

LOCAL POPULATION DIFFERENCES IN PHENOTYPIC PLASTICITY: PREDATOR-INDUCED CHANGES IN WOOD FROG TADPOLES

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Abstract. Taxa that are divided into separate populations with low levels of interpopulation dispersal have the potential to evolve genetically based differences in their phenotypes and the plasticity of those phenotypes. These differences can be due to random processes, including genetic drift and founder effects, or they can be the result of different selection pressures among populations. I investigated population-level differences in predator-induced phenotypic plasticity in eight populations of larval wood frogs (*Rana sylvatica*) over a small geographic scale (interpopulation distances of 0.3–8 km).

Using a common-garden experiment containing predator and no-predator environments, I found population differences in behavior, morphology, and life history. These responses exhibited a habitat-related pattern: the four populations from closed-canopy ponds did not differ from each other in any of their phenotypes whereas the four populations from open-canopy ponds did differ from each other in these traits. This phenotypic pattern matches the pattern of competitors and predators found in these two types of ponds. Based on two years of pond surveys, the four closed-canopy ponds contained very similar competitor and predator assemblages while the assemblages of the four open-canopy ponds were more diverse and highly variable among open-canopy ponds. When combined with past studies, which demonstrate that predators and competitors select for alternative behavioral and morphological traits, these patterns suggest that the population differences may have arisen via natural selection and not via random mutation or drift.

In a second experiment, I cross-transplanted two of the populations into each other's ponds to determine if the populations were locally adapted to the conditions of their native pond (using low and high competition crossed with the presence or absence of a lethal predator). The populations continued to exhibit phenotypic differences, and one of the two populations tested exhibited superior growth in its native pond. This suggests that some wood frog populations are adapted to the local conditions of their natal pond and that localized selection by predation and competition may be the underlying mechanism. Collectively, these experiments indicate that taxa that are divided into discrete populations and face different predator and competitor environments can evolve different phenotypically plastic responses.

Key words: anuran; phenotypic plasticity; population; predator; *Rana sylvatica*; tadpole; wood frog.

INTRODUCTION

The ability of an organism to produce alternative phenotypes in response to environmental change is often an adaptive strategy to minimize loss of fitness in a more harsh environment or to maximize fitness in a more favorable environment (Bradshaw 1965, Schlichting 1986, Sultan 1987, West-Eberhard 1989, Scheiner 1993). Thus, phenotypically plastic responses are thought to evolve due to disruptive selection for alternative phenotypes across temporally or spatially heterogeneous environments. If individuals are organized into distinct populations that differ in the environmental heterogeneity that they experience and there is low dispersal among populations, then we should

observe the evolution of population-specific norms of reaction (Via and Lande 1985, 1987).

A number of organisms exhibit population differences in reaction norms. For example, plant populations can exhibit unique responses to temperature, photoperiod, flooding, reduced resources, and heavy metals (Hiesey 1953, Cook and Johnson 1968, Morrison and Myerscough 1982, McGraw and Antonovics 1983, Scheiner and Goodnight 1984, MacDonald et al. 1988, Schlichting and Levin 1988, Schmitt 1993, Dudley 1996). Similarly, many animal populations exhibit different reaction norms in response to temperature, salinity, and reduced resources (Berven et al. 1979, Berven 1982a, b, 1987, Berven and Gill 1983, Trexler et al. 1990, Trexler and Travis 1990, Moran 1991, Riha and Berven 1991, Bernardo 1994). Whereas we have many examples of population-specific responses to changes in abiotic conditions, we have fewer examples of population-specific responses to changes in biotic conditions.

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Predator-induced plastic responses are one type of biotic condition that has received a great deal of empirical attention at the species level, but much less attention at the population level. Predators (including herbivores) cause prey to alter their behavior, morphology, life history, and physiology in an attempt to reduce the risk of predation (Havel 1987, Sih 1987, Harvell 1990, Lima and Dill 1990, Karban and Baldwin 1997, Tollrian and Harvell 1999). Because predators typically are heterogeneous over space and time and different predators should select for different prey responses, we would expect prey populations to evolve unique plastic responses to predators, providing that interpopulation mixing is low. A number of studies have documented population differences in predator-induced behavior (Giles and Huntingford 1984, Dodson 1988, Riechert and Hedrick 1990, Neill 1992, De Meester 1993, Mathis et al. 1993, Blazquez et al. 1997, Laurila 2000). However, I am aware of only a few studies that have examined population differences in predator-induced morphology (Barry and Bayly 1985, Spitze 1992, Lardner 1998). Whether population differences in predator-induced morphology are a common phenomenon in other taxonomic groups remains to be tested.

Amphibians are a taxonomic group that provides excellent potential for investigating population differences in predator-induced phenotypic plasticity. First, amphibians alter their life history (Skelly and Werner 1990), behavior (Petranka et al. 1987, Kats et al. 1988, Huang and Sih 1990, 1991, Werner 1991, Skelly 1992, Relyea and Werner 1999), and morphology (McCullum and Van Buskirk 1996, Van Buskirk et al. 1997, Van Buskirk and Relyea 1998, Relyea 2000, 2001a) in the presence of predators. Second, the presence of amphibian predators varies tremendously over time and space (Collins and Wilbur 1979, Dale et al. 1985, Gibbons et al. 1997; E. E. Werner, R. A. Relyea, D. K. Skelly, K. L. Yurewicz, unpublished data). Third, some amphibians are known to exhibit high fidelity to their natal ponds (Berven and Grudzien 1990). Whereas amphibian populations are known to exhibit genetically distinct responses to temperature and food resources over large geographic distances (20–1000 km; Berven et al. 1979, Berven 1982a, b, 1987, Berven and Gill 1983, Semlitsch et al. 1990, Riha and Berven 1991, Bernardo 1994, Maurer and Sih 1996), it is unknown whether they may be genetically distinct over small geographic scales (where gene flow should be greater) nor whether they can be genetically distinct for anti-predator responses.

To address these questions, I examined eight populations of larval wood frogs for differences in predator-induced phenotypic plasticity using a common-garden experiment. I then conducted a cross-transplant experiment to address whether these phenotypic differences among the populations might be adaptive differences. This system was chosen for two reasons. First, wood

frogs are found in a wide variety of habitats (i.e., incorporating a wide variety of abiotic and biotic conditions including differences in the predators that are present; Collins and Wilbur 1979, Skelly et al. 1999). Second, wood frogs show high philopatry to their natal ponds (Berven and Grudzien 1990) which should encourage the evolution of population differences in many traits including those related to predator resistance (Van Buskirk and Relyea 1998, Relyea 2001a, c). Based on these data, I hypothesized that wood frog populations raised in the presence and absence of predator cues would exhibit different morphological, behavioral, and life-historical reaction norms.

