

CONVERGENCE OF LIFE-HISTORY PHENOTYPES IN A TRINIDADIAN KILLIFISH (*RIVULUS HARTII*)

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Convergent evolution is characterized by the independent evolution of similar phenotypes within similar selective environments. Previous work on Trinidadian killifish, *Rivulus hartii*, demonstrated repeatable life-history differences across communities that differ in predation intensity. These studies were performed in rivers located on the south slope of Trinidad's Northern Range Mountains. There exists a parallel series of rivers on the north slope of these mountains. As on the south slope, *Rivulus* is found across a gradient of fish predation. However, the predatory fish species in north-slope rivers are derived from marine families, whereas south-slope rivers contain a predatory fish fauna characteristic of the South American mainland. If predator-induced mortality and the associated indirect effects are the causal factors selecting for life-history patterns in *Rivulus*, and these are similar in north- and south-slope rivers, then the specific predatory species should be interchangeable and we would expect convergence of life-history phenotypes across slopes. Here, we characterize the life-history phenotypes of *Rivulus* from north-slope communities by measuring number of eggs, egg weight, reproductive allotment, reproductive tissue weight, and size at maturity. We find similar patterns of life-history divergence across analogous predator communities. Between slopes, minor differences in *Rivulus* life-history traits exist and one potential cause of these differences is the abundance of *Macrobrachium* prawns in north-slope rivers.

KEY WORDS: Adaptation, convergent evolution, predator-prey.

Convergent evolution, when independent lineages repeatedly evolve similar phenotypic characteristics, provides some of the strongest nonexperimental support for adaptation by natural selection (Bell and Foster 1994; Schluter and Nagel 1995). The reason for this power is the proposed cause and effect relationship between selection and the resulting phenotype combined with evidence that the same phenotype evolved repeatedly and independently in similar circumstances (Reznick and Bryga 1996; Reznick et al. 1996). Some of the best-known examples of convergence are the repeated evolution of reduced armor plating in freshwater three spine stickleback from armored marine ancestors (Bell and Foster 1994; Colosimo et al. 2005), the niche partitioning and evolution of specialized body forms in *Anolis* lizards

following the colonization of islands in the West Indies (Losos et al. 1998), and the evolution of increased coloration in male guppies released from predation pressures (Endler 1980, 1982, 1983). Evidence for convergence of the life-history phenotype in response to repeatable features of the environment has been sought in only a handful of cases (Reznick et al. 1996; Johnson 2001; Duponchelle et al. 2008). In this article, we evaluate the evidence for life-history convergence in a Trinidadian killifish (*Rivulus hartii*) from communities with contrasting, independently derived suites of predators.

Trinidad is unique among Caribbean islands in that until recently it was connected to South America, and today only 12 km separate it from the Orinoco Delta in Venezuela (Kenny

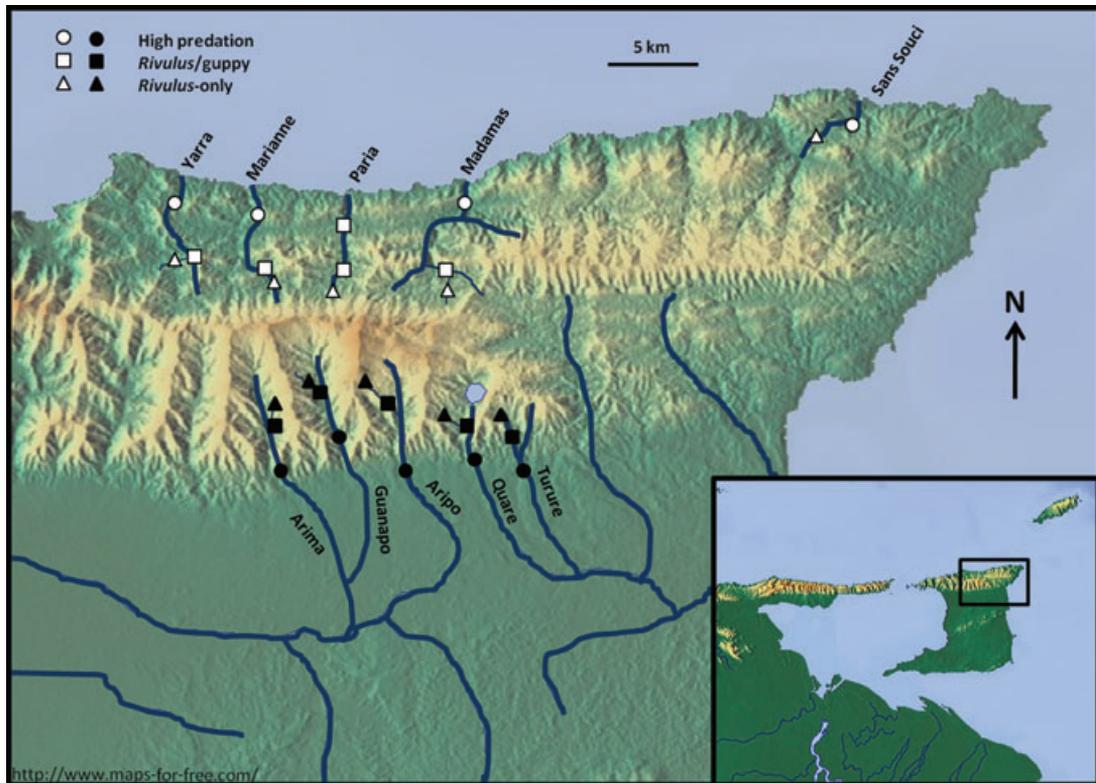


Figure 1. Map of the Northern Range Mountains of Trinidad, showing the north and south flowing drainages that were sampled. Open symbols indicate sites from north-slope rivers (current study) and solid symbols indicate sites from the south-slope survey (Walsh and Reznick 2009).

2008). Approximately 1500 years ago a rising sea level covered the land bridge connecting Trinidad to the mainland. A conspicuous feature of Trinidad's topography is the Northern Range Mountains cutting across the northern half of the island. Although the terrestrial fauna of Trinidad is almost exclusively South American (Continental), the crest of the Northern Range separates two fairly distinctive freshwater fish communities (Figs. 1 and 2). A series of rivers running in parallel drain from the south slope of the Northern Range and pass through Trinidad's interior. These rivers contain a relict fish fauna characteristic of the South American mainland. Predatory species include the cichlids (*Crenicichla alta* and *Aequidens pulcher*), the sardines (*Astyanax bimaculatus* and *Hemibrycon taeniurus*), and the wolf fish (*Hoplias malabaricus*). The north slope of the Northern Range Mountains contains a replicate series of rivers that drain directly into the Caribbean Sea. This series of rivers contains a fish fauna derived from a marine environment. Predatory species found in these rivers are the mountain mullet (*Agonostomus monticola*), gobies and sleepers (*Eleotris pisonis*, *Gobiomorus dormitor*, and *Dormitator maculatus*), and prawns (*Macrobrachium carcinus* and *M. crenulatum*).

Due to its exceptional colonization ability, a killifish, *R. hartii* is widely distributed throughout Trinidad where it occurs in fresh-

water habitats ranging from wide lowland rivers, with predatory fish species, to narrow headwater streams where they are the only fish species present (Gilliam et al. 1993). Previous work has made use of the fact that *Rivulus* are found across different fish communities to test predictions from life-history theory (Walsh and Reznick 2008, 2009, 2010). This work was performed on rivers draining the south slope of the Northern Range. Here, *Rivulus* are typically found in: (1) High predation (HP) sites with several piscivorous species, (2) *Rivulus/guppy* (RG) sites where *Rivulus* occur with guppies (*Poecilia reticulata*), and (3) *Rivulus*-only (RO) sites where *Rivulus* is the only fish species. HP sites tend to be found in the lower portion of rivers, RG sites tend to be further upstream, and RO sites are nearly always furthest upstream in tributaries where other species have failed to colonize.

