

ORIGINAL ARTICLE

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Gene flow and local adaptation in a sunfish-salamander system

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Abstract There is increasing evidence that populations may not be well adapted to their local environments, and as a result, recent interest has focused on understanding factors that constrain adaptive evolution. This study presents data suggesting gene flow may constrain the ability of larvae of the streamside salamander *Ambystoma barbouri* to avoid predation by fish via escape behavior and life history tactics. Streamside salamander larvae face conflicting selection pressures in different streams. Some streams are ephemeral, where larvae should be active to feed, grow, and reach metamorphosis before stream drying. Other streams contain predatory fish, where larvae should be generally inactive to avoid predation. Previous work has shown that streamside salamander larvae exhibit ineffective antipredator behavior by having inappropriately high activity levels with fish, resulting in high predation in laboratory and field experiments. This study investigated the possibility that gene flow from larvae in ephemeral habitats may reduce the escape performance of larvae from populations with fish and alter their life history characteristics to increase their susceptibility to fish predation. I assayed escape behavior (speed, acceleration, and duration of escape) and life history characteristics (hatching date, size, stage) associated with predator avoidance among laboratory-reared larvae from four populations. As predicted, two populations (one with fish and the other fishless and ephemeral) connected by gene flow were not significantly different in almost all assays. In contrast, larvae from an isolated population with fish had significantly stronger

escape behaviors and delayed hatching than both an isolated population that lacked a history of fish co-occurrence and the population with fish but gene flow from a fishless population. These results support theory suggesting that gene flow can constrain adaptive evolution.

Key words Gene flow · Local adaptation · Life history · *Ambystoma barbouri*

Introduction

Recent interest has developed in understanding factors that constrain adaptive evolution (Cheverud 1984; Maynard Smith et al. 1985; Arnold 1992). Although animals are often studied with the assumption that they are at an adaptive optimum (Krebs and Davies 1991; Roff 1993), there is increasing empirical evidence that organisms are not always well adapted. Thus, it is important to understand the mechanisms that act as evolutionary constraints (Maynard Smith et al. 1985; Arnold 1992).

One important source of evolutionary constraints are conflicting selection pressures, and the foraging-anti-predator tradeoff is a common example. Organisms are frequently faced with conflicting demands of avoiding predation, where food is often unavailable, and feeding, which increases exposure to predators (Werner 1986; Sih 1987; Werner and Anholt 1993).

Conflicting selection pressures of feeding and predator avoidance may constrain adaptive evolution of larvae of the streamside salamander, *Ambystoma barbouri* (Sih et al. 1988, 1992; Maurer and Sih 1996). Relative to an ancestral sister taxon, *A. texanum*, larvae of *A. barbouri* have evolved increased activity, apparently in response to higher overall habitat ephemerality (Maurer and Sih 1996). Increased activity is correlated with increased feeding and developmental rates crucial for reaching metamorphosis before stream drying

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(Maurer and Sih 1996). However, unlike their ancestor that exists exclusively in fish-free aquatic environments, *A. barbouri* larvae also coexist with predatory fish in some streams, where decreased activity and use of refugia is beneficial (Petranka 1983; Sih et al. 1988, 1992). Streamside salamanders respond to the presence of fish and even fish chemical cues accordingly (Sih and Kats 1991), but they are still relatively active and suffer high predation in both natural streams and laboratory experiments (Sih et al. 1992). One key behavior that helps explain such high predation rates is larval emergence from refugia, a reflection of the foraging-antipredator tradeoff (Sih et al. 1992).

