Wikelski 2002). Within individuals, RMR has been shown to be repeatable over extended periods of time and across life-history stages (McCarthy 2000; Nespolo and Franco 2007). Thus, the constraints of RMR are likely consistent throughout an animal's life span. Yet, empirical studies often fail to find a significant correlation between RMR and growth rate (see table 1 of Burton et al. 2011). We also did not find a correlation between individual RMR and growth rate. The lack of a correlation suggests that their evolutionary trajectories are unlikely to place direct constraints on one another. However, the relationship between RMR and growth rate may be complex. Because RMR and growth rate are products of the production and allocation of energy, the mitochondrial and nuclear genomes both contribute to these phenotypes. Moreover, recent evidence suggests that intergenomic epistasis underlies variation in metabolic phenotypes through genotype–genotype–environment interactions ($G \times G \times E$) (Arnqvist 2010). Thus, RMR and growth rate could be tied to $G \times G \times E$ but testing these complex interactions is beyond the scope of this study.

Plasticity and rapid evolution in novel environments

Finite energy budgets are thought to constrain phenotypic evolution through compulsory trade-offs in

life-history traits (Stearns 1992). The ability to colonize a novel environment depends on how well phenotypes can be paired with the environment. Plasticity is undoubtedly the first response by individuals colonizing a new environment and determines the range of phenotypes selection acts on. When the environment induces adaptive plasticity, the strength of selection is by definition reduced because the phenotypes shift toward the new optimum (e.g., Price et al. 2003). In contrast, when the plasticity in non-adaptive, the strength of selection is stronger because the environment includes a greater phenotype–environment mismatch and adaptation then requires that selection overcomes the plastic response (Grether 2005; Ghalambor et al. 2007; Conover et al. 2009). Examples of adaptive plasticity, particularly in physiological traits, are rare and found primarily in morphological traits (e.g., Day et al. 1994; Day and McPhail 1996; Wund et al. 2008; reviewed by Conover et al. 2009). Yet, we showed that the HP population of guppies had lower RMR than did their naturally derived descendants in the low-predation environment. When we experimentally replicated that process in the introduced populations, we found that adaptive plasticity in RMR evolved from a non-plastic ancestor. This response shows how plasticity can evolve as a by-product of directional selection in different environments (e.g., Via and Lande 1985; Gotthard and Nylin 1995; Via et al. 1995; Czesak et al. 2006).

In contrast, non-adaptive plasticity is usually found when environmental pressures (e.g., temperature, growing season, and abundance of resources) moderate physiological rate processes (Grether 2005; Conover et al. 2009). Thus, selection favors individuals better capable of developing under adverse conditions, and when released from the environmental cue, the genotype produces elevated values of a trait and exhibits countergradient variation (e.g., Conover and Present 1990; Conover and Schultz 1995; Grether 2005). In this study, growth rate showed non-adaptive plasticity and a countergradient response between divergent populations, with the HP population having the fastest intrinsic growth rate despite the tendency for cues from a predator to suppress growth rate. However, when experimentally transplanted into low-predation streams, the ancestral HP population must have harbored sufficient additive genetic variation for selection to overcome the phenotype–environment mismatch and the introduced populations rapidly diverged in low-predation environments.

Conclusions

We found that RMR and growth rate show genetic divergence between natural HP and LP populations of guppies. These differences appear to reflect the evolution of adaptive divergence, given that the introduced populations rapidly diverged in parallel patterns toward the phenotypes of the LP population. Both traits also exhibited a plastic response to being reared in the presence or absence of cues from a predator. RMR in the introduced populations evolved adaptive plasticity that led to cogradient variation; growth rate exhibited non-adaptive plasticity that produced countergradient variation in response to the novel environment. Although, we found evidence that both traits evolved rapidly and in the direction expected, RMR only diverged in the derived environment (absence of cues from a predator) whereas growth rate exhibited a greater degree of divergence and diverged in both rearing environments. These results suggest that patterns in phenotypic plasticity and their influence on underlying genetic variance can alter the range of phenotypes exposed to selection, and likely contribute to the rate of phenotypic evolution in novel environments.

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