Study system

Wood frogs are common throughout eastern North America. They typically breed in the early spring and oviposit in ponds that lack fish due to the high predation risk that fish pose (Collins and Wilbur 1979, Hopey and Petranka 1994, Relyea 2001c). The eggs typically hatch within 1–2 wk and the tadpoles experience rapid growth (Wilbur 1977, Murray 1990). Ecologists have only recently discovered that wood frog tadpoles are capable of tremendous behavioral and morphological plasticity in response to both aquatic predators and aquatic competitors. In response to predators, tadpoles become less active and develop relatively large tails and small bodies; this suite of traits makes tadpoles more resistant to predation at the cost of decreased growth (Relyea 2000, Relyea and Werner 2000, Relyea 2001a, b). In response to competitors, tadpoles become more active and develop relatively small tails and large bodies; this suite of traits makes tadpoles less resistant to predation at the benefit of increased growth (Relyea 2002). After growing for ~6 wk, the tadpoles metamorphose into terrestrial frogs. After achieving sexual maturity, wood frogs live in forest habitats, only returning to the ponds to breed and complete their life cycle.

METHODS

Common-garden experiment

The common-garden experiment determined whether eight populations of wood frog larvae exhibited different phenotypes in the presence and absence of predators. Each population was represented by three egg masses that were collected from ponds located on the University of Michigan's Edwin S. George Reserve (ESGR) and the neighboring Pinckney Recreation Area between 17 and 19 April 1996. The three egg masses very likely were the offspring of different parents; in Michigan, 92% of breeding male wood frogs breed with only one female and 85% of breeding females breed with only one male (Howard and Kluge 1985). Typically, population studies would use a mixture of tadpoles from at least 10 sibships. However, this study was designed with multiple objectives that precluded

the use of sibship mixtures (e.g., Van Buskirk and Relyea 1998, Relyea 2001b). Therefore, I used three separately reared sibships. Thus, the responses from each sibship served as a single replicate of the population. In this way, all populations were replicated three times. This design is less likely to find population differences where they truly exist, given that the mean response from three sibships should be more variable than the mean response from replicated mixtures of 10 sibships. Thus, the design employed in the common-garden experiment was a conservative test for population differences.

Earlier observations indicated that wood frogs inhabit both open- and closed-canopy ponds (ponds shaded by surrounding trees). These two types of ponds differ in a number of biotic and abiotic characteristics. For example, Werner and Glennemeier (1999) found that open-canopy ponds have higher sunlight, higher productivity, higher temperatures, and higher dissolved oxygen than closed-canopy ponds. As a result, resources in an open-canopy pond are primarily photosynthesis-based whereas resources in a closed-canopy pond are primarily detritus-based. Open-canopy ponds also contain abundant submergent and emergent macrophytes whereas closed-canopy ponds typically have no macrophytes, presumably due to the shading effect of the overhead canopy (R. Relyea, *personal observation*). While the two types of ponds vary in their abiotic characteristics, there is similar variability among ponds within each type (Werner and Glennemeier 1999; E. E. Werner, R. A. Relyea, D. K. Skelly, K. L. Yurewicz, *unpublished data*).

Whereas open- and closed-canopy ponds have consistent abiotic differences, they have inconsistent biotic differences. Open-canopy ponds have highly variable assemblages of larval anurans (potential competitors) among ponds while closed-canopy ponds rarely contain other species of larval anurans due to low-quality food (Werner and Glennemeier 1999; E. E. Werner, R. A. Relyea, D. K. Skelly, K. L. Yurewicz, *unpublished data*). Similarly, the composition of predators among open-canopy ponds is highly variable while the composition of predators among closed-canopy ponds is similar (E. E. Werner, R. A. Relyea, D. K. Skelly, K. L. Yurewicz, *unpublished data*; also see *Methods: Quantifying the local predator and competitor environments*). Because both competition and predation favor different behavioral and morphological traits (see *Introduction*), tadpole populations from ponds that contain consistent competitor and predator assemblages (closed-canopy ponds) should evolve similar behavioral and morphological phenotypes; tadpole populations from ponds that contain different competitor and predator assemblages (open-canopy ponds) should evolve different behavioral and morphological phenotypes.

I conducted the common-garden experiment using four populations from open-canopy ponds (Cattail

Marsh = 16 395 m², Buffer Zone Marsh = 14 525 m², Gravel Pit Pond = 41 108 m², and Pearl Two Pond = 3500 m²) and four populations from closed-canopy ponds (Southwest Woods Pond = 863 m², Dreadful Hollow = 409 m², West Woods Big Pond = 657 m², and Silver Lake Three Pond = 432 m²). I tried to select ponds that spanned different regions of the study area and a range of sizes. Because closed-canopy ponds are necessarily smaller ponds, one cannot easily unconfound the two factors. However, many of the differences between open- and closed-canopy conditions can be explained by the effects of shading rather than by small pond size (Werner and Glennemeier 1999).

Wood frog eggs were placed into separate 50-L wading pools that contained aged well water and allowed to hatch. All egg masses hatched by 2 May 1996. On 6 May 1996, hatchlings from each egg mass were split into predator and no-predator pond mesocosms at a density of 110 tadpoles per tank (42/m²). Mean mass of individuals per sibship ranged from 15 to 23 mg. A sample of 20 tadpoles from each egg mass was set aside to determine if there was any mortality due to handling the tadpoles. After 24 h, every sibship experienced 100% survival, indicating that handling the tadpoles had no immediate effect on their survival. These surviving tadpoles were then preserved in 10% formalin for later analysis of initial differences in morphology.

The pond mesocosms consisted of cattle watering tanks (set up on 26 April 1996) containing 1000 L of well water, 300 g of leaves (primarily *Quercus* spp.), an aliquot of plankton from a nearby pond, and 25 g of rabbit chow to serve as a source of nutrients and an initial food source. Each tank was equipped with four predator cages constructed of 10 × 10-cm slotted plastic drain pipe, covered on both ends with fiberglass screening. Tanks were covered with fiberglass screen lids to exclude terrestrial predators and ovipositing insects and then randomly assigned predator and population treatments in a completely randomized experimental design. Predator treatments contained a single larval dragonfly (*Anax* spp. in their penultimate instar) in each of the four cages. These predators coexist with wood frogs in many natural ponds (Van Buskirk and Relyea 1998) and were collected from nearby experimental ponds. Predators were fed ~300 mg of larval wood frogs three times per week; in tanks containing empty cages, the cages were lifted to equalize disturbance among treatments. Predator feeding was terminated on 8 July 1996 because the tadpoles in most tanks were metamorphosing.