Most often, the foraging-antipredator tradeoff is investigated within populations, but the conflicting selection pressures of habitat ephemerality and predation also exist among streamside salamander populations. Gene flow from ephemeral populations apparently constrains antipredator behavior in some *A. barbouri* populations where larvae coexist with predatory fish (Storfer and Sih 1998). In these streams, there was a positive and significant correlation between both the strength of the antipredator response and duration of survival in a predation experiment and genetic and geographic isolation from fishless streams (Storfer and Sih 1998). In general, gene flow is thought to homogenize populations, preventing local differentiation by selection and swamping local adaptation (Mayr 1963; Slatkin 1985, 1987; Templeton 1986). This important theoretical work is now being backed by an increasing body of empirical evidence that suggests that gene flow can compromise adaptive evolution relative to survival (Stearns and Sage 1980), life history traits such as clutch size (Dhondt et al. 1990), antipredator behaviors and diet choice (Riechert 1993), and the degree to which individuals are cryptic (Kettlewell 1956; Camin and Ehrlich 1958; King and Lawson 1995; Sandoval 1994).

Prey can reduce the duration of their exposure to predators by altering life history switch points (Werner and Gilliam 1984; Werner 1986). One important life history switch point in amphibians is hatching, and predation risk should influence the timing of hatching (Shine 1978). The purpose of the present study was to provide detailed analyses of the role of gene flow in influencing escape behavior (speed, acceleration, and duration) and life history traits (hatching characteristics) associated with predator avoidance in four populations of *A. barbouri*. Unlike the previous work, larvae were reared individually and in family structure; thus, the contribution of genetics can be addressed. The four populations were chosen based on relative isolation levels; two populations, one with fish and one fishless and ephemeral, are connected by gene flow. The other two populations (with and without fish) are relatively isolated from their nearest neighbor population with the opposite selection pressure (Table 1).

If gene flow is swamping local adaptation, the two populations connected by gene flow should not differ significantly for escape behaviors and life history traits

Table 1 Gene flow differences among the four populations. Pairwise geographic distance and estimated number of migrants shared per generation (Nm) between Raven Run and Chandler Creek. For Albert Moore, the pairwise geographic distance and number of migrants shared per generation with its nearest fishless neighbor are shown. Similarly, for Sligo, the pairwise geographic distance and gene flow estimate to the nearest population with fish are presented. In all cases, 95% upper and lower confidence intervals (CI) are presented for number of migrants per generation based on bootstrapping with 1000 replicates (GDA version 1.6; Lewis and Zaykin 1996)

Population	km	Nm	Upper 95% CI	Lower 95% CI
Raven Run– Chandler Creek	1	2.022	4.467	1.139
Albert Moore	14	0.805	1.548	0.512
Sligo	51.7	0.242	0.572	0.103

associated with predator avoidance. Conversely, isolated populations should be closer to their optimum because selection, in the absence of gene flow (and genetic drift), should drive populations to local adaptive peaks (Wright 1931, 1988; Slatkin 1985, 1987). Thus, the isolated population with fish should have increased escape response and increased changes in life history traits that enhance predator avoidance relative to the isolated fishless population or a population with fish and gene flow from the fishless population. Similarly, the isolated fishless population should have increased changes in life history traits associated with ephemerality relative to the fishless population with gene flow from the population with fish.

Methods

Study sites

Salamander eggs were collected from four populations in and around the Lexington, Kentucky, area. Raven Run (downstream portion) represented a population of larvae that coexist with fish and are connected by gene flow to a fishless population, Chandler Creek (Table 1). Albert Moore is a population of salamanders that coexist with fish and that is relatively genetically and geographically isolated from ephemeral, fishless populations, while Sligo is an isolated fishless and ephemeral population (Table 1). Gene flow was determined with an allozyme study of nine polymorphic loci among 15 known streamside salamander populations within a 50-km radius of Lexington (Fig. 1 in Storfer and Sih 1998), and generally followed predictions from isolation-by-distance models (Storfer 1999).