Different ecological and predatory interactions occur in these three community types that alters *Rivulus* survival, and growth rate. *Rivulus* in HP sites experience a higher monthly mortality rate, decreased population density, and increased growth rate relative to *Rivulus* from sites in which they are alone (Gilliam et al. 1993; Fraser et al. 1999, A. I. Furness unpubl. data). The increases in mortality and lower abundances of *Rivulus* in HP sites are likely due to predation by piscivorous fish, whereas the increase in growth represents a hypothesized indirect effect of



Figure 2. The aquatic fauna of Trinidadian streams. (A) Female *Rivulus hartii*, (B) *Gobiomorus dormitor*, (C) *Eliotris pisonis*, (D) *Macrobrachium crenulatum*, (E) *Hoplias malabaricus*, (F) *Crenicichla alta*, (G) Female *Poecilia reticulata*. B, C, and D are predators found in north-slope rivers, whereas E and F are predators found in south-slope rivers. Guppies (*P. reticulata*) and *R. hartii* are found across both slopes.

predation mediated through enhanced per capita food availability (Walsh and Reznick 2008). In RG sites *Rivulus* are found at densities lower than in RO sites (Gilliam et al. 1993; Walsh et al. 2011), which is likely due to guppy predation on juvenile *Rivulus* (D. F. Fraser and B. A. Lamphere unpubl. data). Furthermore, *Rivulus* in sites that contain guppies exhibit an increased rate of growth (Walsh et al. 2011) that is almost certainly a result of the reduced *Rivulus* densities. This is because *Rivulus* transplanted from RO sites to RG sites showed an almost immediate increase in growth rate (Walsh et al. 2011). These data suggest that guppies do not negatively affect adult *Rivulus* through competition, or alternatively, the reduction in *Rivulus* density (caused by the presence of guppies) more than offsets for any competitive interactions between these two species, thus leading to the observed increases in *Rivulus* growth rate. These contrasting patterns of mortality, density, and individual growth have the potential to cause life-history divergence (e.g., Charlesworth 1980).

A survey of *Rivulus* life-history phenotypes across five rivers found that *Rivulus* from HP sites exhibited increased fecundity, increased reproductive allocation, decreased egg size, and a smaller size at maturity (Walsh and Reznick 2009). At the opposite end of the spectrum *Rivulus* from RO sites exhibited significantly lower fecundity, decreased reproductive allotment, increased egg size, and a larger size at maturity. With the exception of size at maturity, *Rivulus* from RG sites showed intermediate trait values when compared to *Rivulus* from HP and RO sites (Walsh and Reznick 2009). Common garden experiments performed on a subset of these communities showed that such differences among *Rivulus* populations are heritable and have a genetic basis (Walsh and Reznick 2008, 2010). These contrasting *Rivulus* life-history patterns indicate important roles for both the direct and indirect effects of predation. Early models of life-history evolution predict that increased rates of predation on large, mature size classes favor the evolution of earlier maturation and increased reproductive

effort, whereas the opposite predictions are made when mortality is increased in young or small size classes (Gadgil and Bossert 1970; Law 1979; Charlesworth 1980). *Rivulus* life-history divergence in HP sites is consistent with these theoretical models, but life-history divergence between *Rivulus* from RG and RO sites is inconsistent with theory that considers the impacts of predation alone. *Rivulus* from RG sites experience a population bottleneck during their larval stage due to guppy predation. Yet, such *Rivulus* show an earlier maturation and increased reproductive effort relative to *Rivulus* from RO sites. Models that include prominent roles for impacts due to density, growth, and resource availability, common indirect effects of predation (Wootton 1994), provide better fits with observed patterns of phenotypic evolution in RG sites.

Our prior comparisons of *Rivulus* life histories utilized rivers along the south slope of the Northern Range Mountains in Trinidad. There exists an analogous series of fish communities in the rivers of the north slope of the Northern Range Mountains (Endler 1983; Fraser et al. 1995; Reznick et al. 1996; Millar et al. 2006). *Rivulus* are found in the same community types (HP, RG, and RO) as in south-slope rivers. Although here the HP localities contain a different set of potential predators that are derived from the connecting marine environment. RG sites again contain *Rivulus* and guppies, as well as the small nonpredatory benthic goby (*Sicydium punctatum*). Lastly, RO sites sometimes have a low density of *Sicydium punctatum* in addition to *Rivulus*.

There is a key difference between north- and south-slope communities that may impact patterns of divergence among *Rivulus* communities and thus the potential for convergent evolution. North-slope rivers drain directly into the Caribbean Sea and prawns from the genus *Macrobrachium* are found throughout these rivers, often at very high densities. In contrast, south-slope rivers are more remote from the sea and contain few *Macrobrachium* prawns. *Macrobrachium carcinus* was observed feeding upon *Rivulus* in the confines of a minnow trap (A. I. Furness pers. obs.) and *Macrobrachium crenulatum* will readily eat guppies (Endler 1983) and *Rivulus* (Fraser et al. 1995) in aquaria. Guppy life-history evolution was shown to be unaffected by prawns (Rodd and Reznick 1991; Reznick et al. 1996). However, guppies are livebearers whereas *Rivulus* are egg layers; if prawns are primarily bottom feeders they may impact *Rivulus* population dynamics through differential mortality on the egg stage. Furthermore, *Rivulus* are found at lower density in north- than south-slope rivers (Fraser et al. 1995, A. I. Furness pers. obs.) and Fraser et al. (1995) hypothesized that this difference could be due to the negative influence of prawns.

Here, we evaluate the evidence for convergent evolution by examining patterns of phenotypic life-history divergence in *Rivulus* from each of three different predation communities (HP, RG, and RO) across five river drainages on the north slope of Trinidad's Range Mountains. If similar differences in predator-

induced mortality (HP sites) and the indirect effects of interactions with guppies (RG sites) are the causal factors selecting for life-history divergence in *Rivulus*, then the specific predatory species should be interchangeable and we would expect to see similar patterns of life-history variation among *Rivulus* communities across slopes. We specifically predict that *Rivulus* from HP localities will mature at smaller size and invest more heavily in reproduction compared to *Rivulus* from RO sites. We also predict that *Rivulus* from sites with guppies will again be intermediate between HP and RO sites. A failure to observe convergence in the life history of *Rivulus* between north- and south-slope rivers may indicate that prawns impact the expression of life-history traits in *Rivulus*.

Materials and Methods

Rivulus hartii (Rivulidae) is a species of killifish found in northern South America and surrounding Caribbean islands (Murphy and Collier 1996; Murphy et al. 1999; Jowers et al. 2008). This species is an egg layer and exhibits external fertilization. There is little sexual dimorphism and both sexes reach an asymptotic size around 100 mm. Their diet consists mainly of aquatic and terrestrial invertebrates (Fraser et al. 1999). In the wild *Rivulus* have been found to produce eggs throughout the year (Fraser and Gilliam 1992).