Quantifying growth, larval period, and morphology

Over the duration of the experiment, three samples of tadpoles were removed to assess changes in phenotypes among populations and predator treatments. I removed samples after 18 d (20 tadpoles), 23 d (40 tadpoles), and 35 d (20 tadpoles). The remainder of the surviving tadpoles (up to 30) were left in the tanks to

metamorphose. The first tadpole metamorphosed after 44 d and the last metamorph appeared after 79 d. All metamorphs were collected and preserved when they had achieved Gosner (1960) stage 44–46 (only a tail stub remaining). Mass and larval period were measured for each metamorph and the means for each tank were used as response variables. Because growth was measured three times, development was measured twice, and survivorship was measured at the end, separate analyses of variance were conducted for each response variable, using repeated measures in the analysis of growth and development.

Three of the preserved samples (hatchling sample, 18-d sample, and the 35-d sample) were used in the analysis of morphology. The effect of larval predators on the morphology of the terrestrial metamorphs is the subject of a separate paper (Relyea 2001b). Wood frog morphology was measured from video images of 10 preserved tadpoles and metamorphs from each sample using Bioscan image analysis software (Optimas, Bothell, Washington, USA). For tadpoles, I took measurements from both the side view (tail fin depth, tail fin length, tail muscle depth, body depth, and body length) and the top view (tail muscle width and body width; see Fig. 1 in Relyea [2000]). For the larger tadpoles of the second and third samples, a glass plate was placed under the tail fin in the side view to provide a more natural, undistorted image. At the time of measurement, tadpoles were also weighed and assigned a Gosner stage.

I analyzed the morphology for each of the four temporal samples. To examine whether the populations and predator treatments had an effect on tadpole and metamorph morphology, I first had to remove differences in morphology due to differences in overall body size. To this end, I regressed the linear dimensions against mass using a single linear regression (after log-transforming both groups of data to improve the linearity of the relationship) and saved the residual values for each response (a standard morphometric procedure, Bookstein 1989). The mean residual response for each tank was used in the analysis. I used a multivariate analysis of variance (MANOVA) to examine the morphological traits because the responses are not independent of each other.

Quantifying activity

The second extraction of tadpoles was conducted after 23 d of exposure to quantify activity of the populations. I removed 40 tadpoles from each tank; 20 tadpoles were immediately preserved while the other 20 were split into two groups of ten. The two groups of 10 were added to separate 8-L tubs of aged well water and tubs were shelved under a bank of fluorescent lights placed on 14:10 light:dark timers. The predator environments experienced in the tanks were maintained in the tubs. For each predator tub, a single *Anax* was caged in screened, 0.25-L plastic cups and fed ~300 mg of

wood frog tadpoles for the duration of the activity trial. No-predator tubs contained empty cups.

The activity trial lasted 7 d. I initially fed the tadpoles a 3:1 ration of rabbit chow:Tetramin fish flakes at a rate of 10% of wood frog body mass per day. This ration proved to be too high and resulted in fouled water by day 2. I changed the water and added a new 5% food ration. I quantified tadpole activity 24 times during the experiment, including all hours of the day. Activity was quantified by slowly approaching a tub and making scan samples (Altmann 1974) of the proportion of active (moving) tadpoles; this is a standard method for measuring tadpole activity (Skelly and Werner 1990, Skelly 1992, Relyea and Werner 1999, Relyea 2000, 2001a). The mean of the 24 observations was used as the response variable for each tub.

The activity trial was analyzed as an analysis of variance. The activity data were not transformed since they met the assumptions of normality and homoscedasticity. For all analyses, I first conducted analyses of variance across all eight populations and then conducted tests across the four open-canopy and four closed-canopy populations.

Cross-transplant experiment

A cross-transplant was conducted in 1998 to determine if the populations differed in their response to competition or predation under the natural conditions of each population's native pond. This experiment applied four treatments to each population, using screened pens and a factorial combination of low and high competition (50 or 100 tadpoles, respectively) with the presence and absence of lethal (uncaged) predators. The treatments were replicated four times in four spatial blocks in a completely randomized block design. Because of the relatively high number of treatments and limited experimental space in natural ponds, only two populations were used (the Buffer Zone Marsh population and the Southwest Woods Pond population) in each of two ponds (Buffer Zone Marsh and Southwest Woods Pond). For each population, I collected ≥ 10 wood frog egg masses that had been recently oviposited in the ponds. The predators in the experiment represented the dominant predators in each of the ponds; larval predaceous diving beetles (*Dytiscus* sp.) were used in the Buffer Zone Marsh pens and larval tiger salamanders (*Ambystoma tigrinum*) were used in the Southwest Woods Pond pens.

Pens were $1 \times 1 \times 1$ m, constructed from 5×5 -cm lumber, and covered with 0.25-mm nylon mesh to exclude predators and other amphibians from entering the pen. The bottom of each pen was additionally covered with aluminum window screen to protect against tearing from rocks and sticks in the benthos. I placed the pens into each pond on 13 May and I added 300 g of dry oak leaves to each pen to serve as a substrate for periphyton growth (oak leaves are a component of the benthos in both ponds to varying degrees). On 16

May, tadpoles and predators were added to each pen (mean tadpole masses: Buffer Zone Marsh = 70.7 mg, Southwest Woods Pond = 51.6 mg). The predators were initially caged to permit the target tadpoles to acclimate to their new environment and to the presence of the predator. On 18 May, the predators were released from their cages. A sample of 20 tadpoles from each population was set aside to assess 48-h handling survivorship; it was 90% for both populations.

The experiment was terminated on 8 June 1998. Prior to termination, a 60-cm (dbh) oak tree fell into Southwest Woods Pond, crushing 11 of the 32 pens and leaving 21 viable pens. Upon termination, tadpoles and predators were sorted from the leaves, counted, and weighed to estimate survivorship and growth rate. A sample of ten tadpoles were preserved for subsequent morphological analysis as described in the common-garden experiment.

Because each pond differed in a multitude of ways including the specific predator treatment used (*Dytiscus* or *Ambystoma*), I analyzed the survivorship, growth, and morphological traits separately for each pond as described for the common-garden experiment. In the analysis of Buffer Zone Marsh, three pens were removed from the analysis: two pens had their predators die during the experiment and one pen suffered an invasion from a predatory giant water bug (*Lethocerus* sp.). Furthermore, because the lethal predators often clipped off the tail tips in failed predation attempts, tail length was an unreliable and highly variable measure; therefore, tail length was excluded from the analysis.