Rearing

Larvae were reared from early egg stages (prior to stage 9, determined in the field, late blastula, approximately 29 h old at 20 °C; Harrison 1969) under standard laboratory conditions. Six clutches of eight eggs were collected from each of the four study populations. Due to early mortality of some eggs, additional eggs were collected from three of the four populations to equalize sample sizes (Raven Run, Chandler Creek, and Albert Moore). Individual eggs were aerated and reared in round plastic containers (18 cm diameter, 5 cm high) with a constant water level (1 l of carbon-filtered water) and a 5-cm-long, 2-cm-diameter piece of PVC pipe

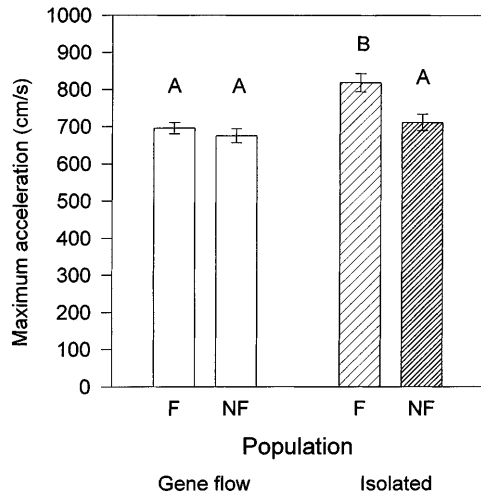


Fig. 1 Maximum acceleration for larvae from different populations. *White bars* represent the two populations connected by gene flow (*F* Raven Run, $n=38$; *NF* Chandler Creek, $n=37$); *hatched bars* represent the two isolated populations (*F* Albert Moore, $n=43$; *NF* Sligo, $n=41$). Shown are means \pm 1 SE. *Different letters* indicate significant differences among means

as refuge. Water was changed twice a week. Larvae were fed controlled amounts of food (*Daphnia magna*) daily. Populations were randomized among shelves in an environmental chamber that mimicked natural temperature (estimated from the average temperature from three streams twice a week) and light regime. Vitalite full-spectrum bulbs were used to illuminate the chamber. Larvae were 21–28 days old during experiments.

Behavioral assays: escape speed, acceleration, and duration

Individual salamander larvae were removed from the environmental chamber and given 5 min to acclimate (in their plastic rearing container) on a standardized grid with 1-cm squares. Individuals were tapped with a blunt probe to initiate movement. Each individual underwent three trials with 5 min between trials. A Sony VHS video camera was used to record salamander movement in constant lighting conditions between 1500–1900 hours to control for temporal fluctuations in activity level. Videotapes were then analyzed at 60 frames per second on a Peak Motus motion analysis system (version 2.1). Distance was calibrated by digitizing two points at the corners of one grid square. Each flight response was cropped one frame before an individual initiated movement and two frames after active tail motion stopped, and duration of the response was recorded. A template was created to digitize salamander movement automatically by tracking the centroid (center point) of an individual. Maximum speed and velocity of each trial were calculated automatically (using distance between centroid points between frames), hand corrected in cases of computer tracking error, and recorded.

If gene flow is swamping local adaptation, then the following predictions should hold. First, larvae from Raven Run and Chandler Creek should show escape speeds that do not differ statistically. Second, salamanders from Albert Moore should be significantly faster than individuals from Sligo or Raven Run. Third, larvae from Chandler Creek should be faster than larvae from Sligo.

Life history assays: age, stage, and size at hatching

Stage at hatching, days until hatching, and size at hatching were compared for eggs collected from the four populations. Larvae

from fishless, ephemeral populations should hatch early to start feeding early to reach metamorphosis before stream drying (Maurer and Sih 1996). Conversely, larvae from populations with fish should hatch later at a more advanced stage with better swimming ability, or perhaps to minimize time spent with predators (Sih and Moore 1993; Moore et al. 1996). Thus salamander larvae from Albert Moore are expected to hatch the latest, and from Sligo to hatch the earliest. Larvae from Raven Run and Chandler Creek should not differ in stage, size, or days until hatching, and individuals from Raven Run should hatch earlier than Albert Moore. Finally, Sligo larvae should hatch earlier and at a larger size and later stage (indicating selection by habitat ephemerality for more rapid development) than those from Chandler Creek.