CHARACTERIZATION OF COLLECTION SITES

Rivulus were collected from five river drainages on the north slope of the Northern Range Mountains in Trinidad. The collection localities and those of an earlier study performed on the south slope (Walsh and Reznick 2009) are indicated in Figure 1. The collections were made from mid-January through early March 2010. This collection period is comparable to that of the south-slope *Rivulus* collections (January and early February 2007). The rivers sampled were the Yarra, Marianne, Paria, Madamas, and Sans Souci. HP localities were located in the main river, tended to be close to the ocean, and were characterized by a wide river, increased depth, and more open canopy (see Table 1). RG localities were found upstream, above barrier waterfalls that excluded strongly piscivorous species. RO localities were found even further upstream in streams where other fish failed to colonize. An attempt was made to collect *Rivulus* from each of these three community types in all five river drainages. However, this was not possible in the Paria and Sans Souci Rivers. The Paria River has a 10-m waterfall approximately 500-m upstream from where it enters the Caribbean Sea. The area below this waterfall has a high density of predatory gobies (*E. pisonis*) and ocean fish. *Rivulus* and guppies were found at extremely low densities and *Rivulus* were not collected. The habitat data from this HP site are included in Table 1 for comparison. The Paria waterfall serves as a barrier to all strongly piscivorous species, so everything upstream of this point

Table 1. Environmental habitat variables at *Rivulus* collection sites. Significant differences among *Rivulus* communities were found for mean river width, mean river depth, and canopy openness.

| Locality | Mean width (m) | Mean depth (m) | Canopy openness (%) | Prawn CPUE | Conductivity (μ S) | Salinity (ppm) | Total dissolved solids (ppm) | Temperature ($^{\circ}$ C) |
|--------------------------------|----------------|----------------|---------------------|---------------------|-------------------------|--------------------|------------------------------|-----------------------------|
| Yarra River | | | | | | | | |
| High predation | 12.8 | 0.48 | 41.7 | 0.0 | 193 | 96 | 135 | 23.4 |
| <i>Rivulus</i> /guppy | 2.2 | 0.28 | 20.1 | 44.7 | 199 | 97 | 139 | 23.5 |
| <i>Rivulus</i> only | 1.4 | 0.16 | 12.0 | 24.8 | 163 | 81 | 113 | 23.0 |
| Marianne River | | | | | | | | |
| High predation | 6.7 | 0.70 | 13.7 | 2.1 | 267 | 133 | 187 | 23.7 |
| <i>Rivulus</i> /guppy | 4.0 | 0.18 | 23.4 | 3.8 | 341 | 172 | 239 | 24.7 |
| <i>Rivulus</i> only | 2.3 | 0.26 | 10.9 | 6.0 | 194 | 97 | 136 | 23.8 |
| Paria River | | | | | | | | |
| High predation | 10.5 | 0.48 | 40.9 | 0.8 | 296 | 145 | 210 | 22.9 |
| <i>Rivulus</i> /guppy (wide) | 11.7 | 0.37 | 41.9 | 30.5 | 327 | 166 | 226 | 22.0 |
| <i>Rivulus</i> /guppy (narrow) | 2.7 | 0.12 | 13.3 | 1.2 | 220 | 107 | 154 | 23.0 |
| <i>Rivulus</i> only | 2.1 | 0.17 | 9.1 | 9.0 | 207 | 106 | 149 | 22.8 |
| Madamas River | | | | | | | | |
| High predation | 20.3 | 0.69 | 54.4 | 10.9 | 226 | 110 | 162 | 24.0 |
| <i>Rivulus</i> /guppy | 3.5 | 0.18 | 19.0 | 12.8 | 221 | 111 | 154 | 22.1 |
| <i>Rivulus</i> only | 1.7 | 0.09 | 8.9 | 4.5 | 204 | 102 | 144 | 23.5 |
| Sans Souci River | | | | | | | | |
| High predation | 5.3 | 0.24 | 18.8 | 8.0 | 363 | 181 | 256 | 25.3 |
| <i>Rivulus</i> only | 2.1 | 0.07 | 10.4 | 28.2 | 356 | 175 | 251 | 25.1 |
| Kruskal–Wallis (H): | 10.66** | 8.57* | 9.50** | 3.380 ^{NS} | 1.86 ^{NS} | 1.82 ^{NS} | 1.86 ^{NS} | 1.69 ^{NS} |

NS = not significant ($P > 0.05$); * $P < 0.05$; ** $P < 0.01$.

is categorized as RG or RO. Two RG sites were chosen from the Paria River. The first RG site was directly above the Paria waterfall, near the Paria River's intersection with the Jordan River. The physical habitat features of this site are very much like that of a typical HP site (open canopy, wide river, increased depth) but without the predatory fish species. The other RG site from the Paria River is approximately 5-km upstream, and contains habitat typical of RG sites from other rivers. The environmental contrast between these two RG sites provides a useful test of the independent contribution of the environment to the life-history phenotype. In the Sans Souci River, a suitable RG site could not be located, so only HP and RO sites are represented from this drainage.

Each site was categorized according to its fish community on the basis of prior work (Endler 1983; Reznick et al. 1996; Millar et al. 2006), thorough inspection of the fish community, and the presence and location of barriers limiting dispersal. Global positioning system (GPS) coordinates were taken at each site (Table S1) and a number of habitat variables were measured (summarized in Table 1). Width and depth measurements were taken at five points approximately evenly spaced throughout the site. Depth measurements were taken in the center of the river. Water chemistry variables (temperature, salinity, total

dissolved solids, and conductivity) were taken at the center of the site using a handheld meter (EC400: ExStik II, Extech Instruments, Nashua, NH). Canopy openness was measured using a concave spherical densiometer (model C, Forestry Suppliers Inc., Jackson, MS). Measurements were made in all four cardinal directions while standing at the site center. Prawn abundance was assayed using a catch per unit effort (CPUE) measure (as in Millar et al. 2006; McKellar et al. 2009). Four collapsible mesh minnow traps (model TR-501, Promar Company, Gardena, CA) were baited with six pellets of dry dog food. The traps were approximately evenly dispersed throughout the site and placed in at least 25 cm of water in areas with low current. After being left undisturbed for 40 min all *Macrobrachium* prawns were counted. Trapping time was converted to a standardized metric in which one unit of effort is equal to 1 h, and the average of four traps was used to compute an overall CPUE for each site.

CHARACTERIZATION OF LIFE-HISTORY PHENOTYPE

At each site *Rivulus* were captured using hand nets. Shortly after collection all female *Rivulus* were euthanized in MS-222 and preserved in 10% formalin. The number of identifiable females in each collection ranged from 19 to 59, and all females from each collection were dissected.

The following data were collected from each female: (1) total length, (2) number of mature eggs, (3) wet weight of mature eggs, (4) somatic dry weight (females total weight minus reproductive tissue and gastrointestinal tract), (5) total dry weight of reproductive tissue, and (6) reproductive allotment, defined as the total dry weight of reproductive tissue divided by somatic dry weight. Eggs greater than 1.65 mm in diameter were considered mature (Walsh and Reznick 2009) and generally there was a clear distinction between mature and immature eggs.

Size at maturity was evaluated by dividing females into 2-mm size classes. The minimum size at maturity was the smallest size class where 50% or more of the females contained at least one mature egg. Using 50% as a criterion eliminates the potential biasing of this estimate due to a single outlier individual.

STATISTICAL ANALYSES

Univariate approach

We compare the life-history phenotypes of north-slope *Rivulus* from different predator communities and rivers using analyses of covariance (ANCOVAs). Dependent variables evaluated were number of eggs, egg weight, reproductive allotment, and reproductive tissue weight. In each of these analyses, community (HP, RG, RO) and river (Yarra, Marianne, Paria, Madamas, Sans Souci) were entered as fixed effects. We argue that treating community and river as fixed effects is justifiable because these are repeatable features of our sampling design (Walsh and Reznick 2009). *Rivulus* life-history trait values generally increase as a function of body size, so total length was included as a covariate for analyses of all life-history traits. For the main analysis, the two RG sites from the Paria River were pooled. However, a separate planned comparison was also performed between these two sites (see Table 1).