Quantifying the local predator and competitor environments

I quantified the composition and density of predators and potential anuran competitors for each pond over a 2-yr period to examine how the wood frogs from different populations face different biotic challenges. This survey work represents a small part of a larger collaborative effort to survey the natural ponds of the ESGR and the neighboring Pinckney Recreation Area (E. E. Werner, R. A. Relyea, D. K. Skelly, K. L. Yurewicz, unpublished data; K. Yurewicz, unpublished data; respectively). While two years of data cannot provide extensive insights into the long-term conditions of each pond, the data do show important general patterns. Density estimates were conducted using 20–40 pipe samples of each pond taken in late May and early June, approximately halfway through the wood frog's larval period. Each pipe samples an area of 0.1 m²; thus, the 20–40 samples can be used to estimate mean densities of taxa per pond. Density estimates exist for seven of the eight ponds for both 1996 and 1997. The eighth pond, Silver Lake Three, was not sampled during these years but was sampled in 1998 for comparison. Larval anurans were identified to species: wood frogs, green frogs (*Rana clamitans*), spring peepers (*Pseudacris*

crucifer), western chorus frogs (*P. triseriata*), gray tree frogs (*Hyla versicolor*), and American toads (*Bufo americanus*). Predators of larval anurans were lumped into broad categories: larval and adult caudatans (salamanders; *Ambystoma* and *Notophthalmus*), dytiscid beetles (larvae and adults), hydrophilid beetles (larvae and adults), larval aeshnid dragonflies, and larval libellulid dragonflies. I conducted a simple analysis of species richness for tadpoles and predators within each year using a nonparametric Kruskal-Wallis test.

RESULTS

The common-garden experiment demonstrated that growth, development, and morphology all differed among populations and between predator treatments. Because there are a large number of responses for each of these two types of variation, I will first present the predator responses and then present the population differences.

Common-garden experiment: responses to predators

Caged predators affected tadpole development, growth, and morphology (Tables 1–3, Figs. 1–3). Tadpoles reared in the presence of predators exhibited slower development by day 18 and took longer to achieve metamorphosis. Predators also reduced growth of tadpoles on all three sample dates ($P < 0.02$) but did not affect growth by the time that metamorphosis occurred due to the extended larval period ($P = 0.678$).

Predators further induced changes in wood frog morphology and activity (Table 3, Figs. 2–3). After 18 d, predator-induced tadpoles developed relatively deeper and longer tail fins ($P < 0.009$), deeper and narrower tail muscles ($P < 0.008$), and shallower and shorter bodies ($P < 0.001$); body width was unaffected ($P = 0.238$). After 35 d of exposure, predators continued to induce deeper tail fins and shorter bodies ($P < 0.001$). Wood frog larvae were less active in the presence of predators ($F_{1,32} = 28.6$, $P < 0.001$).

Common-garden experiment: population differences

Tadpoles grew from the start of the experiment until at least day 35 and then decreased their growth by 60% when they metamorphosed into juvenile frogs (Table 1, Fig. 1). At the start of the experiment, there were no population differences in mass across all eight populations nor were there any population differences in mass within each canopy type ($P > 0.05$). In the first sample (day 18), there was a marginally significant population difference across all eight populations ($P = 0.063$); growth differed among open-canopy populations ($P = 0.018$) but not among closed-canopy populations ($P = 0.429$). In the next two samples, populations did not differ in growth, even within canopy type ($P > 0.3$). At metamorphosis, open-canopy populations differed in growth ($P = 0.019$) but closed-canopy populations did not ($P = 0.159$).

Development of wood frog tadpoles also differed

TABLE 1. Results of the repeated-measures ANOVA for the growth rate of eight populations reared in a common-garden experiment in both the absence and presence of caged predators.

| Source | df | F | P |
|--|--------|-------|--------|
| Between subjects | | | |
| Population | 7, 32 | 0.4 | 0.911 |
| Predator | 1, 32 | 73.1 | <0.001 |
| Population \times Predator | 7, 32 | 2.1 | 0.074 |
| Within subjects | | | |
| Time | 3, 96 | 571.8 | <0.001 |
| Time \times Population | 21, 96 | 1.6 | 0.075 |
| Time \times Predator | 3, 96 | 24.6 | <0.001 |
| Time \times Population \times Predator | 21, 96 | 0.8 | 0.695 |

among populations (Table 2, Fig. 2). The eight populations did not exhibit overall significant differences in their 18-d Gosner stage nor in their developmental rate to metamorphosis; however, there were differences within each canopy type. Both developmental measures differed among the open-canopy populations but not among the closed-canopy populations.

There were no significant activity differences across all eight populations ($F_{7,32} = 0.52$, $P = 0.811$; Fig. 2). The open-canopy populations exhibited a nearly significant interaction between the populations and the predator environment ($F_{3,16} = 2.8$, $P = 0.073$) because two populations strongly responded to the presence of predators while the other two populations did not. In

contrast, there were no activity differences among the closed-canopy populations ($F_{3,16} = 0.3$, $P = 0.834$) nor did the populations interact with the predator treatments ($F_{3,16} = 0.9$, $P = 0.449$).

Tadpole morphology also exhibited population differences (Table 3, Fig. 3). The initial sample of tadpoles exhibited no significant difference across all eight populations; however, the analysis by canopy type indicated differences in tail muscle width among the open-canopy populations ($P = 0.032$) but not among the four closed-canopy populations. By day 18, there were significant morphological differences; open-canopy populations differed in morphology (tail depth, $P = 0.011$; muscle depth, $P = 0.041$; and body length, $P = 0.005$) but closed-canopy populations did not. By day 35, there were still differences among the open-canopy populations but not among the closed-canopy populations. The open-canopy populations differed in muscle width and muscle depth ($P < 0.01$) and these traits interacted with the predator treatments.

Cross-transplant experiment

The cross-transplant experiment, using more natural conditions, confirmed that local populations of wood frogs differ in their phenotype and performance. The morphological phenotypes of the two populations showed consistent differences when raised in either pond (Table 4). When reared in Buffer Zone Marsh,

