

RAPID EVOLUTION OF ESCAPE ABILITY IN TRINIDADIAN GUPPIES (*POECILIA RETICULATA*)

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Abstract.—Predators are widely assumed to create selection that shapes the evolution of prey escape abilities. However, this assumption is difficult to test directly due to the challenge of recording both predation and its evolutionary consequences in the wild. We examined these events by studying natural and experimental populations of Trinidadian guppies, *Poecilia reticulata*, which occur in distinct high-predation and low-predation environments within streams. Importantly, in the last two decades several populations of guppies have been experimentally introduced from one type of predatory environment into the other, allowing measurements of the consequences of change. We used this system to test two hypotheses: First, that changes in predatory environments create phenotypic selection favoring changes in escape ability of guppies, and second, that this selection can result in rapid evolution. For the first test we compared escape ability of wild caught guppies from high- versus low-predation environments by measuring survival rates during staged encounters with a major predator, the pike cichlid *Crenicichla alta*. We used guppies from three streams, comparing two within-stream pairs of natural populations and three within-stream pairs of an introduced population versus its natural source population. In every comparison, guppies from the high-predation population showed higher survival. These multiple, parallel divergences in guppy survival phenotype suggest that predatory environment does create selection of escape ability. We tested our second hypothesis by rearing guppies in common garden conditions in the laboratory, then repeating the earlier experiments using the F₂ generation. As before, each comparison resulted in higher survival of guppies descended from the high-predation populations, demonstrating that population differences in escape ability have a genetic basis. These results also show that escape ability can evolve very rapidly in nature, that is, within 26–36 generations in the introduced populations. Interestingly, we found rapid evolutionary loss of escape ability in populations introduced into low-predation environments, suggesting that steep fitness trade-offs may influence the evolution of escape traits.

Key words.—Genetic divergence, geographic variation, phenotypic selection, *Poecilia reticulata*, predator-prey interaction.

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Many fields of biology assume that predators are the primary factor shaping the evolution of prey escape abilities. However, this causal hypothesis is difficult to test due to the challenge of measuring selection by predators and the evolutionary consequences for prey in a single, natural system. Several studies demonstrate that predators create phenotypic selection favoring enhanced escape ability of prey (e.g., Brodie 1992; Dugatkin 1992; McCollum and Van Buskirk 1996; Watkins 1996), and others show that genetically based differences in escape ability among related prey populations or species are correlated with predatory environment (e.g., Seghers 1974; Sinervo and Losos 1991; Huntingford et al. 1994; Magurran et al. 1995). If these patterns result from causal effects of predators on prey evolution, then changes in predatory environments should result in evolutionary changes in prey escape abilities (McPeck et al. 1996; McPeck 1997). Our study tests this hypothesis experimentally in a natural vertebrate system.

The study examines wild populations of guppies, *Poecilia reticulata*, from freshwater streams on the island of Trinidad, in the southern Caribbean. These populations occur in distinct high-predation (high mortality) and low-predation (low mortality) environments, typically downstream and upstream, respectively (reviews in Endler 1995; Magurran et al. 1995; Reznick et al. 1996a; Houde 1997; Rodd and Reznick 1997). Individual streams are largely geographically isolated from one another, and genetic studies of guppies indicate that pop-

ulations are more closely related within than between streams (reviews in Magurran et al. 1995; Houde 1997). Thus, the metapopulation in each stream represents a unique evolutionary lineage and metapopulations in separate streams provide replicate assays of the effects of divergent predation environments. Also, the composition of the predator communities varies across streams, allowing the effects of specific predators to be partitioned from the effects of the generalized predation environment (e.g., Reznick et al. 1996a). Importantly, two decades ago several populations of guppies were introduced from one type of predation environment into the other within streams, providing experimental tests of hypotheses on the causal relationship between environment and evolution (e.g., Reznick et al. 1990).

Previous studies of Trinidadian guppies from high- versus low-predation environments document differences in a diverse range of traits, including color and life-history patterns and reproductive and foraging behaviors (see above reviews). Guppies also display population differences in several anti-predator behaviors (Seghers 1973; Breden et al. 1987; Magurran et al. 1992, 1995), yet little is known about the abilities of Trinidadian guppies to survive predatory attacks (Seghers 1973). The results cited above suggest that guppies from high-predation environments will show greater escape abilities, and that these abilities will evolve in guppy populations following changes in predation environment. We test these possibilities as follows.

The first stage of the study tests the hypothesis that divergent predatory environments create divergent phenotypic selection of guppy escape ability. In these experiments, we

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TABLE 1. Characteristics of the study populations. Source population indicates the original source of guppies in introduced populations. Stream size refers to size at the study site. Grid references from Universal Transverse Mercator Grid, Zone 20, 1:25000 topographical maps (Land and Surveys Division, Trinidad and Tobago Government, 1978).