To monitor hatching, the environmental chamber was checked several times a day throughout the experimental period. Upon hatching, Harrison's (1969) stage of each larva was determined by observing individuals under a Wild dissecting scope at $\times 6$. Larval snout-vent length and number of days from collection until hatching were also recorded.

Data analysis

Statistical analyses were performed with SYSTAT version 6.1 for Windows (SPSS 1996). Initially, effects of hatching variables (date, stage, and size) and escape variables (speed, acceleration, and duration) were tested with multivariate analyses of variance (MANOVAs). The main effect in each MANOVA was significant and to protect α levels in the overall test, ANOVAs were conducted with a Bonferroni-corrected α (i.e., hatching had three variables, so $\alpha=0.05/3$ or 0.0167). For life history traits, populations were compared with nested ANOVAs with clutch source nested within population. Analysis of covariance (ANCOVA) tests were performed with larval snout-vent length as the covariate on speed, acceleration, and duration-of-escape response. Bonferroni-corrected Fisher's LSD multiple comparison tests were used to determine whether there were differences among means in the four populations. Because there were three trials in the speed, acceleration, and duration-of-escape responses, the intraclass correlation was calculated as a measure of repeatability (Lessells and Boag 1987; Boake 1994; Falconer and McKay 1996). Power tests were performed in all analyses to test the robustness of the equality of trait means for larvae from Raven Run and Chandler Creek. In assays where Sligo and Albert Moore differed significantly, the predicted effect size for the power analysis was the difference between Albert Moore and Sligo; in assays where the two populations did not differ significantly, the average percent difference for the significant differences between Albert Moore and Sligo was used as the predicted effect size (7.9%).

Results

Behavioral assays: escape speed, acceleration, and duration

Because mean repeatabilities for each of the three larval traits were low (0.336 for velocity, 0.041 for acceleration, and 0.358 for duration), with repeatability ranging from $r=0.024$ (for acceleration in Chandler Creek where $F=1.057$ and $df=26,48$) to $r=0.549$ (for duration in Albert Moore where $F=4.102$ and $df=27,50$) for traits in each population, I used the maximum of the three trials for each individual for population-level comparisons. The overall MANCOVA was significant (Rao's $R=3.57$, $df=9,370$, $P=0.003$). Maximum velocity did not differ among the four populations ($F=1.72$,

$df=3,24$, $P=0.17$). In addition, variation among clutches did not explain a significant amount of variation in maximum velocity ($F = 1.41$, $df=24,131$, $P = 0.115$). Only one prediction was met; the two populations connected by gene flow were equal [mean maximum velocity for Raven Run = 35.051 ± 0.817 (SE) cm/s; Chandler Creek = 34.89 ± 0.781 cm/s], and power for this test was high (0.99).

In contrast, significant variation among populations in maximum acceleration was detected ($F=8.82$, $df=3,24$, $P=0.001$; Fig. 1). Again, clutches did not explain a significant proportion of the variation in acceleration ($F=1.31$, $df=24,131$, $P=0.17$). However, population-level differences in acceleration met three of the four predictions. The two populations connected by gene flow were equal ($P=0.54$, power=0.56). The isolated population with fish had a significantly higher acceleration than both the isolated fishless population ($P=0.002$, Bonferroni $\alpha=0.016$) and the population with fish and gene flow from the fishless population ($P=0.001$, Bonferroni $\alpha=0.013$). Against one prediction, there was no difference between the two fishless populations (Chandler Creek and Sligo, $P=0.271$).

There were no differences in maximum duration of escape response among the four populations ($F=1.53$, $df = 3,24$, $P = 0.21$; mean maximum duration of escape for Raven Run = 1.406 ± 0.139 s, Chandler Creek = 1.274 ± 0.109 s, Albert Moore = 1.39 ± 0.110 s, Sligo = 1.534 ± 0.144 s). The two populations connected by gene flow did not differ statistically, but power was low (0.1). No pairwise differences between populations were significant.