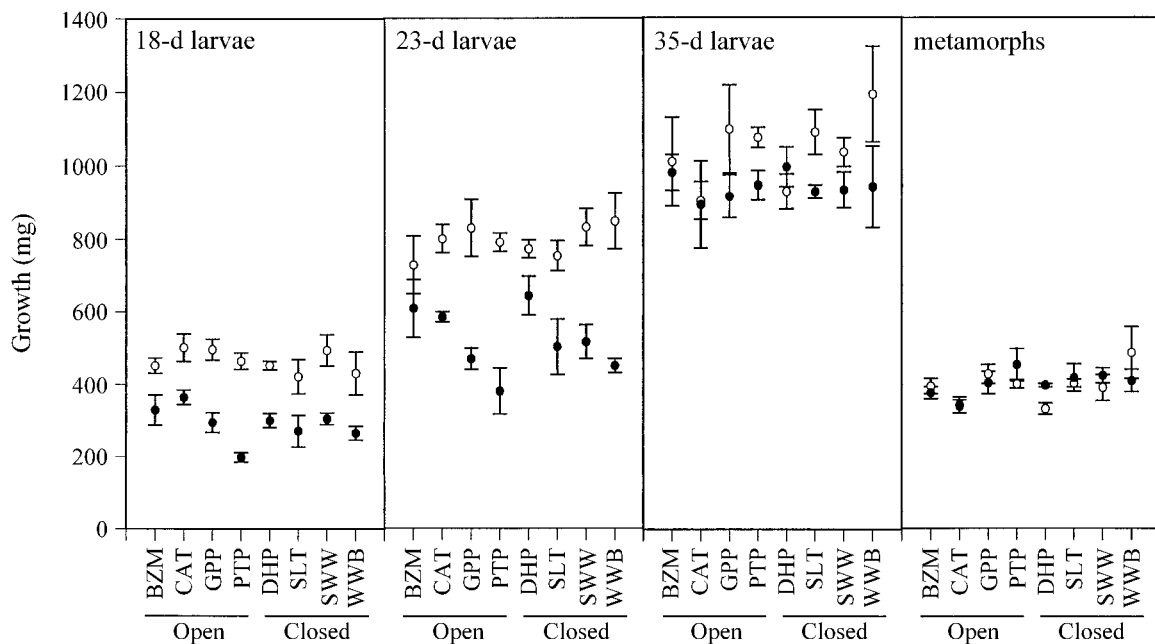


FIG. 1. Mean growth of eight populations of wood frogs reared in a common-garden experiment in either the absence (open symbols) or presence (closed symbols) of caged predators. Tadpoles were weighed after 18, 23, and 35 d of exposure to the treatments and again at metamorphosis. "Open" and "Closed" refer to open-canopy ponds and closed-canopy ponds. Ponds are abbreviated as follows: BZM = Buffer Zone Marsh, CAT = Cattail Marsh, GPP = Gravel Pit Pond, PTP = Pearl Two Pond, DHP = Dreadful Hollow Pond, SLT = Silver Lake Three Pond, SWW = Southwest Woods Pond, and WWB = West Woods Big Pond. Error bars represent ± 1 SE.

TABLE 2. ANOVA results for the development of eight populations reared in a common-garden experiment in both the absence and presence of caged predators.

| Dependent variable and source of variation | All eight populations | | | Open-canopy populations | | | Closed-canopy populations | | |
|--|-----------------------|-------|--------|-------------------------|------|--------|---------------------------|------|--------|
| | df | F | P | df | F | P | df | F | P |
| 18-d Gosner stage | | | | | | | | | |
| Predator | 1, 32 | 132.2 | <0.001 | 1, 32 | 81.7 | <0.001 | 1, 32 | 55.9 | <0.001 |
| Population | 7, 32 | 1.6 | 0.166 | 7, 32 | 3.2 | 0.049 | 7, 32 | 0.8 | 0.490 |
| Predator × Population | 7, 32 | 0.6 | 0.729 | 7, 32 | 1.3 | 0.317 | 7, 32 | 0.4 | 0.773 |
| Developmental rate (1/larval period) | | | | | | | | | |
| Predator | 1, 32 | 92.4 | <0.001 | 1, 32 | 49.9 | <0.001 | 1, 32 | 44.6 | <0.001 |
| Population | 7, 32 | 1.5 | 0.208 | 7, 32 | 3.5 | 0.040 | 7, 32 | 0.5 | 0.713 |
| Predator × Population | 7, 32 | 1.5 | 0.195 | 7, 32 | 3.6 | 0.038 | 7, 32 | 0.5 | 0.708 |

the Buffer Zone population developed wider tail muscles ($P = 0.026$) than the Southwest Woods population, particularly in the presence of the predator (Fig. 4). Further, the two populations differed in how they altered their body length when competition was increased (i.e., a population-by-competition interaction, $P = 0.047$). The addition of predators resulted in surviving tadpoles from both populations possessing deeper tail fins and shorter bodies.

When reared in Southwest Woods Pond, the two populations continued to exhibit different morphology (Fig. 5). The Buffer Zone population exhibited shallower bodies ($P = 0.035$), deeper and wider muscles

($P < 0.02$), and deeper tail fins (particularly when experiencing competition, $P = 0.046$) than the Southwest Woods population. The lethal predator resulted in surviving tadpoles having deeper tails and shorter bodies ($P \leq 0.0001$). Competition did not affect tadpole morphology (Table 4).

In addition to differences in morphology, the two populations also differed in relative performance when reared in each other's pond (Table 5). When the populations were reared in Buffer Zone Marsh (Fig. 6), predation reduced both survivorship and growth ($P < 0.0001$) whereas competition only reduced tadpole growth ($P = 0.025$). There also was a population-by-predator interaction on survivorship, caused by a difference in survivorship between populations in the absence of predators ($P = 0.009$) but not in the presence of predators ($P = 0.821$). That is, in Buffer Zone Marsh, the Buffer Zone population survived better than the Southwest Woods population when dytiscid predators were absent. When the populations were reared in Southwest Woods Pond (Fig. 7), predation by tiger salamanders reduced survivorship ($P = 0.0001$) and competition reduced tadpole growth ($P = 0.020$). However, the two populations did not differ in how they grew or survived in the face of the four competition and predation treatments.

Quantifying local predator and competitor densities

The results of the pond survey indicated that open- and closed-canopy ponds differed qualitatively in their composition of predators and potential competitors (Figs. 8–9). Open-canopy ponds contained more diverse assemblages of tadpoles than closed-canopy ponds in both years (1996, $P = 0.026$; 1997, $P = 0.013$). Closed-canopy ponds contained exclusively wood frogs whereas open-canopy ponds contained up to five other species of larval anurans. Similarly, open-canopy ponds had a higher diversity of predators in both years (1996, $P = 0.022$; 1997, $P = 0.036$). Closed-canopy ponds contained primarily caudatans and dytiscid beetles (primarily *Acilius* sp., not *Dytiscus* sp.). In contrast, open-canopy ponds contained these two predator taxa as well as aeshnid dragonflies, libellulid dragonflies, and hydrophilid beetles.

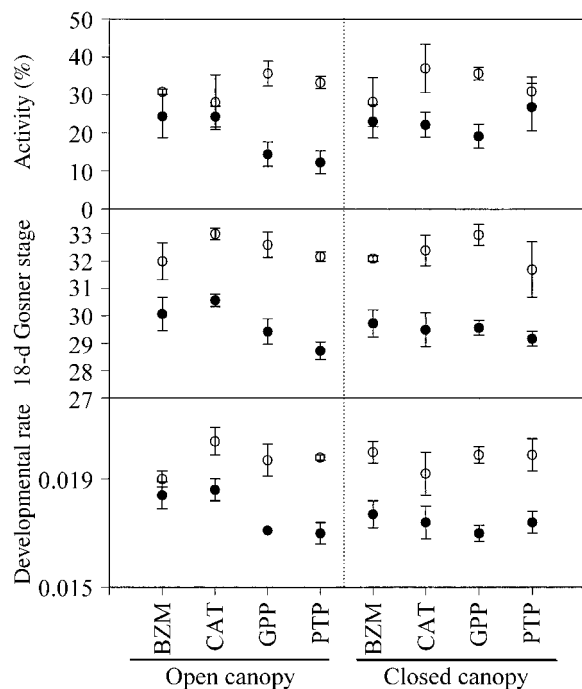


FIG. 2. Activity, 18-d Gosner stage (development after 18 d of treatment exposure), and developmental rate to metamorphosis (1/larval period) of eight populations of wood frogs that were reared in a common-garden experiment in either the absence (open symbols) or presence (closed symbols) of caged predators. Population abbreviations are as in Fig. 1. Data are means \pm 1 SE.

