

Fine-scale local adaptation in life histories along a continuous environmental gradient in Trinidadian guppies

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Summary

1. Theoretical models of life-history evolution predict a continuum of fast to slow life histories, yet most of empirical support for this theory comes from studies that have considered dichotomous environments (i.e. high vs. low food, presence or absence of major predators). Although this approach has been very successful in identifying the signature of local adaptation, it might limit our ability to identify the causes of underlying patterns of phenotypic variation. By studying the variation in life-history traits along continuous gradients, we can gain better insight into the diversity of adaptations exhibited by natural populations.

2. We studied the evolution of life-history traits along a gradient of predation pressure in the Trinidadian guppy (*Poecilia reticulata*). Six localities along the Guanapo–Caroni River drainage were selected with respect to their predator community, going from upstream localities where guppies only coexist with a single gape-limited fish predator, to lowland sites where guppies coexist with a complex fish community. Along this gradient, we characterized the field pattern of phenotypic variation in age and size at maturity and reproductive effort. Further, to determine the genetic basis of this variation, we measured these traits in second-generation laboratory-born fish from the same localities sampled in the wild.

3. In nature, we found a fine-scale pattern of phenotypic variation in most life-history traits that paralleled the continuous predation gradient. In the laboratory, we observed that reproductive allocation and brood size progressively decrease while age at maturity and interbrood interval progressively increase with a reduction in the predator community, suggesting a genetic basis to the parallel patterns observed in the field for reproductive allocation and offspring number.

4. However, there were some exceptions to the observed pattern of variation. Females from one low-predation locality matured younger and reproduced more frequently than expected based upon the simple nature of the fish community. We also found significant differences between our field and laboratory results for embryo size, suggesting that this trait is highly plastic.

5. Our results imply that local adaptation in guppies occurs at a finer scale than has previously been shown. Furthermore, while our results are consistent with predator-driven life-history variation, we also find patterns of plasticity that would not be apparent in the traditional dichotomous approach.

Key-words: common garden, fecundity, phenotypic plasticity, *Poecilia reticulata*, predator–prey interactions, reproductive allocation

Introduction

Divergent natural selection can drive adaptive divergence in conspecific populations exploiting heterogeneous habitats

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(Mazer & Damuth 2001). In particular, the proliferation of life-history strategies that evolve under divergent ecological conditions has been of central interest to evolutionary biologists (Roff 1992; Stearns 1992; Charlesworth 1994). A great body of theory has been developed around the role of age-specific mortality in shaping life histories (Gadgil & Bossert 1970; Law 1979; Charlesworth 1994). High ratios of

adult to juvenile extrinsic mortality are predicted to select for faster life histories, where individuals mature younger and smaller, have increased allocation to reproduction, reproduce more frequently, have larger litters and have accelerated rates of senescence (Gadgil & Bossert 1970; Law 1979; Roff 1992; Stearns 1992; Charlesworth 1994). Extensive empirical support exists for these predictions (Roff 1992; Stearns 1992). For example, differences in adult to juvenile survival result in variation in the age at maturity and reproductive effort in brook trout, *Salvelinus fontinalis* (Hutchings 1993) and freshwater amphipods, *Hyaella azteca* (Wellborn 1994), and reduced adult mortality results in lower reproductive investment and reduced rates of senescence in Virginia opossums, *Didelphis virginiana* (Austad 1993), and in water fleas *Daphnia pulex* (Dudycha 2001).

However, despite the theoretical emphasis on a continuum of fast to slow life histories (Stearns 1992), most empirical work has focused on population comparisons at the extreme ends of an ecological gradient. For example, few studies have compared life-history traits of populations along an environmental gradient to test if traits diverge to produce a continuous variation that tracks the environmental gradient or if traits show a threshold response (Piché, Hutchings & Blanchard 2008; Walsh & Reznick 2008, 2010; Kawakami *et al.* 2011; Mariac *et al.* 2011; Tomkins *et al.* 2011). Yet, understanding how continuous variation across environmental gradients is generated and maintained has important implications to our understanding of adaptive evolution and the processes that lead to speciation (Endler 1977).

Populations of Trinidadian guppies (*Poecilia reticulata*) have served as a model system to understand how spatial environmental variation leads to divergent selection pressures and adaptive divergence between populations (Endler 1995; Magurran 2005). In particular, Trinidadian guppies show a repeated pattern of life-history divergence in response to differences in predation risk (Endler 1995). Guppies that co-occur with predators are younger and smaller at maturity (Reznick 1982a; Reznick, Butler & Rodd 2001), produce more offspring per litter (Reznick & Endler 1982), produce smaller offspring (Reznick 1982b), and allocate more resources to each reproductive event (reproductive allocation; Reznick 1982a; Reznick, Rodd & Cardenas 1996) compared to guppies in depauperate communities that experience reduced predation pressure. These patterns are consistent with predictions from theory that models how life histories evolve in response to mortality risk (Gadgil & Bossert 1970; Law 1979; Charlesworth 1994). Similarly, guppies have been an important study system for other traits thought to be under divergent selection from predators, including various antipredator behaviour (Seghers 1974; Magurran *et al.* 1992; Godin & Davis 1995; Ghalambor, Reznick & Walker 2004; Magurran 2005; Walker *et al.* 2005; Botham *et al.* 2006; Huzinga, Ghalambor & Reznick 2009), morphology (Langerhans & DeWitt 2004; Alexander *et al.* 2006) and colouration (Endler 1991; Magurran & Seghers 1994; Endler & Houde

1995; Houde 1997). Because many of these phenotypic differences have been shown to have a genetic basis, these results collectively provide strong evidence for local adaptation to high- and low-predation communities (Magurran 2005).

Despite the continuous nature of spatial environmental variation throughout the guppies' distribution, the aforementioned traits have been repeatedly studied under a dichotomy of high vs. low-predation localities. High-predation localities tend to be higher-order, low-elevation streams, containing a diverse fish community that includes multiple potential predators of guppies. On the other hand, low-predation localities often are headwater streams, with a depauperate fish community, usually including only a killifish, *Rivulus hartii*, which is considered an occasional predator of small guppies (Endler 1978; Reznick & Endler 1982). In addition to the differences in fish community composition, high- and low-predation localities differ in a number of physical and chemical characteristics that affect primary productivity and thus influence food availability for guppies (Reznick, Butler & Rodd 2001). For example, high-predation localities tend to have open canopies that increase primary productivity, causing predation risk to co-vary with reduced competition (Reznick, Butler & Rodd 2001). Thus, the inability to disentangle the effect of predation from the effects of correlated environmental variables, such as resource availability, complicates simple causal relationships between predation and divergent traits (Johnson 2002).