Population	Predation level	Source population	Mountain slope	Stream size	Grid reference
1 Aripo natural downstream	High	—	South	Large	PS 942 778
2 Aripo natural upstream	Low	—	South	Large	PS 937 805
3 Aripo introduced midstream	High	2	South	Large	PS 936 798
4 Aripo introduced upstream	Low	1	South	Small	PS 931 800
5 El Cedro natural downstream	High	—	South	Small	PS 896 784
6 El Cedro introduced upstream	Low	5	South	Small	PS 893 794
7 Yarra natural downstream	High	—	North	Large	PS 797 928
8 Yarra natural upstream	Low	—	North	Small	PS 834 878

compare escape abilities of wild caught guppies from high-versus low-predation environments by measuring their survival rates when placed in artificial pools with *Crenicichla alta*. We predict that survival will be greater in guppies from high-predation environments, that is, that ability phenotype previously diverged between guppy populations as a consequence of their different selective environments. We examine divergence between populations within three streams, first comparing escape abilities between natural populations to determine the historical pattern of divergence. We then compare abilities between natural source populations and their experimental, descendent populations, introduced 15–20 years earlier into new predatory environments, to determine the consequences of such change. The second stage of the study tests the hypothesis that phenotypic selection has resulted in evolution of escape abilities. We rear guppies from a subset of the original populations in common garden conditions in the laboratory, then repeat the earlier experiments using the F₂ generation. Differences in survival among the F₂ populations would suggest a genetic basis and indicate rapid evolutionary divergence of escape abilities in Trinidadian guppies.

MATERIALS AND METHODS

Study Populations

We studied guppies from three streams in the Northern Range Mountains of Trinidad. These streams contain sets of barrier waterfalls that confine large piscivorous fish to downstream regions. Guppies frequently occur both above and below waterfalls, in distinct high-predation (downstream) and low-predation (upstream) populations (reviews in Magurran et al. 1995; Reznick and Bryga 1996; Reznick et al. 1996a; Houde 1997). The suite of aquatic predators impacting guppies differs between streams on the north and south facing slopes of the mountains. On the south slope, upstream guppies primarily occur with only one other fish, *Rivulus hartii*, a small omnivore that feeds primarily on invertebrates and occasionally on juvenile guppies (Liley and Seghers 1975; Mattingly and Butler 1994). Downstream, south slope guppies occur with several large predatory fish, a principal guppy predator being the pike cichlid *Crenicichla alta* (Liley and Seghers 1975; Endler 1978). In contrast, on the north slope, upstream guppies occur with *Rivulus* and predatory prawns (*Machrobrachium crenulatum* and *M. faustinum*), and downstream guppies occur with large piscivorous fish of marine

origin, including gobies (*Gobiomorus dormitor*) and mullet (*Agonostomus monticola*), and not *Crenicichla* (Liley and Seghers 1975; Endler 1978).

We studied guppies from two south slope streams, the Aripo and the El Cedro, and one north slope stream, the Yarra (Table 1). Within each stream, we studied guppies from both high-predation and low-predation populations, and on each visit to the study sites we confirmed the presence of the predators listed above. In the south slope rivers, our study populations included both natural populations and several introduced populations, which had experienced recent changes in predation environment due to the introductions. In the Aripo, *Crenicichla* were introduced in 1981 into a midstream population of guppies that previously lacked predatory fish other than *Rivulus* (D. Reznick, pers comm.) (Table 1, Population 3). Also in the Aripo, downstream guppies were introduced in 1976 into an upstream tributary that previously contained *Rivulus*, but no guppies (Endler 1980) (Table 1, Population 4). Lastly, in the El Cedro, downstream guppies were introduced in 1981 into upstream regions containing only *Rivulus* and *Aquidens pulchar* (Reznick and Bryga 1987) (Table 1, Population 6). *Aquidens* is another smaller predator that may eat juvenile guppies, although Seghers (1973) found no guppy remains among the stomach contents of 42 *Aquidens* from south-slope rivers. We studied guppies from each of the introduction sites and source populations in 1996 and 1997 (26–36 generations after introduction, according to life-table estimates of generation time, Reznick et al. 1997). The study populations were selected to provide five within-river comparisons of guppies from high-predation versus low-predation populations: two comparisons among natural populations (Table 1, Aripo 1 vs. 2, Yarra 7 vs. 8), two comparisons of a high-predation source population with a low-predation introduced population (Aripo 1 vs. 4, El Cedro 5 vs. 6), and one comparison of a high-predation introduced population with a low-predation source population (Aripo 3 vs. 2).

Escape Ability of Wild Caught Guppies

This study compares the ability of wild caught, adult guppies from high and low-predation populations to escape predation when placed in artificial pools with *Crenicichla alta*. The experiments involved color marking six guppies from each of the two populations to be compared, placing these 12 guppies in a *Crenicichla* enclosure, allowing the cichlid

TABLE 2. Results of multifactor ANCOVA of survival differential for each set of comparisons. The factor separation event refers to the cause of geographical separation between populations (i.e., natural vs. introduction events) in a comparison. All 1996 comparisons involved population pairs separated by introductions. Results of two-way ANOVA/ANCOVA of all possible two-factor combinations of these variables showed similar main effects and no significant interaction terms (see Methods, Statistical Analyses). Data for wild caught guppies combined in subsequent analyses. *Indicates covariate.