Differences between community types were evaluated for each of the dependent variables using Fisher's LSD comparisons. Residuals were checked to ensure that the assumptions of normality and homogeneity of variances were upheld. Number of eggs and reproductive tissue weight were logarithmic transformed and reproductive allotment was square root transformed. Four fish that were greater than 68 mm in total length and contained only a single egg were not included in the analyses. These individuals were statistical outliers (standardized residuals more than 1.5 times the interquartile range) for one or more life-history traits. Furthermore, fish of this size with a single egg were likely replenishing their egg supply after recently depositing a full clutch of eggs; this is because the majority of fish of this size contained numerous eggs and the individuals with a single egg were exceptions to the norm. The assumption of homogeneity of slopes was upheld for all analyses. Each population had only a single estimate for the minimum size of maturity, so a nonparametric Kruskal–Wallis test was used to compare differences across communities. All

statistical analyses were performed in PASW Statistics 18 or SAS 9.2.

Multivariate approach

A discriminant function analysis was used to classify north-slope *Rivulus* populations according to community type on the basis of the combined features of the life history. The independent or predictor variables used were least square site means for number of eggs, egg weight, and reproductive allotment. Separation among community types was evaluated by quantifying the Mahalanobis distances between group centroids.

The Paria River populations were not included in this analysis because the discriminant function uses the combined life-history features of each population as a basis for cross classifying all other populations, and is thus highly sensitive to “outlier” populations. In four of the rivers the life-history trends were consistent, and in many respects the Paria River is different from that of the others. For example, the Paria River contained a RG site that had habitat features typical of other HP sites. Including the Paria River populations, given that they exhibit life-history features that are opposed to the trends in the other four rivers, makes the discriminant function relatively uninterpretable and largely obscures what are otherwise very clear and consistent trends. As a result of removing the Paria River populations, we note that the discriminant analysis is used as a descriptive tool highlighting the convergence of *Rivulus* life-history phenotypes across slopes, rather than as a classic statistical test to formally evaluate a hypothesis.

A discriminant function was used to classify *Rivulus* populations from the south slope (as in Walsh and Reznick 2009), allowing for a qualitative comparison between the multivariate separation among community types across the two slopes. Again, least square site means were used for each of the predictor variables (life-history traits) and the covariate (length) was adjusted to the same level for both slopes. The discriminant function performed on *Rivulus* populations from the south slope was then used to classify *Rivulus* populations from the north slope. This amounts to a test of the degree of life-history convergence across slopes. The reasoning behind this analysis is that if predation community (interactive effects of predators, environment, and resources) is the major determinant of *Rivulus* life-history patterns, and these are similar on both the north and south slope, then we should see convergence of the life-history phenotype that would manifest itself as a high success rate in cross-classification.

Environmental factors contributing to the life-history phenotype

It is possible that habitat features of a specific site could select for *Rivulus* life-history trait(s) that do not neatly fall into the HP, RG, and RO trichotomy. To explore between-site variation in *Rivulus* life-history traits, we used general linear models that included

Table 2. Analyses of *Rivulus* life-history traits using general linear models (*F* values). (A) Includes all populations from each of the five rivers. (B) Comparison of the two low predation sites in the Paria River.

| | df | Number of eggs | Egg weight | Reproductive allotment | Reproductive tissue weight |
|---------------------|----|----------------|-------------|------------------------|----------------------------|
| (A) All populations | | | | | |
| Covariate: | | | | | |
| Total length | 1 | 186.984*** | 299.763*** | 45.261*** | 521.365*** |
| Main effects: | | | | | |
| River | 4 | 4.421** | 22.368*** | 8.548*** | 6.225*** |
| Community | 2 | 15.129*** | 39.011*** | 22.256*** | 15.171*** |
| River × Community | 6 | 2.260* | 13.675*** | 3.252** | 3.443** |
| Error MS (df) | | 0.093 (290) | 0.149 (290) | 0.209 (290) | 0.039 (290) |
| (B) Paria River | | | | | |
| Covariate: | | | | | |
| Total length | 1 | 33.419*** | 46.311*** | 4.121* | 122.794*** |
| Main effect: | | | | | |
| Site | 1 | 11.513*** | 5.247* | 19.366*** | 30.511*** |
| Error MS (df) | | 0.069 (43) | 0.116 (43) | 0.150 (43) | 0.018 (43) |

NS = not significant ($P > 0.05$); * $P < 0.05$; ** $P < 0.01$.

predation community, percent canopy openness, and prawn CPUE. We were interested in determining which of these variables explains a significant amount of variation for each life-history trait, and how much; to this end we report the *F* statistic and effect size of each habitat variable. The effect size (partial eta-squared) describes the “proportion of total variation attributable to the factor, (excluding) other factors from the total nonerror variation” (Pierce et al. 2004). All analyses used log-transformed site means for the habitat variables (canopy openness and prawn CPUE) and least square site means for the life-history traits. Predation community was entered as a fixed effect and the habitat variables from each site were included as covariates. This general linear model procedure is equivalent to multiple linear regression with each of the three explanatory variables entered into the model. In this case, however, the method employed is preferable. This is because predation community is a categorical variable with three levels (HP, RG, RO); in multiple linear regression a dummy coding scheme would be required and this complicates interpretation when only the overall effect of predation community is of interest.

Results

North-slope *Rivulus* show life-history differentiation among predator communities (Tables 2 and 3, Fig. 3). The greatest divergence is between RO and HP populations. RG populations exhibited life-history values that were intermediate between *Rivulus* from RO and HP sites, but usually closer to the RO populations (Table 3, Fig. 3). The Paria River exhibited a different pattern than that of other rivers. *Rivulus* from the RG (narrow) site had low values for life-history traits (number of eggs, reproductive allotment,

and reproductive tissue weight) relative to other RG sites, and the Paria RO site showed unusually high values for number of eggs, reproductive allotment, and reproductive tissue weight (Table 3). In contrast to other rivers, the egg weight for the Marianne RO population was lower than that of either the HP or RG populations from this river (Table 3). This one anomalous result caused a highly significant river by community interaction for egg weight (Table 2A). These atypical results are further explored in the discussion section, “Explaining variation in life-history traits.”

Community effects

Rivulus from HP populations were characterized by a greater number of eggs, lower egg weight, greater reproductive allotment, and higher reproductive tissue weight than *Rivulus* from RO populations (Table 3, Fig. 3). All of these differences were significant at the 0.05 level and the degree of contrast between these communities is worth noting. *Rivulus* from HP localities exhibited a 74% increase in number of eggs, a 15% reduction in egg weight, and a 54% increase in reproductive allotment relative to RO fish.

Rivulus from RG sites showed a phenotype intermediate to that of HP and RO fish (Table 3, Fig. 3). *Rivulus* from RG sites showed a lower number of eggs, greater egg weight, lower reproductive allotment, and lower reproductive tissue weight relative to HP fish; although Fisher's LSD tests revealed that only the differences between number of eggs and egg weight were statistically significant (Table 3). These differences translate to a 31% decline in number of eggs, a 15% increase in egg weight, and a 12% decline in reproductive allotment.