Several reasons exist for why past research has focused on the simple dichotomy between high- and low-predation communities and dominated the study of adaptation in guppies. First, these paired comparisons match the natural distribution of guppy populations in some drainages, where the transition from high- to low-predation habitat occurs abruptly among adjacent communities separated by a single waterfall barrier (e.g. Aripo River; Reznick 1982a). Second, variation in life-history traits is consistent and repeatable between guppies collected from localities that are easily characterized by the fish community (i.e. only *Rivulus* in low-predation localities and the presence of *Crenicichla* in high-predation localities; e.g. Reznick & Bryga 1996). Thus these contrasts have proved an effective way to assess the effects of predation on local adaptation. Finally, the focus on this dichotomy is in part the consequence of an emphasis on statistical power and efficiency. The dichotomy approach has been very productive, and virtually every attribute studied in guppies has suggested local adaptation (Endler 1995). However, this approach also risks falling short of revealing the diversity of adaptations displayed by natural populations of guppies.

In his first comprehensive exposition on the role of predation in shaping the evolution of male colouration in guppies, Endler (1978) described a gradient of fish communities that ranged from diverse communities downstream to those that contained only a single species of fish, *R. hartii*, in the headwater streams. Between these extremes lay a fairly regular sequence of communities in which there is a stepwise deletion of some species as one moves from the most diverse communities downstream to the least diverse upstream. Cor-

responding with this stepwise deletion of predators, Endler (1978) described a parallel sequence of change in male colouration, which implies that the individual species of predators had unique effects in shaping male colouration and that the difference between high- and low-predation populations was the cumulative effect of the predator community. This gradient in male colouration in guppies suggests that local adaptation in this species occurs at a much finer scale than has been traditionally addressed in the guppy system. Thus, while the high- and low-predation dichotomy has served well as a basis for studying adaptation in guppies, it does not represent the full diversity of communities experienced by Trinidadian guppies and may not represent the full spectrum of local adaptation exhibited by guppies. More importantly, if natural selection occurs at smaller spatial scales along this habitat gradient, we might be under-exploiting the opportunities that are available for using guppies as a model for studying how and why certain aspects of the phenotype evolve.

Here, we report how guppy life histories vary along a gradient defined by changes in the assemblage of fish species, with stepwise deletions of potential predators from higher- to lower-order streams. We examined life-history traits of guppies from six sites along the Guanapo River in the Northern Range Mountains of Trinidad (Fig. 1). The two end points along this river represent typical high- and low-predation localities previously considered in the bulk of the existing literature on guppy evolution. In addition to these localities, we examined guppies from three other fish communities that lie between the traditional high- and low-predation localities both in terms of location and in terms of the complexity of the predator community. We also incorporated a locality from the Caroni River, downstream of the confluence of the Guanapo and Caroni Rivers, which extends the gradient to incorporate a community with additional predators not typically included in past studies of high-predation communities.

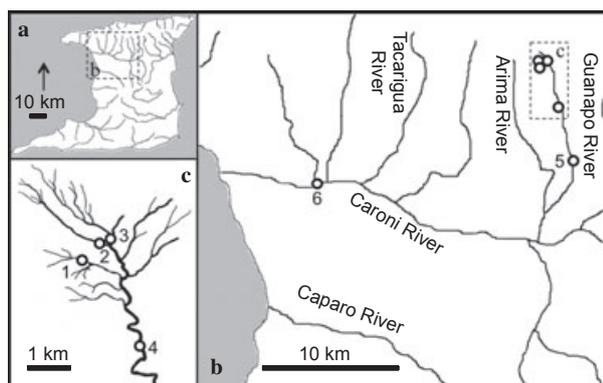


Fig. 1. Localities sampled along the Guanapo–Caroni drainage. (a) Study area within the island of Trinidad, (b) detail of the Guanapo–Caroni drainage showing the localities studied, (c) detail of the headwaters of the Guanapo River. Localities sampled are (1) Taylor; (2) Caigual; (3) Guanapo Low; (4) Guanapo Middle; (5) Guanapo High; and (6) Caroni.

Materials and methods

CHARACTERIZATION OF SAMPLING LOCALITIES

The Guanapo River is part of the larger Caroni River drainage on the south slope of the Northern Range Mountains of Trinidad, West Indies. Gilliam, Fraser & Alkins-Koo (1993) conducted a detailed survey of the fish communities along the Guanapo River, beginning near its confluence with the Caroni River and ending at its headwater tributaries (Fig. 1). At each sampling locale, Gilliam, Fraser & Alkins-Koo (1993) conducted a detailed survey of the fish community and characterized several environmental characteristics. In doing so, they have provided the most detailed description of the gradient of fish communities for any river drainage in Trinidad. This gradient is defined by changes in the assemblage of fish species, with stepwise deletions of potential predators from higher- to lower-order stream, and by correlated changes in the physical and biotic environment. To confirm the gradient in potential predation pressure on guppies initially reported by Gilliam, Fraser & Alkins-Koo (1993), we consulted other published surveys (Kenny 1995; Phillip 1998) and carried out visual surveys. In addition to the predator community, we also characterized the abiotic environment by establishing six stations, one every 50 m, along the stream at each of these six localities. At each station, we measured stream channel width and took three measures of stream depth (at one-fourth, one-half and three-fourths of the stream width). We recorded temperature, dissolved oxygen and pH at each station. We took four canopy openness readings at each station using a spherical densiometer (one reading in the direction of each cardinal point). These variables were always measured in the afternoon and taken in April 2010.

COLLECTION SITES AND HUSBANDRY OF LABORATORY STOCKS

Between January and April of 2008 and 2009, which corresponds with the dry season in Trinidad, we collected between 40 and 60 female guppies from each of the six localities with the goal of comparing life-history traits of guppy females along this gradient, both in the field and in common garden assays. Half of the females from each locality were immediately euthanized with an overdose of MS-222 (ethyl 3-aminobenzoate methane sulphonic acid salt; Sigma-Aldrich, St. Louis, MO, USA) and preserved in 5% formalin to quantify field differences among localities in life-history traits. The second half of the collected females were transported to the laboratory at Colorado State University and used in a common garden study to determine the genetic basis of the life-history variation.