Source	Wild caught 1996			Wild caught 1997			F ₂ guppies		
	df	F	P	df	F	P	df	F	P
Separation event	—	—	—	1	12.53	0.001	1	0.62	0.44
Trial cichlid	2	0.51	0.61	7	1.44	0.22	2	0.03	0.97
Trial rank order*	1	0.07	0.80	1	0.38	0.54	1	0.85	0.37
Guppy size*	1	5.01	0.045	1	0.04	0.85	1	2.57	0.12
Marking color	1	0.03	0.87	1	2.19	0.15	1	0.55	0.47
Guppy gender	—	—	—	—	—	—	1	0.48	0.50
Error	12			36			23		

Dependent variable: survival differential.

to capture half of the guppies, and then scoring the identity of the guppies remaining. To clarify terminology, we consider a *trial* to be one such encounter between a cichlid and 12 guppies, and a *comparison* to be the full set of trials comparing a given pair of low- versus high-predation populations. We used the following random block design for our central set of comparisons, conducted in 1997. Each comparison consisted of eight trials, one trial with each of eight different cichlids. We conducted six comparisons, for a total of 48 trials, and used the same eight cichlids for the six comparisons, randomizing the order that each cichlid encountered the comparisons. The experiment was designed to prevent possible differences among cichlids or trial orders from confounding differences among comparisons. The design also allowed statistical tests of effects of cichlid identity and trial order on the absolute outcome of comparisons; we detected no such effects (Statistical Analyses; Table 2). In 1996 we conducted three pilot comparisons, a subset of the six conducted in 1997. These studies consisted of 5–8 trials per comparison for a total of 18 trials, and used each of three cichlids in 2–4 trials per comparison.

The experiments were conducted in screened, open-air rooms at Verdant Vale Research Station in April 1996, and at Simla Research Station in April 1997, in the Arima Valley, Trinidad. *Crenicichla* were captured with hook and line from local rivers separate from those from which guppies were sampled. Captured cichlids measured 110–190 mm SL, and were housed in circular plastic pools (diameter 1.8 m) filled with water to a depth of 20 cm. The pools were divided into quarters with fine plastic screen; we housed one cichlid in each quarter. We provided each cichlid with two shelters built of rocks, but otherwise kept the pools empty of debris. Each cichlid enclosure was initially stocked with five guppies. These stock guppies originated either from streams not represented in the experiments (1996) or from an artificial pond at Simla that contained a mixed, breeding population of guppies from streams throughout Trinidad (1997). The cichlids began eating the guppies three to ten days following capture, and then typically ate all guppies present within several hours. Thereafter, on days that the cichlids were not used in experiments, each cichlid was given 4–5 stock guppies. We began the experiments 8–10 days following capture, and completed all experiments within four weeks of capturing the cichlids.

Guppies for the trials were collected from streams with hand held dip nets and used in trials within four days of collection. Prior to the trials, the guppies were housed in plastic aquaria (20 × 30 × 20 cm high) and fed three times daily with commercial tropical fish food. On the day before a trial, guppies from the two populations to be compared were anesthetized (in water containing 230 mg/l tricaine methanesulfonate [MS-222] and 115 mg/l sodium bicarbonate), measured to the nearest 0.1 mm, and marked with a spot of acrylic latex paint (red or black) injected into the caudal peduncle (method from Reznick et al. 1996b). In half of the trials in a given comparison, the high-predation guppies were marked red and the low-predation guppies black; colors were reversed in the remaining trials. The colors remained clear and unequivocal throughout the trials.

After marking, the 12 guppies in a trial (six from each population) were placed together in a 500 ml recovery chamber, fed, and left undisturbed overnight. Any guppy that appeared unhealthy the next morning was replaced with a spare that had been marked at the same time (4% of the guppies were replaced overall, never more than two per trial). The trial guppies were then released in the cichlid enclosure. The cichlids hid during the release, emerging 4–20 minutes later. The enclosures were monitored at regular intervals, and when as near as possible to a total of six guppies remained, these guppies were removed and their colors recorded. The trials lasted 15–240 min from time of release to removal of the guppies.

Guppies were size matched within trials, such that means of the two populations differed by less than 1.0 mm (mean difference = 0.1 mm ± 0.4 sd, $n = 66$ trials). All but two comparisons used only adult female guppies (14–22 mm SL), which lack natural color and fin shape variation. One comparison (Aripo 1 vs. 4, Table 1) was conducted twice in 1997, once as a set of eight trials using all females and then again using all males (14–17 mm SL), to examine the possible influence of gender; none was found (see Statistical Analyses). A second 1997 comparison (Yarra 7 vs. 8) consisted of six trials of females and two of males, due to low population sizes in the wild. The comparison result showed similar significance whether the male trials were included or excluded. Thus, for simplicity and consistency with the other, single-sex comparisons, we included only the six female trials in the central analyses of this comparison.