There were modest life-history differences between RG and RO fish. Specifically, RO fish were found to have 19% fewer

Table 3. Life-history parameters of *Rivulus* from each site. Least square means (SE) are reported for number of eggs, egg weight, reproductive allotment, and reproductive tissue weight. See text for details of the size at maturity calculation. The egg weight measurement is a wet weight while that of reproductive allotment and reproductive tissue weight are dry weights. Values under the “Community Comparisons” are the least square means for the different community types. Superscript letters (a, b) represent the results of Fisher’s LSD tests or Kruskal–Wallis test (size at maturity).

| River and fish community (gravid females/total females) | Size at maturity (mm) | Number of eggs | Egg weight (mg) | Reproductive allotment (%) | Reproductive tissue weight (mg) |
|--|--------------------------|---------------------------|--------------------------|-------------------------------|------------------------------------|
| Yarra River | | | | | |
| High predation (13/19) | 35 | 12.63 (1.80) | 3.42 (0.11) | 7.72 (0.57) | 19.20 (2.32) |
| <i>Rivulus</i> /guppy (3/25) | NA | 8.57 (3.77) | 3.90 (0.23) | 3.95 (1.19) | 13.70 (4.85) |
| <i>Rivulus</i> only (18/29) | 31 | 6.85 (1.53) | 4.27 (0.09) | 3.65 (0.48) | 12.94 (1.97) |
| Marianne River | | | | | |
| High predation (26/34) | 33 | 8.51 (1.28) | 3.79 (0.08) | 5.11 (0.40) | 14.83 (1.64) |
| <i>Rivulus</i> /guppy (21/40) | 39 | 6.34 (1.41) | 4.11 (0.08) | 4.65 (0.45) | 13.15 (1.82) |
| <i>Rivulus</i> only (27/40) | 35 | 5.26 (1.24) | 3.52 (0.07) | 3.14 (0.39) | 8.96 (1.60) |
| Paria River | | | | | |
| <i>Rivulus</i> /guppy (wide) (36/40) | 31 | 9.90 (1.09) | 3.32 (0.07) | 6.94 (0.42) | 18.66 (1.07) |
| <i>Rivulus</i> /guppy (narrow) (10/37) | 45 | 4.18 (1.87) | 3.64 (0.12) | 3.68 (0.71) | 9.61 (1.83) |
| <i>Rivulus</i> only (15/26) | 43 | 8.43 (1.70) | 3.58 (0.10) | 4.67 (0.54) | 16.59 (2.18) |
| Madamas River | | | | | |
| High predation (9/29) | 37 | 12.85 (2.29) | 3.07 (0.14) | 5.23 (0.72) | 19.81 (2.95) |
| <i>Rivulus</i> /guppy (46/48) | 33 | 6.09 (0.95) | 3.87 (0.06) | 4.40 (0.30) | 12.66 (1.23) |
| <i>Rivulus</i> only (29/59) | 39 | 5.27 (1.20) | 4.13 (0.07) | 3.19 (0.38) | 10.63 (1.55) |
| Sans Souci River | | | | | |
| High predation (25/44) | 33 | 8.87 (1.42) | 2.97 (0.09) | 3.80 (0.45) | 13.05 (1.83) |
| <i>Rivulus</i> only (30/44) | 37 | 4.97 (1.18) | 3.51 (0.07) | 3.52 (0.37) | 10.04 (1.52) |
| Community Comparisons | | | | | |
| High predation | 34.5 (1.0) ^a | 10.71 (0.86) ^a | 3.31 (0.05) ^a | 5.46 (0.27) ^a | 16.72 (1.11) ^a |
| <i>Rivulus</i> /guppy | 37.0 (3.2) ^a | 7.35 (1.06) ^b | 3.81 (0.06) ^b | 4.81 (0.34) ^a | 13.97 (1.36) ^{ab} |
| <i>Rivulus</i> only | 37.0 (2.0) ^a | 6.16 (0.62) ^b | 3.80 (0.04) ^b | 3.63 (0.20) ^b | 11.83 (0.79) ^b |

eggs, a 25% lower reproductive allotment, and a 15% lower reproductive tissue weight, but only the difference in reproductive allotment was significant. *Rivulus* egg weight from these two community types was nearly identical (Table 3).

There were no differences in *Rivulus* size at maturity among predator communities (Kruskal-Wallis, $H_2 = 0.654$, $P = 0.721$).

River effects

For all life-history traits, save size at maturity, there was a significant effect of river. The Yarra River exhibited the highest life-history values for number of eggs and egg weight, whereas the Paria River exhibited the highest reproductive allotment and reproductive tissue weight (Table S2). For three of four life-history traits, the Sans Souci River had the lowest values (Table S2).

Paria river contrast

There were significant differences between the two RG sites in the Paria River for all life-history traits (Table 2B). *Rivulus* from the RG site with habitat features typical of a HP locality (hereafter “wide” site) showed a significantly higher number of eggs,

lower egg weight, higher reproductive allotment, and higher reproductive tissue weight than *Rivulus* from the more typical RG locality. Furthermore, the values of the life-history traits in *Rivulus* from the RG (wide) site are comparable to those of HP sites in other rivers (Table 3). The *Rivulus* life-history differences between these two sites are striking in part because the RG population with habitat features typical of other RG sites exhibited unusually low values for most life-history traits (Table 3).

Multivariate analyses

The discriminant function analysis separated north-slope *Rivulus* populations on an axis characterized by populations with a high number of eggs, low egg weight, and high reproductive allotment versus those with a low number of eggs, large egg weight, and low reproductive allotment (Fig. 4A). This axis (Discriminant Function 1) accounted for 96.7% of the discriminating power in the model. The discriminant function successfully classified nine of 11 north-slope populations to their proper community type on the basis of the combined life-history features. An analysis of the Mahalanobis distance between group centroids indicated that there was a significant difference between RO and HP populations