We established laboratory stocks from 20 to 30 wild-caught adult females collected from the same six localities to characterize genetic differences among populations. Guppies store sperm, so each female carried viable sperm from one or more males with whom she had mated before capture. Guppy females were individually housed in either 10-L glass tanks (Taylor, Guanapo Low, Middle and High, Caroni) or 1.5-L tanks in recirculating systems (Taylor, Caigual and Guanapo High). All fish were kept on a 12 : 12 light cycle and at 25 ± 1 °C at Colorado State University. There were no differences in life-history traits between fish reared in 10-L glass tanks vs. 1.5-L recirculating tanks (all $P > 0.05$; although there was a significant interaction between population and rearing environment for offspring number, $P = 0.05$). Differences in offspring number among high- and low-predation fish were reduced, although qualitatively maintained, in 1.5-L recirculating tanks compared to 10-L glass tanks (Appendix S1 in Supporting Information). Because this interaction

would tend to mask evidence of a gradient in offspring number, we are confident that patterns of variation in offspring number among populations represent real genetic differences and, if anything, underestimate phenotypic divergence.

Throughout the experiment, all fish were kept on a quantified food diet. Each tank was fed twice daily (AM: Tertramin™ tropical fish flake paste, PM: hatched *Artemia* cysts), and each week, food levels were adjusted to reflect the increase in size of fish and the number of fish in each tank following previous protocols (Reznick 1982a; Reznick *et al.* 2004). The maximum amount of food provided to adults during the course of this experiment (25 µL) matched the high food level used previously by Reznick (1982b) and Reznick *et al.* (2004).

To minimize potential maternal and environmental effects, the laboratory study was carried out on the second-generation (G2) laboratory-born fish of wild-caught females. All wild-caught females gave birth after being isolated in the laboratory; however, the ultimate number of second-generation families derived from each wild-caught female varied among populations, with two populations being represented by <20 families (Guanapo Low = 16 and Guanapo Middle = 18). Up to 10 offspring from each G1 litter were reared together until they were old enough to be accurately identified and separated by sex (approximately 28 days). The accumulation of dark pigments in the anal area and elongation of the anal fin was used to classify females or males respectively following Reznick (1982b). Once sexed, a single female from each family was individually housed in a 10-L glass tank or a 1.5-L recirculating tank. When G1 females were large enough to be sexually mature (approximately 20 mm standard length), they were each paired randomly with an unrelated G1 male from the same population. The same protocol was repeated for G2 fish. However, as soon as they were separated by sex, G2 females were crossed weekly to determine their age at first parturition (Reznick 1982a). Thereafter, females were re-mated after each reproductive event to estimate the reproductive frequency and effort of each population. Males were added to the female tank in the evening after the PM feeding and removed the following morning before the AM feeding so that they never interfered with the food rations given to the females.

We contrasted the life histories of the field-collected and laboratory-reared individuals from each locality to gain insight into the environmental and genetic basis of variation among populations. Phenotypic values of the laboratory-reared G2 generation, propagated from wild-caught females, are interpretable as reflecting genetic differences among localities, whereas the phenotypic values of field-collected individuals should reflect both genetic and environmental effects. Thus, by contrasting these two data sets, we gain insight into the degree to which any differences between localities are because of environmentally induced plastic responses or fixed genetic differences.

LIFE-HISTORY CHARACTERIZATION

Field data

We characterized phenotypic variation in life-history traits among wild populations of guppies following previously published methods (Reznick & Endler 1982; Reznick, Rodd & Cardenas 1996). Briefly, each formalin-preserved female from our field collection was dissected and scored for the number of embryos and their stage of development following Haynes (1995). We then removed the gastrointestinal tract from each female and dried the somatic and reproductive tissues, including developing embryos, in a drying oven at 80 °C until mass was stable. From these females, we measured: (i) number

of developing embryos; (ii) mean dry weight of developing embryos and (iii) reproductive allocation, while controlling for stage of development. Mean dry weight of developing embryos was estimated by dividing the total weight of the brood by the number of embryos. Reproductive allocation (RA) was determined as the dry mass of the brood divided by the sum of the dry masses of the somatic tissue of the female and the brood. RA is thus the proportion of the total dry mass of the mother that consisted of developing embryos.

Laboratory data

In the laboratory life-history assay, we quantified: (i) age and weight at first parturition in females; (ii) interbrood interval; (iii) number of offspring in the first three broods; (iv) dry weight of offspring in the third brood and (v) reproductive allocation (proportion of total dry mass that consists of developing embryos, calculated from the dry mass of the third brood). Interbrood interval was calculated as the average time between the first three reproductive events. Upon the third parturition, females and their offspring were euthanized using an overdose of MS-222 and preserved in 200-proof ethanol. Females were then dissected, and the somatic tissue, the reproductive tissue and the preserved offspring were dried at 80 °C until mass was stable as described above for wild-caught fish. Mean lean offspring weight and reproductive allocation were calculated based on the third brood of each female.

Statistical design and analysis

We analysed field and laboratory data separately using an analysis of covariance (ANCOVA) design, including locality of origin as a categorical fixed effect. We log-transformed dry offspring mass so the data more closely approximated a normal distribution. Fecundity in guppies has been shown to increase with female size, so we included female dry mass as a covariate to model patterns of reproductive allocation, offspring number and offspring size (Reznick, Rodd & Cardenas 1996). We also included stage of development as a covariate when analysing reproductive allocation and embryo mass from wild-caught females, because offspring mass decreases as development progresses (Reznick & Endler 1982). We observed heterogeneity of slopes for reproductive allocation among wild-caught populations when we included developmental stage as a covariate (Table 2). Reproductive allocation declined as development progressed in all populations except for Guanapo High, where RA increased with development. To make inferences about the difference among populations in RA, we conducted two *ad hoc* analyses. First, by eliminating Guanapo High from the data set, we determined that the interaction of reproductive allocation with developmental stage was no longer significant and that the rank order of the remaining populations was not affected by excluding the Guanapo High population. Second, as developmental stage had a significant but small negative effect in reproductive allocation (parameter estimate = -0.006), we removed stage of development as a covariate, which did not affect the rank order of reproductive investment among populations. In Table 2, we present the result of all three analyses, but we only discuss the results of the model that included all populations without stage of development in the analysis, as results from the other models would not change our interpretation. Female size was the only covariate included when analysing offspring mass and reproductive allocation from laboratory-reared females.

We tested the effect of the deletion of predators along the environmental gradient using an *a posteriori* Tukey's HSD pairwise comparison test. By doing this analysis, we gained greater statistical power to

determine not just if there was variation among the study localities, but also if this variation paralleled the predation gradient.