Guppy Breeding in the Laboratory

To examine the genetic basis of population differences in escape ability, we reared guppies in common garden conditions in the laboratory for two generations, then measured escape ability of the F_2 guppies. We used the four populations from the Aripo River (Table 1) for this phase of the study. We initiated each laboratory population with 12–15 adult male and 20–25 adult female guppies, collected in April 1996 and transferred within 10 days to our laboratory in Irvine, California. As adult female guppies store sperm, the total paternal contribution to the laboratory population probably exceeded that of the collected males. Guppies were housed in ten gallon ($26 \times 50 \times 30$ cm high) glass aquaria at 24–26°C, the temperature of the study streams in Trinidad (Reznick and Endler 1982) and exposed to a 12:12 light:dark cycle. Guppies were fed once or twice daily with commercial tropical fish food. Throughout the study, we endeavored to maintain equal fish densities, feeding and maintenance conditions in all aquaria, except that we housed wild caught fish at half the density of the F_1 and F_2 generations. Initially, each population of wild caught fish was divided between two aquaria. After four weeks, gravid adult females were transferred to maternity chambers fixed inside three additional aquaria per population, designated for the F_1 generations. Each plastic maternity chamber ($20 \times 9 \times 9$ cm high) housed 2–4 adult females. Guppies are live-bearers, and the chambers had slotted bottoms that allowed newborn, but not adult guppies to escape. Females were kept in these chambers until they gave birth (2–6 weeks), then returned to their original aquaria and not re-used. We used 16–21 wild caught females to produce each F_1 population, which contained 90–120 individuals each, with approximately equal numbers of males and females. We reared the F_1 guppies to maturity, then generated the F_2 populations as above, but using 25–35 F_1 females as dams and four aquaria for each F_2 population.

Escape Ability of F_2 Common Garden Guppies

We compared escape ability among the four Aripo populations of laboratory-raised, F_2 guppies, in trials designed to replicate those conducted in Trinidad. Specifically, we compared the natural high- versus natural low-predation populations, the introduced high- versus natural low-predation populations, and the natural high- versus introduced low-predation populations. We used the same experimental protocol to conduct these trials as used for the wild caught guppies, with the following additional details. We used three *Crenicichla* in the trials, two collected in April 1997 and used in the 1997 Trinidad trials, and the third collected in August 1997. The cichlids were transported to our laboratory in California within four weeks of capture, and housed in one of the pools that had been used in the Trinidad trials. The pool was maintained at 24–26°C and exposed to a 12:12 light:dark cycle. When not involved in trials, the cichlids were fed 2–3 domestic guppies, 2–3 times a week (*Crenicichla* only eat live prey). In the week prior to a trial, the cichlids were fed 4–5 guppies every other day, and not fed on the day before the trial. Trials were conducted no more than every other day. Trial guppies were selected from groups of fish collected from all four of the F_2 tanks per population. In

order to obtain sufficient sample sizes, the trials were conducted at two month intervals between January and September 1998, as the F_2 guppies matured into the appropriate size classes. Trials from each comparison were conducted at each two month interval, to avoid confounding comparison with interval. Each comparison examined both male and female guppies, with separate trials for the sexes, and $n \geq 5$ trials per sex. Within comparisons, each set of five trials per sex used each of the three cichlids at least once. Within this framework the order of cichlid use was randomized. Mean size difference between guppies of different populations within trials was $0.2 \text{ mm} \pm 0.3 \text{ sd}$, $n = 31$ trials.

Statistical Analyses

We initially analyzed the data in three separate sets, that of wild caught 1996, wild caught 1997, and F_2 guppies. Our results for each trial of these experiments consist of the number of surviving guppies (effectively survival rates) for each of the two populations in the trial. We additionally calculated *survival differential* for each trial: number of surviving guppies from the high-predation population less the number from the low-predation population. Our central analysis tested the hypothesis that survival differential was greater than zero for each population comparison. Before performing this central analysis we conducted the following preliminary examinations of the data. Each of the three datasets was examined for normality. We detected no departures, excepting one outlying trial result in the F_2 data, discussed below. We next examined possible effects of factors controlled by the random block design of the experiment, trial order, cichlid identity, and guppy marking color that we predicted would not affect survival differential. We also examined effects of guppy mean size and gender, factors that were matched within trials, and effects of *separation event* (the cause of the geographical separation between populations in a comparison, i.e., natural vs. introduction events), as visual observations of the data suggested survival differentials differed between these two types of comparisons. The six factors were examined in separate analyses of covariance (ANCOVA) for the 1996, 1997, and F_2 datasets. The models were constructed by first examining all possible pairwise interaction terms in a series of separate two-factor models comprised of two main effects plus the interaction. None of these interactions terms were significant, and further models were limited to main effects only. Table 2 presents the results of multifactor models that include all main effects. Results of the two-factor models were similar for all effects. Trial order was initially coded and tested in two forms: as trial date, and as rank order of the trial for the cichlid (i.e., each cichlids first trial receives rank order one, etc.). Results were similar for both forms and we present results for rank order only. Gender was not an appropriate factor in the 1996 and 1997 ANCOVAs as the 1996 comparisons used only females and the 1997 used only females in all but one comparison. One-way ANOVA of the 1997, Aripo 1 versus 4 comparisons (comprised of one all-male and one all-female comparison) indicated no gender effect on survival differential ($F_{1,14} = 0.44$, $P = 0.51$). The above analyses supported the expectation that trial order, cichlid identity, guppy marking color, and guppy gender did

TABLE 3. Comparisons of escape ability among wild caught guppies from high-predation versus low-predation populations, when placed in pools with *Crenicichla*. Trials began with six guppies per population, and except as noted involved female guppies only. Comparisons with $n > 8$ trials combine data from 1996 and 1997. Population identity numbers from Table 1. Data are mean \pm one SD, P -values from paired t -tests.