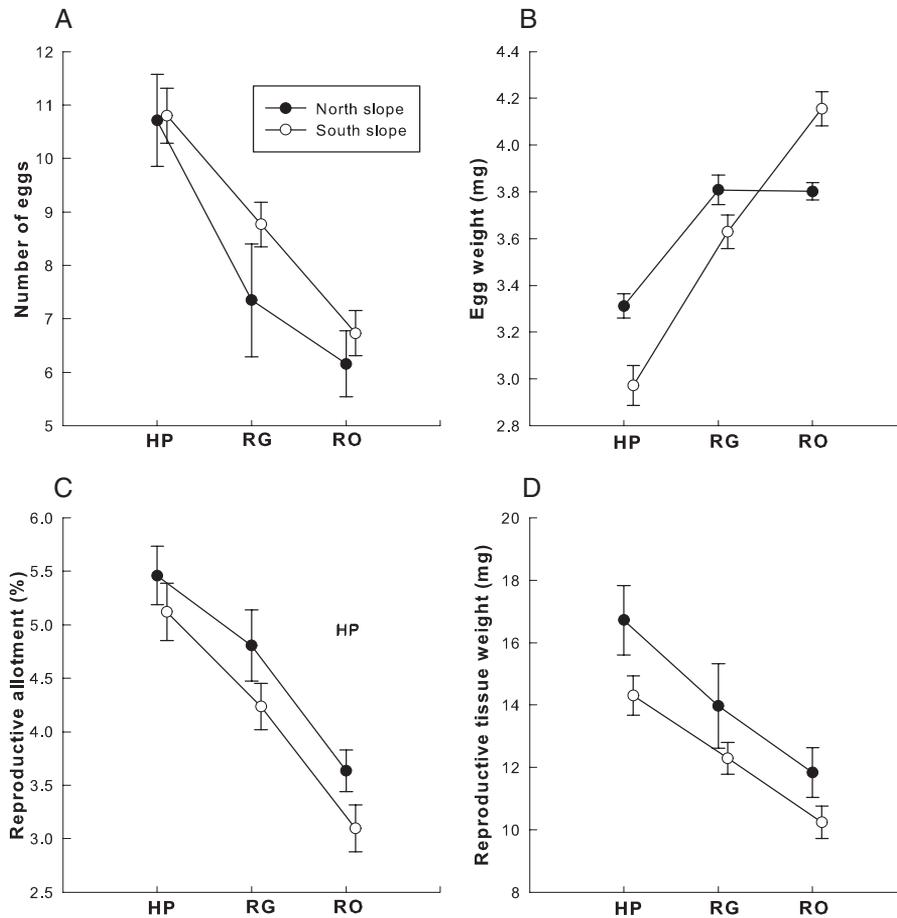


Figure 3. Least square means (\pm SE) of *Rivulus* life-history traits from the three community types on the north and south slope. The covariate in the south-slope analyses was adjusted to the same level as that of the north slope. (A) Number of eggs; (B) egg weight (mg); (C) reproductive allotment (%); (D) reproductive tissue weight (mg). In each graph high predation—HP, *Rivulus*/guppy—RG, and *Rivulus* only—RO.

($F_{3,6} = 6.57$, $P = 0.025$). The difference between RG and HP populations was marginally nonsignificant ($F_{3,6} = 3.78$, $P = 0.078$), whereas the difference between RO populations and RG populations was not significant ($F_{3,6} = 0.45$, $P = 0.73$).

A separate discriminant function performed on south-slope *Rivulus* populations performed equally well, classifying 10 of 12 populations correctly on the basis of community (Fig. 4B). The factor structure matrices of the north- and south-slope analyses are presented together to facilitate qualitative comparison (Table 4). The factor structure coefficients for the first discriminant function are in the same direction across both slopes (Table 4), indicating that populations are separated in a qualitatively similar manner (Fig. 4). Egg weight makes the largest classification contribution for south-slope populations, whereas for north-slope populations it is egg number that makes the largest contribution.

As a final informal test of *Rivulus* life-history convergence the south-slope discriminant function was saved and used to cross-classify north-slope populations. Seven of 11 populations were classified to the correct community type. Three of the four

misclassifications involved RG populations misclassified as RO (or vice versa) thus highlighting the fact that considerable overlap exists between these two community types.

Environmental factors contributing to the life-history phenotype

Across sites, predation community and canopy openness were found to explain significant variation in *Rivulus* number of eggs. Canopy openness alone was found to explain significant variation in *Rivulus* reproductive allotment and reproductive tissue weight (Table 5). Prawn CPUE was marginally nonsignificant in the analysis of size at maturity. In the analysis of egg weight and proportion of gravid females, all three habitat variables were nonsignificant.

Discussion

Rivulus found across a series of predation communities in rivers located on the north slope of the Northern Range Mountains in Trinidad show consistent patterns of phenotypic life-history

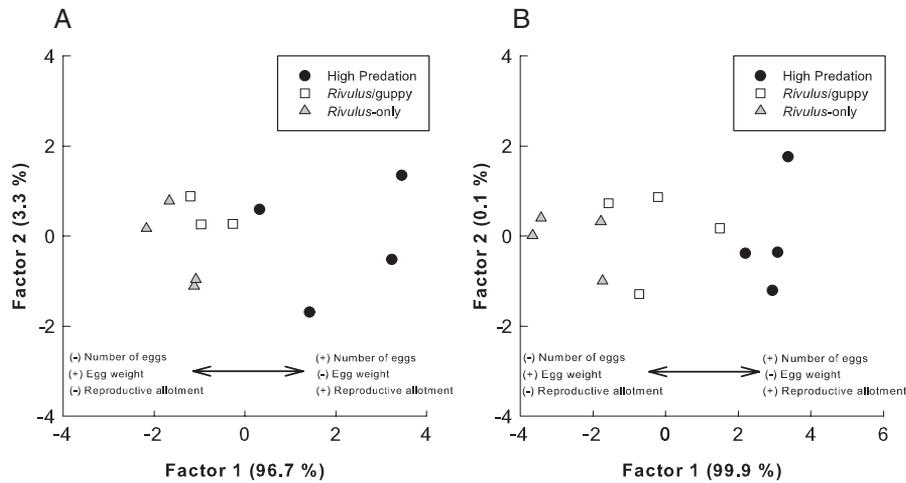


Figure 4. Plot of discriminant scores when (A) north-slope and (B) south-slope *Rivulus* populations are categorized according to community. Closed circles = high predation; Open squares = *Rivulus/guppy*; Gray triangles = *Rivulus* only. Factor 1, which accounted for the vast majority of variation, separated communities characterized by a low number of eggs, increased egg weight, and low reproductive allotment and those with a high number of eggs, decreased egg weight, and high reproductive allotment.

divergence. *Rivulus* phenotypes from HP sites were characterized by more and smaller eggs, higher reproductive allotment, and greater reproductive tissue weight. RO sites exhibited the opposite spectrum of traits and RG sites were intermediate. Such patterns of divergence are consistent with our prior studies on *Rivulus* from rivers on the south slope of the Northern Range Mountains (Fig. 3) (Walsh and Reznick 2009). Not only did north- and south-slope *Rivulus* communities follow the same qualitative patterns of life-history traits, but a discriminant function generated from the south-slope populations was, for the most part, equally successful at cross-classifying north-slope populations on the basis of community type. All of the above data indicate that predation community and correlated environmental differences are major determinants of life-history evolution in *R. hartii*, and the same general patterns were found on both slopes despite the predatory species being different.

Table 4. Values from the canonical discriminant function analyses performed on *Rivulus* populations from north- and south-slope rivers. Discriminant Function 1 (DF1) accounts for 96.7 and 99.9 percent of variance in north- and south-slope rivers, respectively.

| Trait | North slope | | South slope | |
|------------------------|-------------|-------|-------------|-------|
| | DF1 | DF2 | DF1 | DF2 |
| Number of eggs | 0.825 | 0.355 | 0.421 | 0.071 |
| Egg weight | -0.493 | 0.846 | -0.900 | 0.381 |
| Reproductive allotment | 0.515 | 0.760 | 0.413 | 0.409 |

WERE THERE ANY DIFFERENCES IN LIFE-HISTORY PHENOTYPES BETWEEN THE TWO SLOPES?

For one of two complementary analyses, south-slope *Rivulus* showed significant differences in size at maturation across fish communities (Walsh and Reznick 2009). Specifically, in HP sites *Rivulus* exhibited the smallest size at maturity, RO sites exhibited the largest size at maturity, and RG sites were intermediate. However, on the north slope there were no significant differences in size at maturity between community types (Table 3). The calculation of size at maturity is sensitive to the number of fish in each collection that are between approximately 30 and 45 mm (the maturity window) and the reproductive state of those fish when preserved. Thus, the lack of difference between north-slope communities for this trait could be due to lack of precision rather than lack of a true difference. Alternatively, this result could be brought about by a difference between north- and south-slope communities, evolutionary contingency, or differences in genetic variation among populations (e.g., Langerhans and Dewitt 2004).

South-slope *Rivulus* showed significant separation between the RG and RO phenotype for number of eggs, egg weight, and reproductive allotment (Walsh and Reznick 2009). For north-slope *Rivulus*, only reproductive allotment was different between these two community types. This is a difference of degree rather than kind; north-slope *Rivulus* from these two community types exhibited the same patterns of life-history traits as south-slope *Rivulus*, the differences were just less. This can best be visualized by comparing the plots of discriminant scores between the two slopes (Fig. 4); RG populations are clustered closer to RO populations on the north, relative to the south slope.