Results

STREAM CHARACTERISTICS

The six localities sampled in this study show a gradient of potential predators along the Guanapo River, with downstream sites containing increasingly diverse predator assemblages relative to upstream sites (Table 1). Beginning at the headwaters, guppies at the Taylor locality co-occur with a second species of fish (*R. hartii*). The Caigual and Guanapo Low localities have two more species (*Rhamdia sebae* and *Synbranchus marmoratus*) in addition to *R. hartii*. Community complexity increases with the addition of four potential predators of guppies at the Guanapo Middle locality (*Aequidens pulcher*, *Astyanax bimaculatus*, *Hemibrycon taeniurus* and *Hoplias malabaricus*). *Crenicichla* spp., which often preys on guppies, is first found in the Guanapo High location. The Caroni River site presents a more complex community with other potential predators (*Eleotris pisonis*, *Dormitator maculatus*, *Polycentrus schomburgkii*, plus avian predation by several species of egrets, *Bubulcus ibis*, *Egretta alba*, *E. thula*; Table 1).

Several physical attributes of the environment also vary along the Guanapo–Caroni drainage. For instance, there is a gradual increase in stream width from upstream to downstream locales (Table 1, Appendix S2, Supporting Information). There is also a steep change in canopy cover from the Guanapo Low to the Guanapo Middle site, shifting from primarily closed canopy to largely open canopy sites. This step in canopy cover is correlated with the presence or absence of

major guppy predators (i.e. Taylor, Caigual and Guanapo Low vs. Guanapo Middle and High, and Caroni). We found a shift in water temperature that tracked canopy cover along the drainage, where closed canopy sites were similar in temperature and cooler than the open canopy sites (Table 1). A sharp increase in water temperature was found between Guanapo Low and Guanapo middle, and water temperature continued to increase downstream to the Caroni site (Table 1). No clear trends were observed for dissolved oxygen or pH (Table 1).

GUPPY LIFE-HISTORY TRAITS

Life-history traits showed significant phenotypic (Table 2) and genotypic variation (Table 3) among the studied localities. Except for size at maturity and offspring size, all other traits showed continuous changes along the studied gradient (Figs 2–6). We expand on these patterns below.

Age and size at maturity

Field estimates of age and size at maturity can only be approximated from the smallest size at which females begin to reproduce and was not possible here because of the poor representation of small females in the samples from most localities. However, in the laboratory, age at first parturition can more accurately be estimated and was found to vary significantly among populations (Table 3). Age at first parturition decreased along the stream gradient, being latest in the locality furthest upstream (i.e. Caigual) and becoming earlier in subsequent downstream sites (Fig. 2). The only locality not in line with this gradual change was the Taylor population, which had an earlier age at maturity than expected given the

Table 1. Description of the localities sampled along the longitudinal gradient of the Guanapo drainage. Predator community represents the new species founded at each locality going from the headwater stream (Taylor) to the Caroni River

Population	Stream order	Temperature (°C)	DO%	pH	Mean width (cm)	Mean depth (cm)	Canopy (% open)	Predator community
1 Taylor	2	23.5 (0.3)	87.5	7.48	187.54 (0.20)	6.77 (0.57)	6.56 (0.19)	<i>Rivulus hartii</i>
2 Caigual	3	23.2 (0.4)	95.1	7.81	204.47 (0.37)	24.98 (0.60)	5.28 (0.13)	<i>R. hartii</i> <i>Synbranchus marmoratus</i> <i>Rhamdia sebae</i>
3 Guanapo Low	4	23.7 (0.4)	91.3	7.75	403.43 (0.32)	30.73 (0.86)	9.90 (0.46)	All the same as Caigual.
4 Guanapo Middle	4	24.9 (0.9)	93.2	7.90	351.67 (0.31)	12.06 (0.40)	72.23 (0.29)	<i>Hoplias malabaricus</i> <i>Aequidens pulcher</i> <i>Astyanax bimaculatus</i> <i>Hemibrycon taeniurus</i>
5 Guanapo High	4	27.3 (0.8)	98.4	7.92	564.40 (0.47)	20.41 (0.59)	72.43 (0.71)	Plus all in the Guanapo Low <i>Crenicichla</i> spp. <i>Cichlasoma bimaculatum</i>
6 Caroni	5	29.4 (1.1)	69.9	7.71	1575 (19.45)	212.67 (58.34)	100.0 (0)	Plus all in the Guanapo Middle <i>Polycentrus schomburgkii</i> <i>Eleotris pisonis</i> <i>Dormitator maculatus</i> <i>Gymnotus carapo</i> Plus all that are in the Guanapo High

Table 2. Statistical results (F -values) for ANCOVA on reproductive allocation, offspring mass (mg) and offspring number in wild-caught females

	d.f.	Reproductive allocation			Offspring number	Offspring size
		Full model	Excluding GH†	W/O DS		
Locality	5	9.41***	11.00***	8.08***	11.30***	17.06***
Female size	1	1.82 ^{NS}	3.98 ^{NS}	0.06 ^{NS}	43.26***	20.55***
Interaction (L × FS)	5	0.26 ^{NS}	0.23 ^{NS}	0.51 ^{NS}	1.07 ^{NS}	1.90 ^{NS}
Developmental stage	1	6.25*	13.55***	–	–	24.97***
Interaction (L × DS)	5	4.41**	1.50 ^{NS}	–	–	1.61 ^{NS}
Residual sums of squares (d.f.)		0.26 (100)	0.26 (85)	0.34 (106)	1686.74 (106)	1.96 (100)
Total sums of squares (d.f.)		0.52 (117)	0.46 (99)	0.52 (117)	5104.27 (117)	13.54 (117)
R^2		0.49	0.49	0.34	0.67	0.86

*0.05 > P > 0.01; **0.01 > P > 0.001; *** P < 0.001.

†Degrees of freedom for population effect in the model excluding fish from Guanapo High population is 4.

Table 3. Statistical results (F -values) for ANCOVA on the life-history traits measured in the third brood of G2 females

	d.f.	Age at first parturition	Size at first parturition	Interbrood interval	Reproductive allocation	Offspring number	Offspring size
Locality	5	22.28***	14.15***	12.25***	29.07***	17.59***	14.64***
Female size	1	–	–	–	8.19**	36.62***	18.64***
Interaction (L × FS)	5	–	–	–	1.14 ^{NS}	1.15 ^{NS}	0.99 ^{NS}
Residual sums of squares (d.f.)		10 837.22 (158)	0.37 (152)	475.06 (154)	0.07 (99)	993.69 (101)	1.04 (100)
Total sums of squares (d.f.)		18 479.49 (163)	0.54 (157)	663.97 (159)	0.22 (110)	2492.06 (112)	2.81 (111)
R^2		0.41	0.31	0.28	0.66	0.60	0.63

*0.05 > P > 0.01; **0.01 > P > 0.001; *** P < 0.001.