Comparison		Number of survivors		Trials (n)	P -value
High-predation population	Low-predation population	High-predation population	Low-predation population		
1 Aripo natural	2 Aripo natural	5.0 \pm 0.8	1.1 \pm 0.6	8	0.0001
7 Yarra natural	8 Yarra natural	5.3 \pm 1.0	1.3 \pm 0.8	6	0.001
3 Aripo introduced	2 Aripo natural	3.5 \pm 1.4	2.3 \pm 1.0	13	0.005
1 Aripo natural	4 Aripo introduced	4.3 \pm 1.3	2.0 \pm 0.9	13	0.0006
1 Aripo natural, males	4 Aripo introduced, males	3.9 \pm 1.1	2.1 \pm 1.1	8	0.02
5 El Cedro natural	6 El Cedro introduced	4.1 \pm 1.3	2.4 \pm 1.2	16	0.003

not influence survival differential (Table 2); therefore these factors were excluded from further analysis. Effects of size are discussed further below.

These preliminary analyses suggested that design differences between years were unimportant, thus we used two-way ANCOVA to investigate the consequences of combining the two datasets of wild caught guppies. We included size as a covariate as guppy size was positively correlated with survival differential in 1996 (Table 2). This analysis detected no effect of year on survival differential (main effect = year $F_{1,62} = 0.51$, $P = 0.48$; main effect = separation event $F_{1,62} = 12.80$, $P = 0.0007$; covariate = size $F_{1,62} = 2.96$, $P = 0.09$). As size retained marginal significance in the combined data, we examined the consequences of removing size effects from the 1996 data using the residuals of the 1996 regression. When combined with the 1997 data, residual corrected and uncorrected 1996 data produced qualitatively identical results in all subsequent analyses, and results of these combined datasets did not differ qualitatively from those for the 1997 dataset alone. Therefore, for simplicity we used the combined set of uncorrected 1996 and 1997 data for our central analyses of wild caught guppies, and excluded size from further analyses.

The F_2 data displayed no effects of the factors discussed above (Table 2), but did contain one trial that appeared anomalous on visual examination of the data. Dixon's test for outliers indicated that the result of the trial was outlying ($n = 10$ trials in the comparison, $r_{11} = 0.667$, $P < 0.01$, Sokal and Rohlf 1981). Given the otherwise normal distributions of the study datasets, we believe that excluding the outlier creates the F_2 dataset most representative of population trends. Nonetheless, we present the data in two forms, including and excluding the outlier, in the central results Table (4), but thereafter present and discuss results for the latter dataset only.

Our central analyses examined the differential escape ability of guppies from high- versus low-predation populations within each comparison. We tested several methods of quantifying this differential. The first compared the number of surviving guppies from each of the two populations using paired t -tests. Two other methods used one sample t -test to (1) compare the percentage of the captured guppies that belonged to the high-predation population with the null expectation of 0.5; and (2) compare survival differential with the null expectation of zero. All methods produced qualitatively

identical results, and for simplicity we present results only of the paired t -tests. We additionally examined differences between comparisons, to determine if survival differential varied with the type of event separating the populations (natural vs. introduction events) or with the generation of guppies (wild caught vs. F_2 guppies). For these analyses we used survival differential as the dependent variable in one-way ANOVA (effects = comparison or separation event) and two-way ANOVA (effects = generation, separation event, and interaction term). Analyses were performed using StatView version 4.0 (SAS Institute, Cary, NC) and SuperANOVA version 1.11 (Abacus Concepts, Berkeley, CA).

RESULTS

Table 3 presents the outcome of comparisons of escape ability among wild caught guppies. In every comparison, wild caught guppies from high-predation populations survived in greater numbers than guppies from low-predation populations. Thus ability phenotype had diverged among guppy populations within both north and south slope streams, within both female and male guppies, and among populations separated because of natural events and because of experimental introductions.

The magnitude of these differences in ability, survival differential, showed several interesting patterns across comparisons. Comparisons of the two sets of naturally separated populations, from the south slope Aripo and north slope Yarra Rivers, showed large survival differentials that were markedly similar (Fig. 1) despite the geographic separation of the streams and the very different composition of their predator communities. The survival differentials for these two comparisons were also greater than those for comparisons involving introduced populations (Fig. 2), suggesting that either the time or type of event separating populations affects the magnitude of their phenotypic divergence. In support of this suggestion, survival differentials of populations pairs separated by introduction events were not different from each other (Fig. 2; one-way ANOVA $F_{2,39} = 1.30$, $P = 0.29$), and were significantly smaller than those for the naturally separated populations (Table 2; also, comparing only the data in Fig. 1 and 2 using one-way ANOVA, $F_{1,54} = 19.29$, $P = 0.0001$).