Life history assays: age, stage, and size at hatching

The MANOVA main effect for three hatching variables was significant (Rao's $R = 5.227$, $df=9,331$, $P < 0.001$). There were significant differences in number of days until hatching among populations ($F=62.67$, $df=3,20$, $P < 0.0001$; Fig. 2a). Larvae from Raven Run hatched significantly earlier than those from Chandler Creek ($P < 0.0001$), against the equality prediction. However, following predictions, individuals from Albert Moore hatched significantly later than either Sligo (about 5.5 days, $P < 0.0001$) or Raven Run (about 3 days, $P < 0.0001$) larvae, suggesting that larvae from Albert Moore have a shorter duration of fish exposure than larvae from other populations. In addition and as predicted, larvae from Chandler Creek hatched significantly later, on average, than larvae from Sligo ($P < 0.0001$).

There were also significant differences in stage at hatching ($F=3.73$, $df=3,22$, $P=0.013$; Fig. 2b). Again, as predicted, larvae from Albert Moore hatched significantly later than those from Raven Run ($P=0.013$, Bonferroni $\alpha=0.013$), but the difference between Albert Moore and Sligo was only marginally significant ($P=0.028$, Bonferroni $\alpha=0.016$). Raven Run and

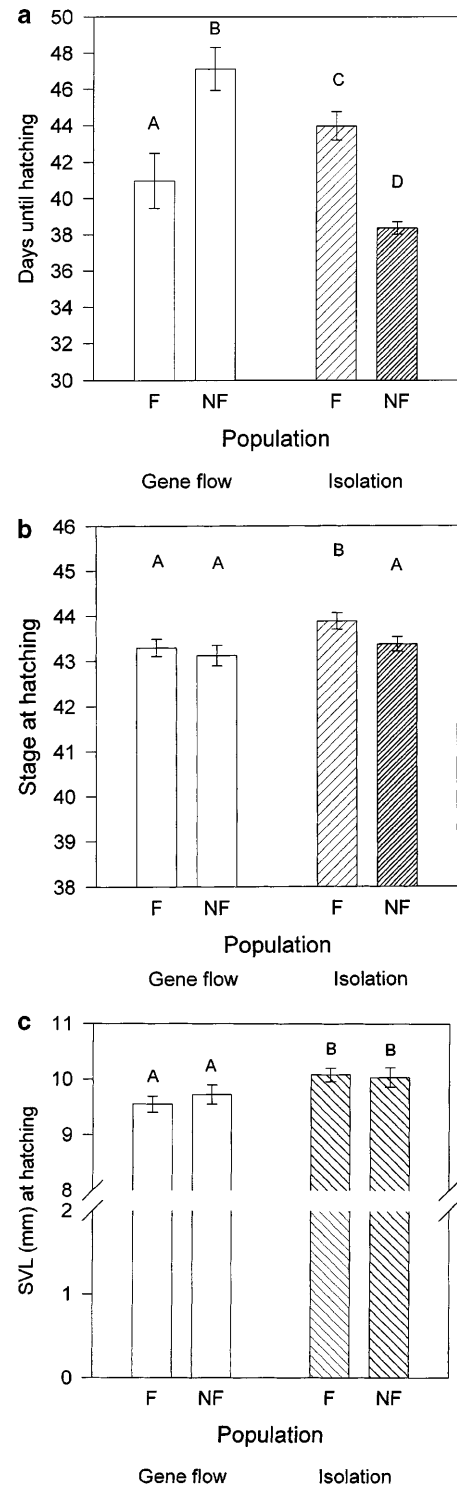


Fig. 2a–c Hatching variables. *White bars* represent the two populations connected by gene flow (F Raven Run, NF Chandler Creek) and *hatched bars* represent the two isolated populations (F Albert Moore, NF Sligo). Shown are means \pm 1 SE. *Different letters* indicate significant differences among means. **a** Days until hatching. (Raven Run, $n=38$; Chandler Creek, $n=37$; Albert Moore, $n=43$; Sligo, $n=41$). **b** Stage of larval hatching. (Raven Run, $n=44$; Chandler Creek, $n=41$; Albert Moore, $n=41$; Sligo, $n=47$). **c** Average size (snout-vent length in mm) of larvae at hatching (Raven Run, $n=43$; Chandler Creek, $n=45$; Albert Moore, $n=42$; Sligo, $n=46$)

Chandler Creek did not differ in stage at hatching ($P=0.49$, power <0.1). The pairwise difference between Chandler Creek and Sligo was also not significant.