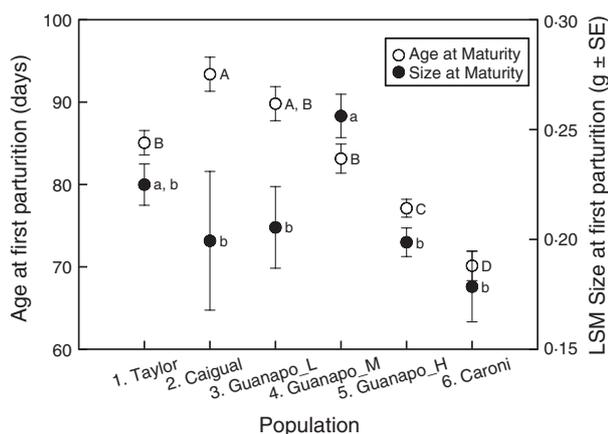


Fig. 2. Mean (\pm SE) age at first parturition (white circles) and adjusted least square mean (\pm SE) wet mass at first parturition (black circles, corrected for female age) of G2 laboratory-reared females. A clear gradient of variation is observed for age at maturity (except for Taylor), but size at maturity does not follow this pattern. Letters by population means represent Tukey's HSD pairwise comparison. Different letters imply significant least squares mean differences between populations at a $\alpha = 0.05$ (upper-case letters for age at maturity, and lower-case letters for size at maturity).

simple nature of the fish community (Fig. 2). Size at first parturition also varied among localities, but was not affected by the gradient (Table 3, Fig. 2). Females from the Guanapo

Middle locality matured at the largest size, while females from the Caroni and Caigual localities matured at the smallest size.

Reproductive effort

Reproductive effort can be separated into: (a) how frequently females reproduce (i.e. interbrood interval), and (b) how much energy females invest in each of their broods (i.e. reproductive allocation). Interbrood interval measured in the laboratory significantly varied among populations (Table 3). Females from the most downstream locality, Caroni, had the shortest interbrood interval compared to all other populations, and interbrood interval gradually increased moving upstream, from high- into low-predation localities (Fig. 3). This resulted in a significant gradient effect and is consistent with predictions that high mortality rates will favour shorter interbrood intervals.

The significant interaction between population of origin and developmental stage in the analysis of reproductive allocation of wild-caught fish (Table 2) limited our ability to make direct comparisons of this trait among localities. In all populations, reproductive allocation declined as development progressed. However, in fish from the Guanapo High locality, reproductive allocation increased with development. When analysing a subset of the data that excluded the Guanapo High samples, developmental stage was a significant

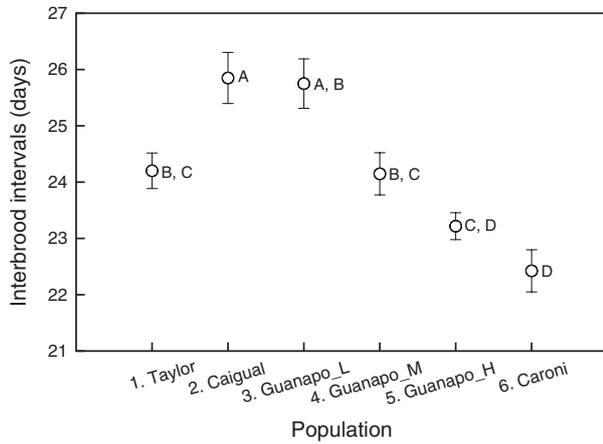


Fig. 3. Mean (\pm SE) interbrood interval of G2 laboratory-reared females. A clear gradient of variation was observed in interbrood interval (except for Taylor), where increasing species richness of the predator community corresponding to more frequent reproductive events. Letters by population means represent Tukey's HSD pairwise comparisons. Different letters indicate significant least squares mean differences between populations at a $\alpha = 0.05$.

covariate, but there was no significant interaction with locality. In this case, we found significant variation among populations in reproductive allocation (Table 2). This result was similar to that obtained when retaining the Guanapo High samples, but ignored developmental stage as a covariate. In both cases, there was a significant gradient effect in reproductive allocation, with RA increasing in locations where piscivorous fish are present (Fig. 4). When this trait was analysed in laboratory-reared females, the results were similar (Table 3), but the pattern of variation along the gradient was more pronounced (Fig. 4). Female size was a significant covariate in the analysis of the laboratory data, with bigger females having greater reproductive allocation (Table 3).

Offspring size and number

The pattern of variation in offspring number was very similar in wild-caught and laboratory-reared fish. Females from the Caroni population had the highest number of offspring, and offspring number gradually decreased along the stream gradient, resulting in a gradient in wild-caught and laboratory-reared fish (Fig. 5). Female dry mass was a significant covariate in both models (Tables 2 and 3), and there were no significant interactions between the independent variables ($P > 0.05$ for all interactions).

There was significant variation among populations in offspring size in both wild-caught and laboratory-reared females (Tables 2 and 3). However, the significant pattern in offspring size observed in offspring of wild-caught females along the studied gradient was not observed in second-generation laboratory-reared guppies (Fig. 6). In the wild, offspring were bigger in the upstream populations than in downstream populations, as would be predicted if there is a trade-off between the size and number of offspring. However, offspring mass in the laboratory was lower in the most upstream and

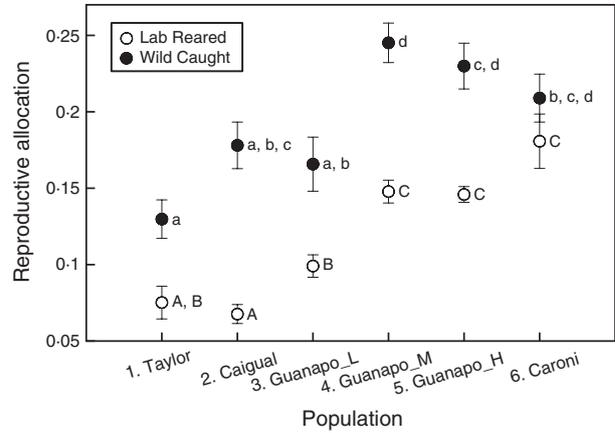


Fig. 4. Mean (\pm SE) reproductive allocation for the third brood in G2 laboratory-reared females (black circles) and wild caught females (white circles). Trends in the laboratory and field data were similar; as the number of potential predatory species increased, females increased reproductive allocation. Although a gradual increase in reproductive allocation from low-predation to high-predation localities was observed in both wild-caught and laboratory-reared females, the pattern was more continuous in laboratory-reared females. Letters by population means represent Tukey's HSD pairwise comparisons. Different letters indicate significant least squares mean differences between populations at a $\alpha = 0.05$ (upper-case letters compare means for laboratory-reared females, lower-case letters compare means for wild-caught females).