Table 4 depicts the comparisons of escape ability among the F_2 , common garden guppies. As with the wild caught

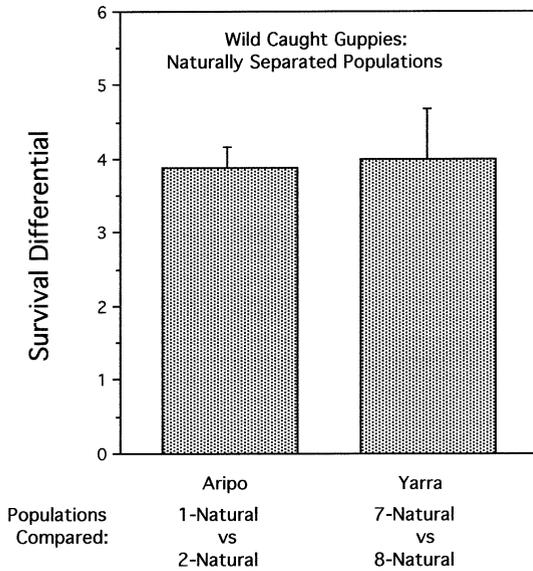


FIG. 1. Differential survival abilities of wild caught guppies from naturally separated populations. Differentials were much greater than zero and nearly identical among Aripo river and Yarra river populations, despite the fact that Yarra guppies had no prior exposure to *Crenicichla*. Survival differential equals the number of trial survivors from the high-predation population minus the number from the low-predation population. Sample sizes as in Table 3, error bars equal one standard error.

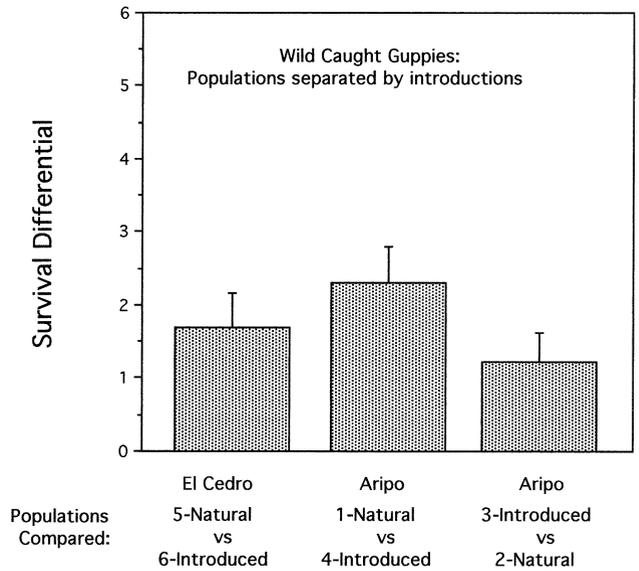


FIG. 2. Differential survival abilities of wild caught guppies from populations separated recently, due to introduction events. Differentials were greater than zero and similar in each of these comparisons, and all were significantly smaller than those for naturally separated populations shown in Figure 1. See text for statistics, also Table 2. Sample sizes as in Table 3, female trials only. Error bars equal one standard error.

guppies, in each comparison F_2 guppies originating from high-predation populations showed higher survival than those from low-predation populations. However, F_2 guppies were not identical to wild caught fish. Among the F_2 , there was no detectable dichotomy of results between the population pairs separated naturally and the pairs separated by introduction events (Figure 3; one-way ANOVA $F_{1,28} = 1.79$, $P = 0.19$; also Table 2), and survival differential among F_2 guppies was less overall than that among the same populations of wild caught guppies (compare Aripo data from Figures 1 and 2 with Figure 3: two-way ANOVA, main effect = generation $F_{1,60} = 11.40$, $P = 0.0013$; main effect = separation event $F_{1,60} = 12.24$, $P = 0.0009$; interaction $F_{1,60} = 2.99$, $P = 0.089$).

DISCUSSION

Wild Caught Guppies: Evidence of Phenotypic Selection

The results provide support for our first hypothesis, that divergent predatory environments create divergent pheno-

typic selection of escape ability in Trinidadian guppies. Guppies that originated from low- rather than high-predation regions of streams were captured by *Crenicichla* at consistently higher rates, regardless of the specific guppy populations being compared, or of guppy gender or home stream (Table 3). Thus, substantial phenotypic divergence in escape abilities had occurred between these populations prior to the study. The geographic separation of the guppy streams and known history of the introduced populations indicate that these divergences occurred independently and in parallel, and therefore that environmental effects on escape phenotype appear quite predictable.

The greater escape abilities of guppies from some high-predation environments might be attributable to prior, direct experience with the study predator. However this possibility cannot explain all the results. As mentioned above the Yarra river is located in a separate drainage system from the other study rivers and contains a different community of predators, notably lacking *Crenicichla*. Yet the survival differential between Yarra guppy populations is similar to that between

TABLE 4. Comparisons of escape ability among F_2 , common garden guppies. Each comparison includes data from both all female and all male trials ($n \geq 5$ trials per gender); gender did not affect trial results (Table 2). The comparison of population 3 versus 2 shows the data in two forms, excluding¹ and including² a strong outlier ($P < 0.01$, see Methods). Population identity numbers from Table 1. Data are mean \pm one SD, P -values from paired t -tests.