A possible explanation for the lessened difference between the RG and RO phenotype on the north slope can be found in

Table 5. Environmental factors explaining variation in *Rivulus* life-history traits across all sites ($n = 14$). The results shown are from general linear models testing the effect of community (high predation, *Rivulus/guppy*, *Rivulus* only), percent canopy openness, and prawn catch per unit effort (CPUE).

| | Community | | Canopy openness | | Prawn CPUE | |
|----------------------------|---------------------|-------------|---------------------|-------------|---------------------|-------------|
| | (F) | Effect size | (F) | Effect size | (F) | Effect size |
| Size at maturity | 0.883 ^{NS} | 0.181 | 0.000 ^{NS} | 0.000 | 3.819 ¹ | 0.323 |
| Proportion gravid | 0.661 ^{NS} | 0.128 | 0.075 ^{NS} | 0.008 | 1.393 ^{NS} | 0.134 |
| Number of eggs | 5.994* | 0.571 | 12.057** | 0.573 | 0.719 ^{NS} | 0.074 |
| Egg weight | 1.470 ^{NS} | 0.246 | 0.543 ^{NS} | 0.057 | 0.306 ^{NS} | 0.033 |
| Reproductive allotment | 0.229 ^{NS} | 0.048 | 8.205* | 0.477 | 1.355 ^{NS} | 0.131 |
| Reproductive tissue weight | 1.118 ^{NS} | 0.199 | 7.546* | 0.456 | 0.091 ^{NS} | 0.010 |

NS = not significant ($P > 0.1$); * $P < 0.05$; ** $P < 0.01$; ¹ $P < 0.1$.

the nature of the biotic communities. Prawns from the genus *Macrobrachium* are abundant in north-slope rivers (Table 1, Millar et al. 2006; McKellar et al. 2009) and may diminish the impact of guppies on *Rivulus* life histories (i.e., the differences between RG and RO sites may be reduced by the presence of prawns). That is, if prawns are responsible for reducing *Rivulus* density in north-slope rivers through predation targeted at the egg or early larval stage, they may be impacting *Rivulus* in a similar fashion as guppies. However, the effect of guppies on *Rivulus* life histories is not completely overshadowed because *Rivulus* from RG sites exhibited consistent, albeit sometimes small, life-history differences compared to RO fish. This result provides further support for the notion that guppies impact *Rivulus* life-history evolution (see Walsh and Reznick 2010, 2011).

Explaining variation in life-history traits: environment versus predation

Predation community correlates with a suite of environmental variables that could potentially impact *Rivulus* life-history evolution. For instance, HP sites tend to be found in the main river and are consequently wider, deeper, and have a more open canopy compared to RG and RO sites. Canopy cover is correlated with primary productivity, which can influence the expression and evolution of life-history traits (Grether et al. 2001; Reznick et al. 2001). The comparison between the two RG sites within the Paria River, with contrasting environmental features, sought to separate the independent effects of environment and predation community on *Rivulus* life-history traits. The main Paria River has habitat features typical of a HP environment, but there are no predatory fish species.

We found that *Rivulus* inhabiting the main Paria River had life-history traits typical of HP sites from other rivers. This suggests that the environment, and likely resource levels, influence the expression of life-history traits in *Rivulus*. We also found that the other Paria RG site (with habitat features typical of other RG

sites: see Table 1) exhibited life-history values that were atypically low compared to sites from all other rivers. In addition, *Rivulus* from the Paria RO site exhibited an unusually high number of eggs and reproductive tissue weight. The causes of these atypical results for the Paria River populations are unknown. It is possible that the two most upstream Paria populations (RG and RO) had been impacted by human activity due to the proximity of these sites to a small town.

In this study, we observed many significant correlations between predation community and life-history traits in *Rivulus* across several independent rivers (Table 3). Such results were expected on the basis of theoretical frameworks (i.e., Charlesworth 1980) and our previous empirical studies (see Walsh and Reznick 2009, 2010, 2011). However, our sampling potentially confounds the impacts due to predators with any environmental variables that are correlated with predation community. To address this issue, we measured many potential confounding environmental variables, such as water temperature, which can affect life-history traits in fish (Houde 1989; Sponaugle et al. 2006). We did not observe significant differences among different fish communities in water temperature, several water chemistry variables, or prawn abundance (Table 1). However, canopy openness covaried with community (Table 1) and explained significant variation in several *Rivulus* traits (e.g., number of eggs, reproductive allotment, and reproductive tissue weight) (see Table 5). Our prior work indicates how the effects of predators and environmental variables such as canopy openness may interact in a complex, nonindependent manner. For example, *Rivulus* in HP sites show the highest adult growth rates (A. I. Furness, unpubl. data) and this effect could be due to both increased resources owing to reduced *Rivulus* densities (as caused by predation) and to increased levels of resources perhaps through higher productivity caused by a more open canopy (Grether et al. 2001). Overall, separating the impacts of predation and the environmental factors that often covary with predation community is a challenge in this system (and most

evolutionary studies of natural populations), and the results of the present study may be influenced by both the presence of predators and canopy cover.

Our prior use of experimental perturbations in natural streams provides strong evidence that guppies are the cause of life-history divergence between RG and RO sites. In 1976 and 1981 guppies were introduced into formerly RO sites. After 25 years, the life-history responses of such *Rivulus* were evaluated relative to upstream “control” (RO) sections of the stream that lack guppies. *Rivulus* from the sites where guppies were introduced exhibited earlier maturity and increased reproductive investment thus demonstrating rapid evolution of these life-history variables in response to the introduction of guppies (Walsh and Reznick 2011). The guppy introduction and RO (control) sites were just tens of meters apart indicating that it was the introduction of guppies (i.e., predation and its indirect effects) and not confounding environmental factors, such as canopy openness that caused this life-history evolution. Consequently, the parallel patterns of divergence observed between RG and RO sites in the north- and south-slope rivers thus argues that the impacts of guppies on *Rivulus* population biology is an important selective pressure.

The case for convergent evolution

To make the case that *R. hartii* shows convergent life-history evolution, a number of points need to be addressed. Life-history evolution in *Rivulus* from the south slope was interpreted in terms of both the direct and indirect effects of predation. It is currently unknown whether similar selective pressures operate in north-slope rivers. However, there are indications that this may not be entirely the case. *Rivulus* density is noticeably lower in north-slope rivers compared to south-slope rivers (Fraser et al. 1995, A. I. Furness pers. obs.). This difference in density was hypothesized to be due to the negative effects of *Macrobrachium* prawns (Fraser et al. 1995). However, it is unknown how effective prawns are at preying upon *Rivulus* in the wild, or if they target particular size classes or even *Rivulus* eggs. It is also worth noting that in all three community types *Rivulus* were often found congregated in side pools slightly removed from the main river, and in these areas they could be locally abundant, even if infrequently found in the main river. That we have demonstrated consistent phenotypic life-history differences across communities is strong evidence that *Rivulus* undergo contrasting selection in these different environments. The details of *Rivulus* population dynamics and ecology in north-slope rivers, and whether they are similar to the better-characterized south-slope communities warrants further study.