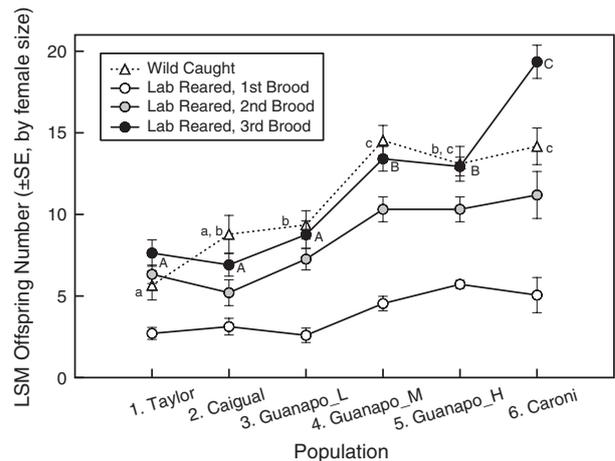


Fig. 5. Least square mean (\pm SE) offspring number (corrected for female mass) produced from the third brood of G2 laboratory-reared females (black circles) and wild-caught females (white circles). Laboratory data replicate the pattern observed in the wild, where the number of potential predatory species is positively correlated with the number of offspring produced per brood. Letters by population means represent Tukey's HSD pairwise comparisons. Different letters indicate significant least squares mean differences between populations at a $\alpha = 0.05$ (upper-case letters compare means for laboratory-reared females and lower-case letters compare means for wild-caught females).

downstream populations and higher in intermediate populations (Fig. 6). In the wild-caught females, stage of development and female size were significant covariates in the analysis of offspring size (Table 3), and there were no

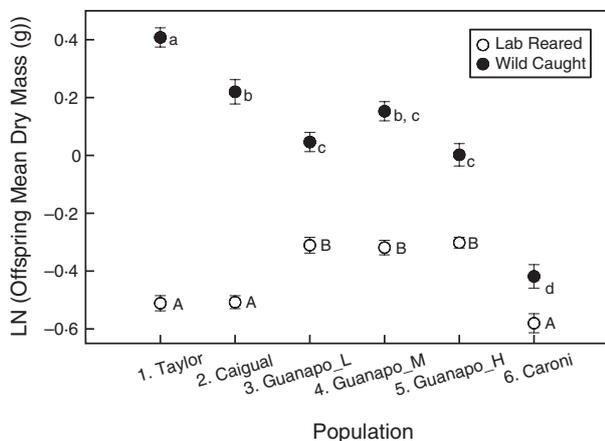


Fig. 6. Least square mean (\pm SE) offspring number (corrected for female mass) produced from the third brood of G2 laboratory-reared females (black circles) and wild-caught females (white circles). Offspring size in the wild follows the expected pattern of larger offspring in low-predation sites and smaller offspring in high-predation sites. However, laboratory data do not reflect this pattern, suggesting this trait can be highly plastic. Letters by population means represent Tukey's HSD pairwise comparisons. Different letters indicate significant least squares mean differences between populations at a $\alpha = 0.05$ (upper-case letters compare means for laboratory-reared females, and lower-case letters compare means for wild-caught females).

significant interactions between the independent variables ($P > 0.05$ for all interactions). As seen in the previous studies, offspring size decreased with stage of development and was positively related to female body mass. In laboratory-reared fish, only female dry mass was a significant covariate (Table 3), and there were no significant interactions between the independent variables ($P > 0.05$ for all interactions).

Discussion

Understanding how continuous phenotypic variation across environmental gradients is generated and maintained has important implications to our understanding of adaptive evolution and the processes that lead to speciation (Endler 1977). Comparisons between species occupying different ends of environmental gradients shed light into the mechanisms driving community structures and distribution limits (Case & Taper 2000; Chase & Leibold 2003; Price & Kirkpatrick 2009). For example, a growth-mortality trade-off drives the distribution pattern of the wood frog (*Rana sylvatica*) and the leopard frog (*R. pipiens*) across a gradient of resource availability (Schiesari, Peacor & Werner 2006). Yet, studies comparing variation among populations are critical to understand the mechanisms behind phenotypic variation and divergence (Endler 1977). Common garden comparisons or molecular approaches can be used to determine the genetic basis of phenotypic variation along environmental gradients. For example, common garden experiments have shown that variation in predation risk along a community gradient has direct and indirect effects on the evolution of life histories in the Trinidadian killifish (*Rivulus hartii*, Walsh & Reznick 2008, 2009, 2010). Similarly, molecular markers have been

used to show that clinal variation in life-history traits in a perennial sunflower (*Helianthus maximiliani*) is the result of local adaptation to spatially heterogeneous environments (Kawakami *et al.* 2011). Moreover, the use of modern genomics has the potential to relate variation in life-history traits to clinal genetic variation (e.g. Mariac *et al.* 2011).

Here, we combined field data and common garden experiments to show how guppy life histories vary along a continuum of predation risk and demonstrate the genetic basis of such variation. Our results reveal a fine-scale continuous gradient in life histories in populations that appear to experience a gradient in predation risk. Previous research on guppies has focused on comparisons between low-predation localities where only guppies and *R. hartii* occur vs. high-predation localities where, in addition to these two species, several other piscivorous fish are present. Comparisons of guppies from these two types of localities have shown that phenotypic and genotypic diversity in life-history traits among guppy populations are correlated with the predator communities to which guppies are exposed. Using a combination of field and common garden data, we show that there is a genetically based gradient in life histories between these two ends of the continuum for all measured traits, except for offspring size and female size at maturity. Below, we discuss these results in the context of the variation in ecological factors along the stream, how ecological factors might favour the evolution of plasticity vs. the evolution of fixed genetic differences and the range of variation expected along the whole gradient.