Comparison		Number of survivors		Trials (n)	P-value
High-predation population	Low-predation population	High-predation population	Low-predation population		
1 Aripo natural	2 Aripo natural	3.9 \pm 1.4	2.1 \pm 1.3	11	0.003
3 Aripo introduced	2 Aripo natural	3.4 \pm 1.0	2.4 \pm 1.0	9 ¹	0.02
		3.2 \pm 1.2	2.7 \pm 1.3	10 ²	0.41
1 Aripo natural	4 Aripo introduced	3.3 \pm 1.4	2.1 \pm 0.9	10	0.04

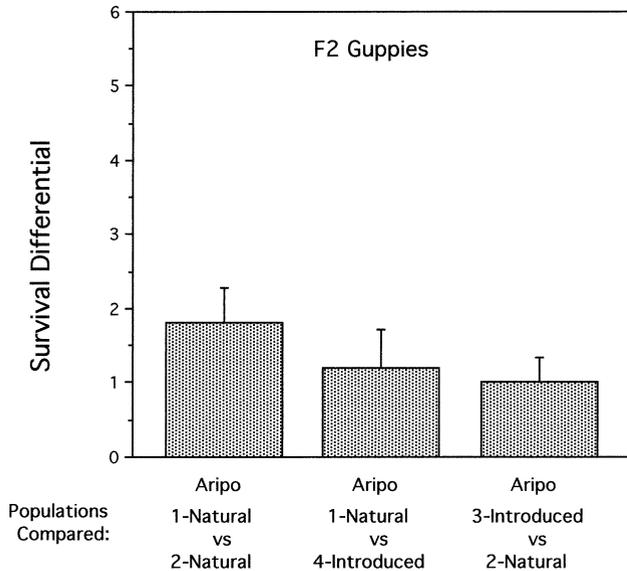


FIG. 3. Differential survival abilities of F_2 , common garden guppies. Differentials were greater than zero and similar in each of these comparisons, and were significantly smaller than the differentials for the same comparisons of wild caught guppies (Figures 1 and 2). See text for statistics. Sample sizes as in Table 4; data for populations 3 vs. 2 excludes outlier. Error bars equal one standard error.

similar, natural populations in the Aripo River (Fig. 1). Thus phenotypic selection may be a generalized consequence of predatory environment and not dependent on the presence of *Crenicichla*.

Our data also suggest that predation level may be the principal source of selection on guppy escape ability. Several environmental factors aside from predation could influence escape phenotypes. In Trinidad, stream size is frequently correlated with predatory environment; streams tend to be small upstream (low-predation) and large downstream (high-predation), potentially confounding predation level with other biotic and abiotic factors (Seghers 1973; Grether et al. 1999; Reznick et al. 2001). To partially mitigate this concern, we collected three of our five pairs of guppy populations from similarly-sized stream areas; both study populations from the El Cedro came from small stream areas, three of the four Aripo populations came from large areas, and the Yarra populations from separate-sized areas (Table 1). As all comparisons showed significant survival differentials (Table 3), the results suggest that possible effects of stream size factors on guppy escape ability may be secondary to the effects of selection resulting from predation level.

Two additional points suggest that predatory environment may be a fairly continuous source of selection of guppy escape abilities. First, gene flow exists among guppy populations within rivers, apparently occurring mainly from upstream to downstream (Magurran et al. 1995; Houde 1997; Becher and Magurran 2000). As guppies from upstream, low-predation environments show poor escape abilities, upstream guppies that migrate must be regularly eliminated from downstream populations in order to preserve the considerable phenotypic divergence that we found. Secondly, each of the three introduced populations that we studied had diverged in phe-

notype from their source populations, in the direction predicted by the change in environment, within the space of 20 years. These data suggest that selection resulting from predators is sufficiently consistent to regularly create as well as maintain population differentiation. This apparent consistency is notable as selection in the wild is frequently considered and has been shown to be both variable and episodic (e.g., Grant and Grant 1989).

Evidence of phenotypic selection of escape ability in Trinidadian guppies fulfills several predictions provided by earlier work. The extensive studies of evolution in these fish demonstrate a remarkably broad influence of predatory environment (reviews above), strongly suggesting that the most direct response to predators, the escape response, should be similarly affected. Also, the presumed anti-predator behaviors of schooling tendency and approach distance (the distance that prey allow predators to approach before fleeing) show evolved differences between guppy populations from different environments (Seghers 1974; Breden et al. 1987; Magurran et al. 1992, 1995). We previously tested the possibility that these behaviors impact guppy escape ability, using high-speed video to record interactions between *Crenicichla* and domestic guppies, and found that increased schooling, approach distance and speed of guppies all improved escape success (O'Steen and Bennett, unpubl. ms.). Together these results prefigured our current findings of differences in escape abilities among Trinidadian guppies.

One provocative result of this study was the loss of escape ability found in the two experimental populations introduced into low-predation environments. This finding suggests either that divergences among populations result entirely from phenotypic selection (i.e., escape ability has no genetic basis, and divergence results solely from predators removing guppies with lesser abilities from the high-predation populations), or that escape ability does have some genetic basis and diminishes in low-predation environments as a consequence of fitness trade-offs or drift. Our results support the latter possibility, as phenotypic divergence was less between populations separated by introduction events than between populations separated naturally (Results, Fig. 1 vs. 2). If phenotypic divergence resulted entirely from environment, then our results would suggest that there must be consistent differences between the low-predation environments of the natural vs. the introduced populations. We know of no such differences. The F_2 experiments (below) also indicate a genetic contribution to escape ability.