We have demonstrated phenotypic divergence of *Rivulus* across different predation communities. Yet, phenotypes are influenced by a combination of genetic and environmental influences. The genetic basis of the life-history differences among *Rivulus* in north-slope rivers is unknown. However, it is very likely that many

of the differences in life-history traits have a genetic basis. This is because *Rivulus* from south-slope rivers exhibited similar life-history patterns across predation communities, and the differences in these life-history traits were found to remain after two generations of rearing in a common laboratory environment (Walsh and Reznick 2008, 2010). In such experiments the environment is held constant, and we assume that any remaining differences among populations represent genetic differences among populations. The use of second-generation fish minimizes confounding environmental and maternal effects.

In these laboratory, common-garden studies *Rivulus* were reared on food levels that sustain the approximate differences in growth between these sites. For instance, mark-recapture studies on *Rivulus* from RG and close by RO sites reveal that *Rivulus* found with guppies grow faster (Walsh et al. 2011); our common-garden experiments incorporated food levels that matched these differences (Walsh and Reznick 2010). The results of our common garden experiments showed that the differences between fish communities depended strongly upon food level (Walsh and Reznick 2008, 2010). *Rivulus* from RG and HP sites produced more eggs and invested more heavily into reproduction than *Rivulus* from RO sites on high levels of food availability, but such trends lessened, disappeared, or reversed on lower food levels (Walsh and Reznick 2008, 2010). Such interactions provide strong evidence for local adaptation to environments that differ in resource availability and also suggest that the indirect effects of predators, mediated through changes in resource availability, shape evolution in *Rivulus* (Walsh and Reznick 2008, 2010). More importantly, the parallel trends of phenotypic divergence observed in this study and our prior work (Walsh and Reznick 2009) and the consistent evidence for local adaptation across several independent rivers on the south slope (Walsh and Reznick 2008, 2010, 2011) suggest that the current results reflect, at least in part, genetically based variation.

Convergent evolution by definition entails independent evolution of the trait or phenotype in separate lineages, rather than it being inherited from a common ancestor (Futuyma 1986; Arendt and Reznick 2008). In our intraspecific study, we have chosen to view each river as if it were an independent replicate nested within slope. The isolation between the north- and south-slope watersheds and different river drainages represents the independent aspect of our study. What is important is the consistent matching of predation community with a given suite of life-history traits, for it is extremely unlikely that any pattern of colonization would result in a near-perfect match between the colonizer(s) life-history phenotype and that being selected for in its new environment. The repetition of the same phenotypic pattern across multiple drainages also strongly suggests natural selection is the cause of such differences, as the stochastic nature of genetic drift would be unlikely to produce consistent patterns of evolutionary change (Endler 1986; Schluter and Nagel 1995). It is more parsimonious

to assume that after *Rivulus* colonize a given river drainage they expand their range and in doing so adapt to the different predation communities they come across. Thus, in general, we would expect *Rivulus* from different predation communities within the same river drainage to be more closely related to each other than to *Rivulus* from the same predation community but in different river drainages.

Two recent molecular phylogenetic studies of *R. hartii* from Trinidad and surrounding islands shed some light on this issue (Jowers et al. 2008; Walter et al. 2011). Walter et al. (2011) found that the greatest amount of genetic differentiation among Trinidadian *R. hartii* corresponds to the north-south split of the Northern Range Mountains, although there was evidence of genetic admixture across slopes in Trinidad's northeast corner. This bolsters the case for convergence; that is, because north- and south-slope *Rivulus* are very distantly related they evolved convergent patterns of life-history divergence independently, presumably in response to similar selection pressures in each river. Two *Rivulus* populations were sampled from the same river drainage in one instance (the main Guanapo River and one of its tributaries) and these populations were found to be more closely related to each other than they were to populations from other rivers (Walter et al. 2011). Walsh and Reznick (2009) found that *Rivulus* from the Guanapo River drainage exhibited the full spectrum of life-history divergence across different predation communities. Thus, the molecular (Walter et al. 2011) and life-history data (Walsh and Reznick 2009) from the Guanapo River indicates independent evolution of divergent life histories within this river drainage, thus supporting the treatment of rivers as independent replicates. Similar molecular patterns in other rivers (that contain the full spectrum of life-history divergence) would argue for independent, convergent evolution of life histories within each river, and would extend the case for convergence from differences between the north and south slopes to rivers found within each drainage. Within river drainages, we would expect low levels of gene flow among different populations—downstream migration could occur as a result of flooding, whereas upstream migration could occur as a result of *Rivulus* colonization ability and penchant for traveling over the damp forest floor to reach headwater streams (Seghers 1978). That sites exhibit significant life-history differences indicates that gene flow is not so great as to obviate local adaptation. Furthermore, Walter et al. (2011) found that *Rivulus* from the two sites within the Guanapo River exhibited significant population-level genetic differentiation at microsatellite loci, suggesting little contemporary gene flow between these two sites.

Conclusions

Despite the geographic separation of the two watersheds and different predatory species, we observed the same general patterns of *Rivulus* life-history divergence across analogous predator

communities in north- and south-slope rivers—that is, *Rivulus* exhibited convergence of the life-history phenotype. Guppies (*P. reticulata*) are found in high- and low-predation habitats in multiple rivers spanning these same slopes, and they likewise exhibit convergence of life histories, despite the predatory species being different across slopes (Reznick and Endler 1982; Reznick and Bryga 1996; Reznick et al. 1996). A few general themes emerge from the parallel study of both species. The first is the generality of predation as an explanation for life-history divergence among different biological communities. Experimental manipulations of guppies and their predators have demonstrated a cause and effect relationship between predator-induced mortality and changes in guppy and *Rivulus* life histories. In 1976 and 1981 guppies native to HP environments were introduced above barrier waterfalls, into previously guppy-free (RO) sections of stream, resulting in the rapid evolution of delayed maturity and reduced reproductive effort (Reznick and Bryga 1987; Reznick et al. 1990). Furthermore, the introduction of guppies caused life-history evolution in *R. hartii* found in these sites (Walsh and Reznick 2011). In a complementary experiment, predation intensity on guppies was increased by introducing predatory pike cichlid, *C. alta*, above a barrier waterfall in the Aripo River that had formerly prevented its upstream movement. Guppies were found below this barrier, with *C. alta* and other predatory species (natural HP site), directly above the waterfall (experimental predator-introduction site), and even further upstream above waterfalls that prevented the introduced *Crenicichla* from moving upstream. After five years, the guppies in the site with introduced *Crenicichla* showed an age and size at maturity statistically intermediate between that of guppies from the upstream and downstream control sites (Reznick 1997). Although the above experiments reveal the generality of predation as an explanation for life-history divergence among different biological communities, a second theme is that in both guppies and *Rivulus* environmental variables that are correlated with predation community provide additional potential causes of life-history evolution. Both species share the same general confounding of predation community and environmental variables; HP sites tend to be higher order rivers and predatory species drop away as one moves further upstream into lower order streams. The overall picture emerging from life-history studies on these two fish species is that their life histories consistently evolve in the same fashion in response to similar selective environments. The consistent matching of phenotype with predation community, and correlated environmental differences, implicates natural selection as the cause of such divergence. Having similar patterns of convergence in two species in the same ecosystem sets the stage for a more robust evaluation of the combined roles of the direct and indirect effects of predators and correlated features of the environment, particularly resource availability, in shaping life-history evolution.

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Supporting Information

The following supporting information is available for this article:

Table S1. Universal Transverse Mercator (UTM) coordinates of north-slope *Rivulus* collection sites.

Table S2. Least square means (SE) of *Rivulus* life-history traits from each river.

Table S3. Correlation coefficients (r) between environmental variables (transformed site means) and life-history traits (Least square means from each site).

Table S4. Correlation coefficients (r) between environmental variables (transformed site means).

Supporting Information may be found in the online version of this article.

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