ECOLOGICAL GRADIENTS IN TRINIDADIAN STREAMS

Endler (1978) showed that colour in male Trinidadian guppies varies gradually from downstream, high-predation localities to upstream and low-predation localities. He proposed that this gradient in colouration was related to gradual changes in the predator community that exerted a graded selective pressure on male colouration. Similar to Endler (1978), we found a graded response in most aspects of the female life history that corresponded to a gradient in predation risk as one moves from headwater tributaries to higher-order streams and rivers. To the extent that it has been evaluated, an increase in predator abundance and diversity is correlated with an increase in mortality rate of guppies (Reznick *et al.* 1996). Thus, we suggest that the observed gradient in life histories is correlated with a parallel gradient in predation risk. Life-history theory predicts that the differences among localities in risk of mortality should in turn select for differences among populations in life-history traits such as age and size at maturity and reproductive effort (Gadgil & Bossert 1970; Law 1979; Charlesworth 1994). While additional studies of other drainages in the Northern Range Mountains are needed to test the generality of these results, our findings are consistent with predictions from life-history theory that gradually increasing predator-induced mortality rates from headwater to lowland streams select for gradually faster life histories.

While our results are consistent with predictions from life-history theory on how increasing predation should shape the life histories, we also recognize that other ecological factors also co-vary along this gradient (Grether *et al.* 2001; Reznick, Butler & Rodd 2001). The most evident changes along this gradient are the size of the stream and the amount of light that reaches the stream (Table 1). These factors are known to translate into higher primary productivity and thus potentially increase food resources available for guppies (Grether *et al.* 2001; Reznick, Butler & Rodd 2001). A second notable factor is the indirect effects of predation (Abrams & Rowe 1996; Reznick *et al.* 1996). Predator consumption results in a reduction in prey abundance, which could drive the evolution of life histories by altering the competitive environment (Gadgil & Bossert 1970; Law 1979). Thus, increased predation pressure could also indirectly affect the strength of density-dependent selection, as those individuals that survive predation will be left with increased resource availability and experience lower intraspecific competition (Abrams & Rowe 1996; Reznick, Butler & Rodd 2001; Bassar *et al.* 2010). It is difficult to make specific predictions of the effects of density-dependent regulation in life-history evolution given that these predictions depend on the specific demographic consequences imposed by predators (Abrams & Rowe 1996). Currently, we lack the information for predicting how life histories will evolve under density-dependent regulation on guppies, but we must bear the environment and the indirect effects of predation in mind as potentially confounding factors that can also influence life-history variation.

GENETIC AND PLASTIC DIFFERENCES IN LIFE HISTORIES

In our laboratory common garden experiment, we observed that reproductive allocation and brood size per brood progressively decrease while age at maturity and interbrood interval progressively increase with decreasing complexity of the predator community. This suggests a genetic basis to the parallel pattern observed in the field for reproductive allocation and offspring number (Figs 2 and 3). An earlier age at maturity and shorter interbrood interval co-varying with increasing predation pressure is predicted from theory (Gadgil & Bossert 1970; Law 1979; Charlesworth 1994) and is consistent with previous comparisons of high- and low-predation populations of guppies (Reznick 1982a; Reznick & Endler 1982). The one locality that did not follow this trend was the Taylor locality where females matured younger and reproduced more frequently than expected based upon the simple nature of the fish community (Figs 2 and 3). Why the Taylor deviates from the continuum will require further study. However, we suspect that other mortality sources besides predation (e.g. water pollution, disease, flash floods) may have selected for faster life histories because detailed mark-recapture studies have found unusually high mortality in the Taylor to other low-predation streams (SW Fitzpatrick, J Torres Dowdall, CK Ghalambor, DN Reznick, WC Funk unpublished data).

A second trait that shows an interesting deviation from the observed gradient in life histories is offspring size (Fig. 5). The field data show that females from all low-predation populations produce large offspring, but as soon as the more effective predators appear in the gradient (e.g. *Hoplias*, characins, and cichlids), offspring size starts to decrease, reaching the smallest sizes in the Caroni River. Such patterns are expected if offspring size and number trade-off against each other. However, this pattern of variation was not observed in our common garden experiments. In the laboratory, females from the two low-predation populations, Taylor and Caigual, produced the smallest but also the fewest offspring. These results for offspring size are interesting for several reasons. In prior comparisons between high- and low-predation guppies, we have consistently observed a compression of the differences in offspring size between laboratory-reared (Reznick 1982a,b; Reznick & Bryga 1996; Reznick *et al.* 2004) and wild-caught fish (Reznick & Endler 1982; Reznick, Rodd & Cardenas 1996).

However, the results here document for the first time an inversion in the pattern of variation of offspring size where low-predation guppies have smaller offspring in the laboratory than high-predation guppies. High-predation guppies usually produce more, smaller offspring than low-predation guppies (Reznick 1982a), whereas, in our laboratory data, we see that high-predation guppies produce more and bigger offspring. Thus, the combination of our laboratory and field data suggests a high degree of phenotypic plasticity in offspring size in the two low-predation populations implying genotype by environment interactions. Prior comparisons between high- and low-predation populations from other drainages did not reveal such extreme genotype by environment interactions when reared in the laboratory under high and low levels of food (Reznick 1982a; Reznick *et al.* 2004). Thus, these results are also evidence of a genetically based decoupling between offspring size and number, such that the expected trade-off between the size and number of offspring is observed in the field but not in the laboratory where resources were controlled.

While we observed that offspring number is similar between the laboratory and the wild, offspring mass tends to be smaller in the laboratory, which, in turn, drives a significant reduction in reproductive allocation in laboratory females compared to wild females. It is difficult to determine what specifically drives the decrease in offspring size and reproductive allocation in the laboratory, as several factors, including resource availability, predation risk, competition, water quality, change between the laboratory and nature. Specifically, food quantity and quality are the most likely causes of this difference, as our restricted food levels could have led to suboptimal energy allocation to reproduction. Evaluating this hypothesis requires replicating the current experiment under different food levels. Indeed, previous common garden experiments testing the effect of food level on life-history traits in guppies found that, while food level did not significantly affect reproductive allocation, it did affect offspring mass (Reznick 1982a,b, Reznick & Bryga 1996).

However, the effect of restricting food was the production of larger offspring (Reznick 1982a,b; Reznick & Bryga 1996). It is possible that the food level we used was higher than typically found by low-predation guppies in the wild and lower than what is encountered by high-predation populations, producing the plasticity pattern observed in our study. However, as no food by predation regime interaction has previously been recorded (Reznick 1982a,b; Reznick & Bryga 1996), further experiments will be needed to determine the cause of these patterns.