Common Garden Guppies: Evidence of Evolution

The data for the F_2 , common garden guppies support the hypothesis that selection created by divergent predatory environments has caused evolutionary divergence of escape abilities among Trinidadian guppy populations. In each comparison among F_2 s, guppies from the high-predation population showed greater survival than those from the low-predation population (Table 4, Fig. 3). Thus, a genetic component underlies the phenotypic divergences among the wild caught guppies, and escape ability can and has evolved. Strikingly, ability evolved within only 15 and 20 years (26–36 generations, Reznick et al. 1997) in the two introduced pop-

ulations studied in the F_2 , and proceeded in the (opposite) directions predicted by the (opposite) changes in predatory environment. This result supports our earlier conclusion that phenotypic selection is both strong and consistent, and also shows that wild populations of guppies contain sufficient genetic variation to evolve rapidly in response.

Evolutionary divergence among populations might have resulted from drift rather than selection, but testing this possibility experimentally would require more introduced populations than currently exist (or are logistically or ethically feasible) in Trinidad. However, we consider drift an unlikely cause of escape ability evolution. First, our current results show marked internal consistency and fulfill the predictions of selection derived both from this study and from the results of other research (discussed above and below). Second, the experimental populations should have been of sufficient size to dampen drift. Populations introduced to low-predation environments were begun with 100–200 guppies and expanded rapidly (Endler 1980; Reznick and Bryga 1987), and the experimental high-predation population resulted from introduction of predators into a large pre-existing guppy population (D. Reznick, pers. comm. 1998). Lastly, the observed rapidity of evolution is consistent with a response to selection.

Our findings complement studies of prey evolution in other systems. Elegant studies of *Enallagma* damselflies indicate that predators create phenotypic selection affecting lamellae size of damselflies (lamellae influence escape success via swimming ability), and that lamellae size evolves following changes in predation level (McPeck et al. 1996; McPeck 1997). Several vertebrate studies also report phenotypic selection of anti-predator traits in nature (Jayne and Bennett 1990; Brodie 1992; Fitzgibbon 1994), or report evolved differences among populations in presumed anti-predator traits (e.g., Garland and Adolph 1991; Sinervo and Losos 1991; Huntingford et al. 1994; Kelley et al. 1997), or demonstrate the value of such traits to prey survival during staged encounters with predators (reviews in McCollum and Van Buskirk 1996; Godin 1997; Smith 1997; Van Buskirk et al. 1997). These studies predict that selection resulting from predators should be widespread and often result in the evolution of prey escape abilities, as found here.

A caveat to this prediction is the possibility that prey respond to changed environments with behavioral or physiological plasticity. Plasticity itself might evolve in response to the change (in addition to or instead of the evolution of absolute escape abilities), or plasticity might provide a sufficient response to the environmental change and mitigate selection. Our results indicate that guppies evolved changes in absolute escape abilities, but allow the possibility that plasticity also contributes to escape phenotype. The survival differentials of the F_2 comparisons were notably smaller than those of the wild caught guppies (Results; Fig. 1–3). These reduced differentials could have resulted from adaptation to laboratory conditions, though we endeavored to minimize such events by keeping population sizes large and rearing only two generations. Alternatively or additionally, relaxed selection in the laboratory could have created the reduced differentials either because guppies with lesser escape abilities were no longer removed from the populations, and/or

because plastic responses to predators were no longer being induced. The latter is an intriguing possibility, as antipredator traits show considerable plasticity in other animals (e.g., Harvell 1986; McCollum and Leimberger 1997; Van Buskirk et al. 1997), notably including stickleback fish that learn escape behavior from conspecifics (Huntingford et al. 1994). Other traits show considerable plasticity in guppies (Reznick 1990; Robinson and Wilson 1995), for example swimming endurance varies in response to water flow rate (Nicoletto 1996), and life history traits vary with local demography, a response that can combine with genetic factors to increase phenotypic differences between populations (Rodd et al. 1997). Documenting the contribution of plasticity to guppy escape abilities should prove useful to understanding the evolution of these traits, and indeed may be favored by selection due to the geographic proximity of differing predatory environments.

Our study suggests one additional factor that may influence prey responses to predation: fitness trade-offs involving escape ability. The F_2 experiments show that release from predation resulted in a striking, evolutionary loss of escape ability in population 4 (Tables 1, 4). The rapidity of this change suggests that selection favored the loss, and thus that escape abilities may be costly to maintain. Certainly such abilities may require that resources be used for vigilance and escapes rather than for foraging and courting. Such trade-offs could lead to the evolution of reduced escape ability in low-predation environments, while limiting the evolution of increased ability in high-predation environments. Similar trade-offs involving antipredator traits are documented in several animals (Harvell 1986; Lively 1986; Spitze 1992; McCollum and Van Buskirk 1996; Brodie and Brodie 1999), and appear to also merit study here.

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