TABLE 3. MANOVA results for the morphology of eight populations reared in a common-garden experiment in both the absence and presence of caged predators.

| Source | All eight populations | | | Open-canopy populations | | | Closed-canopy populations | | |
|------------------------|-----------------------|----------|----------|-------------------------|----------|----------|---------------------------|----------|----------|
| | df | <i>F</i> | <i>P</i> | df | <i>F</i> | <i>P</i> | df | <i>F</i> | <i>P</i> |
| Initial tadpole sample | | | | | | | | | |
| Population | 49, 55 | 0.9 | 0.611 | 21, 6 | 3.8 | 0.046 | 21, 6 | 0.5 | 0.879 |
| 18-d sample | | | | | | | | | |
| Predator | 7, 26 | 90.1 | <0.001 | 7, 10 | 63.6 | <0.001 | 7, 10 | 49.0 | <0.001 |
| Population | 49, 136 | 2.7 | <0.001 | 21, 29 | 4.1 | <0.001 | 21, 29 | 1.5 | 0.171 |
| Predator × Population | 49, 136 | 0.9 | 0.735 | 21, 29 | 1.5 | 0.142 | 21, 29 | 0.6 | 0.850 |
| 35-d sample | | | | | | | | | |
| Predator | 7, 26 | 41.7 | <0.001 | 7, 10 | 21.0 | <0.001 | 7, 10 | 19.4 | <0.001 |
| Population | 49, 136 | 1.3 | 0.150 | 21, 29 | 2.5 | 0.013 | 21, 29 | 0.5 | 0.948 |
| Predator × Population | 49, 136 | 0.7 | 0.918 | 21, 29 | 2.0 | 0.041 | 21, 29 | 0.4 | 0.991 |

Note: Values are the Wilks' lambda *F* statistic and the associated *P* values.

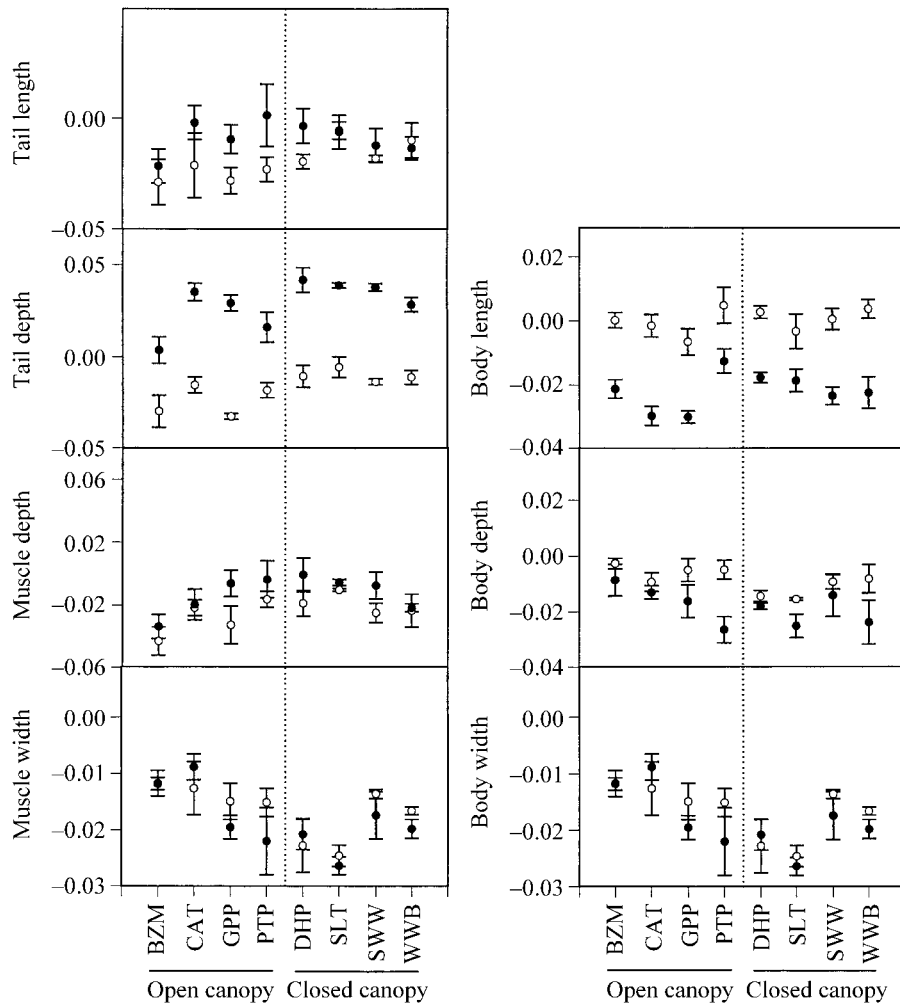


FIG. 3. Relative morphology of eight populations of wood frog tadpoles after 18 d of exposure to either the absence (open symbols) or presence (closed symbols) of caged predators. Differences in morphology due to differences in overall size were removed by regression of mass against each dimension. Population abbreviations are as in Fig. 1. Data are means \pm 1 SE of residuals from regressions of log(linear morphological dimensions) against log(mass).

TABLE 4. MANOVA results examining the effect of population of origin, competition, and predation on the morphology exhibited by larval wood frogs reared in either Buffer Zone Marsh or Southwest Woods Pond.

| Source | df | F | P |
|-------------------------------------|-------|------|---------|
| Buffer Zone Marsh | | | |
| Population | 8, 13 | 3.1 | 0.035 |
| Predator | 8, 13 | 50.5 | <0.0001 |
| Competition | 8, 13 | 1.0 | 0.470 |
| Population × Predator | 8, 13 | 2.4 | 0.079 |
| Population × Competition | 8, 13 | 3.8 | 0.016 |
| Predator × Competition | 8, 13 | 2.1 | 0.119 |
| Population × Predator × Competition | 8, 13 | 3.2 | 0.031 |
| Southwest Woods Pond | | | |
| Population | 8, 6 | 7.4 | 0.013 |
| Predator | 8, 6 | 7.4 | 0.012 |
| Competition | 8, 6 | 1.3 | 0.384 |
| Population × Predator | 8, 6 | 0.7 | 0.670 |
| Population × Competition | 8, 6 | 0.5 | 0.827 |
| Predator × Competition | 8, 6 | 2.0 | 0.214 |
| Population × Predator × Competition | 8, 6 | 0.4 | 0.886 |

Note: Values are multivariate Wilks' lambda statistics.