Furthermore, size at hatching differed significantly among the four populations, and three of four predictions were met ($F=5.77$, $df=3,23$, $P=0.001$; Fig. 2c). Salamanders from Raven Run and Chandler Creek did not differ in hatching size ($P=0.8$, power = 0.75). Larvae from Albert Moore hatched significantly larger than those from Raven Run ($P=0.003$, Bonferroni $\alpha=0.016$), but not Sligo ($P=0.84$). Larvae from Chandler Creek hatched at a smaller size than those from Sligo ($P < 0.0001$).

All three assays showed significant effects of family (clutch) source on variation in days until hatching ($F=25.63$, $df=20,135$, $P < 0.0001$), stage at hatching ($F=3.33$, $df=22,147$, $P < 0.0001$), and size at hatching ($F=3.33$, $df=22,147$, $P < 0.0001$).

Discussion

Escape behavior

Some experiments suggest that faster amphibian larvae have a higher survival rate than slower larvae (Hileman and Brodie 1994; Watkins 1996). The morphology of ambystomatid larvae suggests that they are generally designed for high acceleration over short distances, but not for constant-velocity swimming (Hoff et al. 1989). Fish, in contrast, generally have good swimming endurance (Webb 1975, 1978). Thus, the best chance of escape from a fish for a larva of *A. barbouri* is probably a quick burst of speed out of the water column and into a refuge. Initial acceleration is crucial for fast starts (Webb 1975, 1978) and may be crucial for such a rapid initial burst away from a predator.

Accordingly, acceleration was often highest at the initiation of larval movement. Significant differences in acceleration among the four populations followed predictions based on the hypothesis that gene flow is swamping local adaptation to fish. Larvae from the isolated population with fish had the highest average acceleration of all four populations. In contrast, in all three assays, the escape response did not differ significantly in the two populations connected by gene flow.

Life history traits

Two studies indicate that embryos of *A. barbouri* significantly delay the stage and size at which they hatch in response to chemical cues from two different predators, green sunfish, *Lepomis cyanellus*, and flatworms, *Phagocotus gracilis* (Sih and Moore 1993; Moore et al. 1996). Flatworms prey only on smaller, less developed streamside salamander larvae and pose no threat to larger ones (Petranka et al. 1987); thus, delayed hatch-

ing should greatly reduce the predation risk posed by flatworms.

The small differences in hatchling size among populations in this study (<1 mm) might also have an effect on escape ability from fish. Delaying hatching in response to predatory fish results in more developed larvae that might have increased escape success or increased ability to resist drift into fish pools (Moore et al. 1996). Empirical work on animal swimming speed generally suggests that larger animals are faster (Schmidt-Nielsen 1984); differences in salamander hatchling size have also been shown to affect swimming ability (C. Wells unpublished data; S.K. Babcock, personal communication).

Delaying hatching may also reduce time of exposure to fish because streamside salamander eggs are laid under rocks and are generally safe from fish predation (Petranka 1983; Kraus and Petranka 1989). This expectation is supported by theory that suggests predation risk should influence timing of hatching (Shine 1978; Sargent et al. 1987). Observed differences in time to hatching were much greater than those observed in hatchling size. However, it remains to be tested whether hatching later reduces exposure to fish in natural streams with occasional fish pools.