What type of selective environment would favour such plasticity in offspring size and number? Life-history theory predicts an increase in reproductive effort as adult mortality increases (Charlesworth 1994), but does not make specific predictions about how resources should be distributed among offspring or patterns of plasticity in resource allocation. In contrast, other theory suggests that the relationship between offspring size and number is determined by offspring fitness, which in turn should influence how parents distribute resources among offspring (Smith & Fretwell 1974; Wilbur 1977). Guppies occurring in low-predation sites are thought to experience reduced resource availability owing to high intraspecific competition and lower primary productivity, such that large offspring have a competitive advantage over smaller offspring (Reznick 1982b; Jørgensen, Auer & Reznick 2011). However, the advantage of large offspring disappears under high resource availability (Bashey 2008). If the low-predation populations studied here experience predictable fluctuations in population density and resource availability, theory predicts such variable environments could favour plasticity in offspring size (Levins 1968; Via & Lande 1985; Moran 1992). We have observed large fluctuations in the population densities of these two low-predation sites that correspond with seasonal flash floods during the Trinidadian wet season (J. Torres-Dowdall personal observation). Thus, one hypothesis for why plasticity in offspring size has evolved in these populations, but not other previously examined low-predation populations, is that these populations experience more variable environments. For example, the marine bryozoan *Bugula neritina* experience a high degree of environmental variation in the field, which selects for increased plasticity in offspring size (Monro, Sinclair-Taylor & Marshall 2010). Similarly, environmental variation in habitat quality (i.e. seed size and resistance) has selected for high levels of plasticity in egg size on the seed beetle *Stator limbatus* (Fox 2000; Fox, Czesak & Fox 2001). Comparative studies of the population dynamics of low-predation populations in combination with laboratory experiments raising guppies under high and low food availability might shed further light on this issue.

Our results strongly suggest that the genetic variation observed along this predation gradient is the result of local adaptation. However, the observed pattern of genetic variation among populations could also be influenced by gene flow (García-Ramos & Kirkpatrick 1997; Barton 2001). Our study system falls along a linear gradient, which potentially has a strong directional axis of dispersal and gene flow. If natural selection favours two extreme ecotypes, a high-predation and

a low-predation ecotype, gene flow along the linear gradient can contribute to the observed pattern of variation. Indeed, although we lack estimates of gene flow in this drainage, we suspect there is an interaction between selection and gene flow, producing the observed pattern of genetic and phenotypic divergence between localities. However, previous studies on guppies have shown that gene flow between populations is highly reduced by physical barriers, including waterfalls and geographical distance (Crispo *et al.* 2006). All of our sites are separated by at least one waterfall that is at least 2 m high, and some of our sample localities are separated by several kilometres. Therefore, the effect of gene flow is probably small. To confirm this prediction, comparing neutral genetic variation (i.e. F_{ST}) and adaptive quantitative variation (i.e. Q_{ST}) along the habitat gradient could provide insight into the relative roles of selection and gene flow in the observed pattern of life-history variation (Barton 2001; Volis & Zhang 2010; Kawakami *et al.* 2011).

EXTENDING THE HIGH-PREDATION LIFE-HISTORY CONTINUUM

In this study, we sampled a population that is beyond the normal range of communities commonly included in the guppy literature. The Caroni River population showed an even faster life history than fish from Guanapo High, a typical *Crenicichla* locality (Figs 2–6). The Caroni life history demonstrates that guppy life-history traits can evolve beyond the range found within prior research on their life-history evolution (Reznick 1982a; Reznick & Bryga 1996; Reznick *et al.* 2004). Although there are large changes in the fish community between the Caroni River and the Guanapo High localities that would suggest increased predation pressure (Table 1), there are also several other factors that vary between these sites, making it difficult to assign causality to the observed changes in life-history traits. The Caroni site is in a much larger river and has a strong anthropogenic influence (WRA 2001). It has lost most of its canopy and is highly eutrophied. In addition, a phenotypically similar close relative of guppies, the swamp guppy (*Poecilia picta*), is present at this site and can potentially compete with guppies for resources affecting the selection pressures guppies' experience. Thus, the Caroni locality, as well as other lowland guppy population, provides an opportunity to explore the high-predation and high resource limits of life-history variation in guppies and how other ecological factors, like interspecific competition, affect life-history evolution.

Conclusion

In this study, we provide evidence for fine-scale local adaptation in life-history traits in response to a gradient in predation pressure. Endler (1978) studied how a similar gradient in predation affects male colouration in guppies, and in general, our results are consistent with his. However, one notable difference here is that Endler found a stronger effect of *Crenicichla* on male colouration than we found for life-history

traits in female guppies. *Crenicichla* is an important diurnal predator with acute vision (Kemp *et al.* 2009); thus, it is reasonable to expect that it would play a larger role in Endler's study. In the current study, mortality rate is proposed to shape life-history evolution, and it appears that other predators are as effective in this regard as is *Crenicichla*. Thus, it is insightful to consider how guppies that experience variation in predation risk respond to different predators. For example, Botham *et al.* (2006) showed that guppies' behavioural response depends on the predator they are exposed to, being the most responsive towards *Crenicichla*. Comparisons of guppies from communities having only a subset of the predators present in typical *Crenicichla* sites could be informative in this regard. Such comparisons could also help to disentangle the effects of co-varying environmental factors that potentially confound all studies attempting to ascribe a particular agent of selection to patterns of phenotypic variation. For example, there is a clear break in life histories on the Aripo River in association with a barrier waterfall and a break in predation risk that is independent of changes in the physical environment (Reznick 1982a), potentially allowing for better separation of predation and resource availability effects. Finally, comparisons along gradients provide a better framework for interpreting the scale at which selection shapes phenotypic and genetic variation. In the current study, if we had only compared the extremes of the gradient (i.e. Taylor vs. Guanapo High), we might have incorrectly concluded that predation pressure was not correlated with the evolution of life histories in guppies from the Guanapo River. However, by looking at the whole gradient, we found strong evidence for predation driving life-history evolution in guppies at a relatively fine scale. Finally, our results are in agreement with an increasing number of studies showing that selection along environmental gradients can produce clinal adaptive genetic variation on life-history traits (e.g. Walsh & Reznick 2008, 2009, 2010; Kawakami *et al.* 2011; Mariac *et al.* 2011; Tomkins *et al.* 2011) and hold promise for better estimates of the scale at which adaptive divergence occurs.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Appendix S1. Effects of rearing conditions (10 L glass tanks vs. 1.5 L flow through tanks) on guppy life-history traits.

Appendix S2. Correlation between predation risk and environmental variables including stream width and depth, canopy cover, temperature and pH.

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