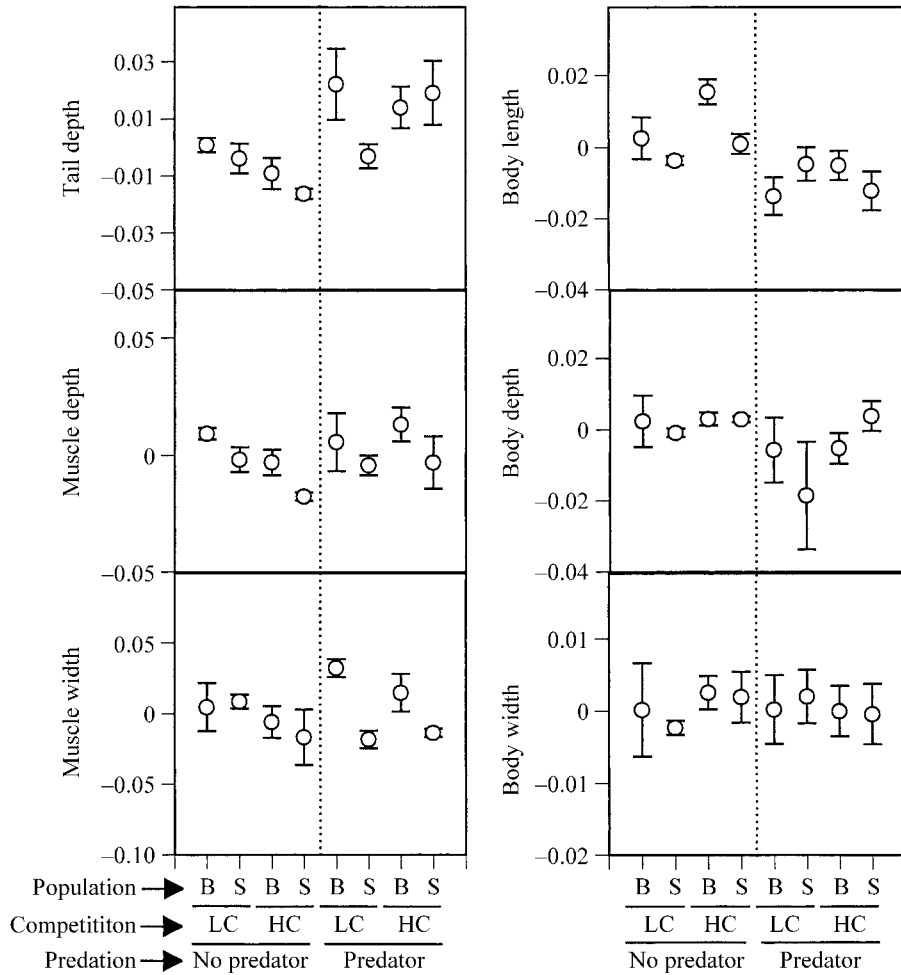


FIG. 4. The relative morphology of larval wood frogs from two populations reared in Buffer Zone Marsh (B = Buffer Zone population, S = Southwest Woods population). The tadpoles were reared under a factorial combination of low and high competition (LC and HC) and the presence and absence of dytiscid beetle predators. Differences in overall size were removed prior to analysis. Data are means \pm 1 SE of residuals from regressions of log(linear morphological dimensions) against log(mass).

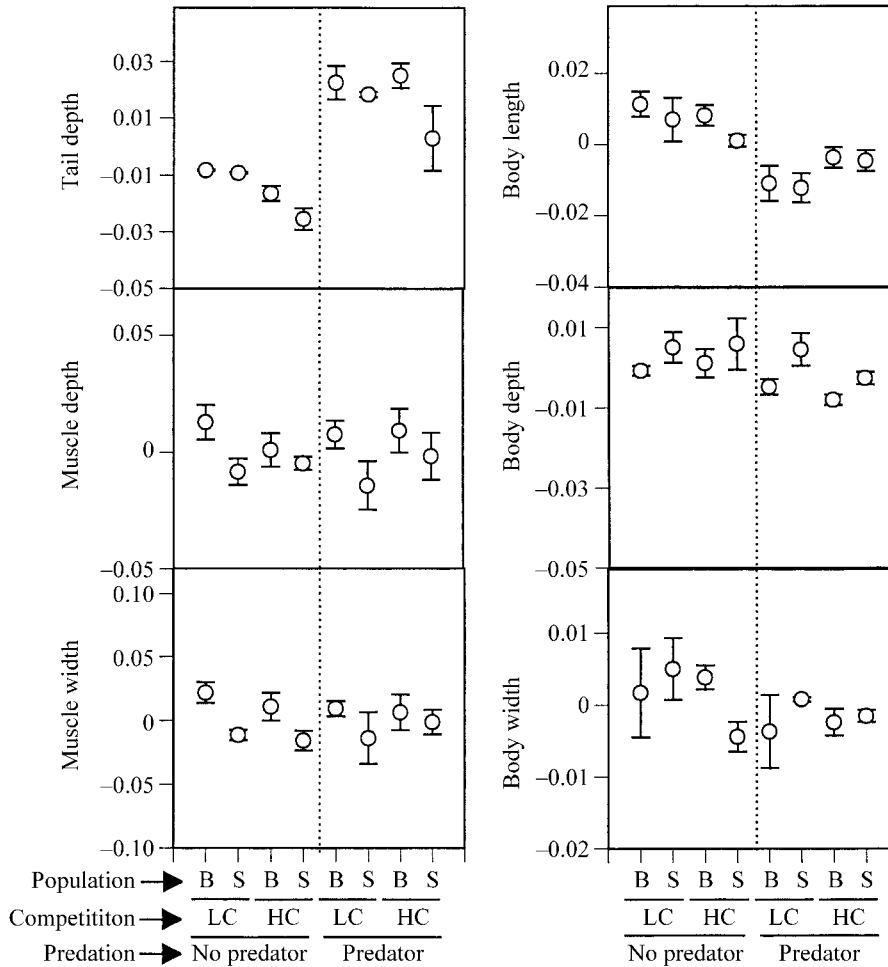


FIG. 5. The relative morphology of larval wood frogs from two populations reared in Southwest Woods Pond (B = Buffer Zone population, S = Southwest Woods population). The tadpoles were reared under a factorial combination of low and high competition (LC and HC) and the presence and absence of ambystomatid salamander predators. Differences in overall size were removed prior to analysis. Data are means \pm 1 SE of residuals from regressions of log(linear morphological dimensions) against log(mass).