Some predictions based on the overall hypothesis that gene flow is swamping local adaptation to fish were supported, with significant differences in stage, age, and size at hatching among the four populations. As predicted, larvae from an isolated population with fish (Albert Moore) hatched at a significantly later stage and date than larvae from an isolated population without a history of fish coexistence (Sligo) or a population with fish and gene flow from a fishless population (Raven Run). The two populations connected by gene flow (Raven Run and Chandler Creek) did not differ in their stage or size at hatching, but against a prediction, these two populations differed significantly in number of days until hatching. However, larvae from the fishless ephemeral population, which should hatch early, hatched significantly later than larvae from the fish population; the two populations were apparently not well adapted in this regard. In general, differences in size and stage in the present study were significant, but small biologically. All eggs were reared in a fishless environment, but if they had been reared in an environment with fish cues, observed differences among populations might have been enhanced.

Gene flow and local adaptation

Gene flow is often conceived as a constructive force that counteracts drift and inbreeding, factors that tend to erode genetic variation in local populations (Slatkin 1985, 1987). Gene flow can also spread adaptive genes or gene complexes among demes. However, three major results from these experiments support gene flow as a force that can constrain adaptive evolution by swamping local adaptation to fish: (1) larvae from an isolated

population with fish exhibited stronger antipredator avoidance (escape acceleration and delayed hatching) than larvae from an isolated population with no history of fish co-occurrence; (2) an isolated population with fish had larvae with increased predator avoidance than larvae from a population with fish that is receiving gene flow from a fishless population, and (3) populations connected by gene flow generally did not differ significantly.

These results have implications for the relative importance of gene flow and natural selection. In theory, with strong selection (in the absence of gene flow and drift) populations should attain the optimum for a trait; there should be no variation in that trait (Endler 1977). Larvae from Albert Moore showed higher escape acceleration and later hatching than the two populations connected by gene flow. Further support came from a previous predation experiment that showed that the isolated population with fish survived longer when exposed to fish than the isolated fishless population (Storfer and Sih 1998). In addition, salamander larvae from this population had increased performance in other behavioral characteristics relative to other populations with both fish and increased rates of gene flow from fishless populations (Storfer and Sih 1998).

When selection pressures differ among populations, high rates of gene flow should swamp local adaptation (Haldane 1930; Shields 1982; Allendorf 1983; Templeton 1986). Indeed, two populations connected by gene flow with the conflicting selection pressures of habitat ephemerality and predation did not differ significantly in all but one assay in these experiments. These results suggest that gene flow from the fishless population into a population with fish may be making some larvae in the population with fish slower or causing them to hatch earlier; larvae from this population are thus potentially subject to higher predation than more isolated populations with fish.

However, there is more limited support for effects of gene flow in the reverse direction, i.e., from the population with fish into the fishless, ephemeral population. Two of six predictions were met that supported the hypotheses that the isolated, ephemeral population should be better adapted in terms of life history characteristics and have slower escape responses than the ephemeral fishless population with gene flow. As predicted, larvae from the ephemeral population with gene flow hatched significantly later and at a smaller size than larvae from the isolated ephemeral population, but larvae from the two ephemeral populations did not differ in stage at hatching. Also contrary to the predictions, there were no significant differences in the three escape response variables between the two ephemeral populations. This lack of difference could be attributable to other factors not addressed in this study, such as terrestrial predators that may influence escape speeds in the isolated ephemeral population.

One implicit assumption when considering the constraining effects of gene flow is that the traits under study have a genetic basis. I suggest that there are genetic components at least to the life history traits

measured in this study because: (1) sibships differed significantly in several assays, and (2) population differences were shown despite rearing in a common, controlled laboratory environment. Previous research has also suggested that the larval antipredator behaviors have a genetic basis (A. Sih unpublished data). However, the evidence for a genetic contribution to *A. barbouri* behavior is inconclusive because early common environmental effects or maternal effects were not eliminated. Ideally, controlled breeding experiments should be conducted in the laboratory to estimate narrow-sense heritability, or the proportion of phenotypic trait variation that is attributable to (additive) genetic differences among individuals (Falconer and McKay 1996).

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