TABLE 5. MANOVA results examining the effect of population of origin, competition, and predation on the growth and survivorship exhibited by larval wood frogs reared in either Buffer Zone Marsh or Southwest Woods Pond.

| Source | df | F | P |
|---|-------|--------|---------|
| Buffer Zone Marsh | | | |
| Population | 2, 19 | 3.2 | 0.061 |
| Predator | 2, 19 | 1375.0 | <0.0001 |
| Competition | 2, 19 | 4.5 | 0.025 |
| Population \times Predator | 2, 19 | 4.1 | 0.033 |
| Population \times Competition | 2, 19 | 2.3 | 0.125 |
| Predator \times Competition | 2, 19 | 1.0 | 0.217 |
| Population \times Predator \times Competition | 2, 19 | 2.1 | 0.149 |
| Southwest Woods Pond | | | |
| Population | 2, 12 | 2.1 | 0.170 |
| Predator | 2, 12 | 23.8 | 0.0001 |
| Competition | 2, 12 | 4.6 | 0.033 |
| Population \times Predator | 2, 12 | 0.5 | 0.629 |
| Population \times Competition | 2, 12 | 0.2 | 0.817 |
| Predator \times Competition | 2, 12 | 1.9 | 0.189 |
| Population \times Predator \times Competition | 2, 12 | 0.5 | 0.619 |

Note: Values are multivariate Wilks' lambda statistics.

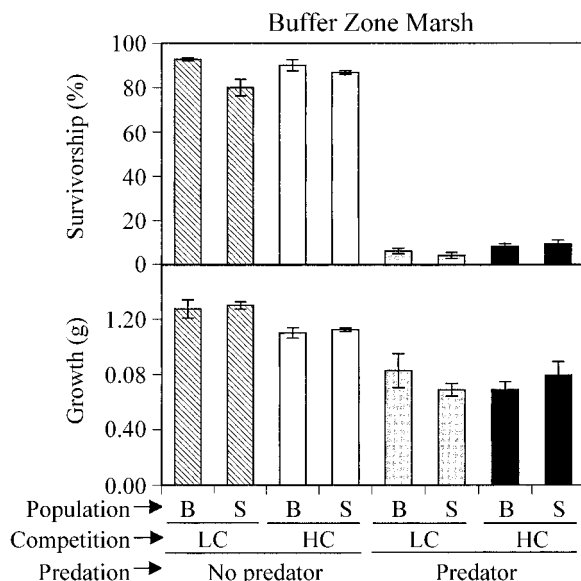


FIG. 6. The survival and growth of larval wood frogs from two populations reared in Buffer Zone Marsh (B = Buffer Zone population, S = Southwest Woods population). The tadpoles were reared under a factorial combination of low and high competition (LC and HC) and the presence and absence of dytiscid beetle predators. Data are means \pm 1 SE.

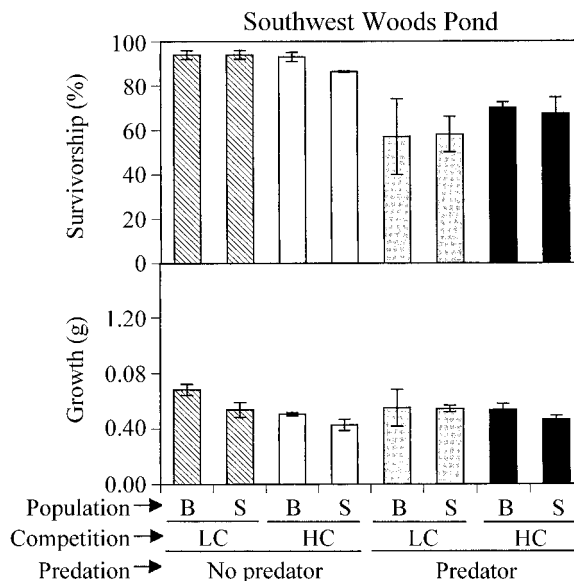


FIG. 7. The survival and growth of larval wood frogs from two populations reared in Southwest Woods Pond (B = Buffer Zone population, S = Southwest Woods population). The tadpoles were reared under a factorial combination of low and high competition (LC and HC) and the presence and absence of ambystomatid salamander predators. Data are means \pm 1 SE.

DISCUSSION

Predator-induced responses in wood frogs

The predator-induced responses of the larval wood frogs were consistent with several past studies. In the common-garden experiment, predators induced wood frog tadpoles to develop relatively deeper tails and shorter bodies. These alternative phenotypes are adaptive responses; tadpoles with deeper tails and shorter bodies survive better in the presence of free (uncaged) predators but grow more slowly in the absence of free predators (Van Buskirk et al. 1997, Van Buskirk and Relyea 1998, Relyea and Werner 2000, R. Relyea, *unpublished manuscript*). Similarly, in the cross-transplant experiment, surviving tadpoles that experienced lethal (uncaged) predators also exhibited relatively deeper tails and shorter bodies. However, lethal predators not only induce morphological changes by emitting chemical cues (Petranka et al. 1987, Kats et al. 1988, McCollum and Leimberger 1997); lethal predators also can cause deeper tail fins and shorter bodies in the surviving tadpoles by reducing intraspecific prey competition (which induces morphological changes) and by killing the prey nonrandomly with respect to morphology (Van Buskirk and Relyea 1998; Relyea, *in review a*). Recent experiments have demonstrated that selection (nonrandom killing) accounts for very little of the total phenotypic change (\sim 5%) while chemical induction and reduced competition account for 95% of the phenotypic change (Relyea 2000). Therefore, while the morphological changes in the cross-transplant ex-

periment may have multiple causes, the changes are consistent with the common-garden experiment and with the previous studies.

Tadpoles also reduced their activity in the presence of predators. Predator-induced reduction in activity has been observed empirically in many taxa (Sih 1987, Lima and Dill 1990) including larval anurans (Petranka et al. 1987, Werner 1991, Skelly 1992, Relyea 2001a) and is believed to be an adaptive response. In the absence of predators, higher activity results in greater foraging effort and faster growth (Skelly 1992, Relyea and Werner 1999), whereas in the presence of predators, higher activity results in higher encounter rates with predators and a greater risk of predation (Gerritsen and Strickler 1977, Skelly 1994, Anholt and Werner 1995).

Because development often is related positively to growth in amphibians (Travis et al. 1987, Blouin 1992), the slower growing tadpoles in the predator treatments experienced slower development. The longer development time of predator-exposed tadpoles allowed them more time to grow at a slower rate and ultimately metamorphose at the same size as tadpoles exposed to no predators. Thus, the predator-induced tadpoles metamorphosed at the same size as the no-predator induced tadpoles, but at a mean of 7 d later (a 12% increase in development time).

Population differences in wood frogs

In the common-garden experiment, the eight populations of wood frogs differed in a large number of

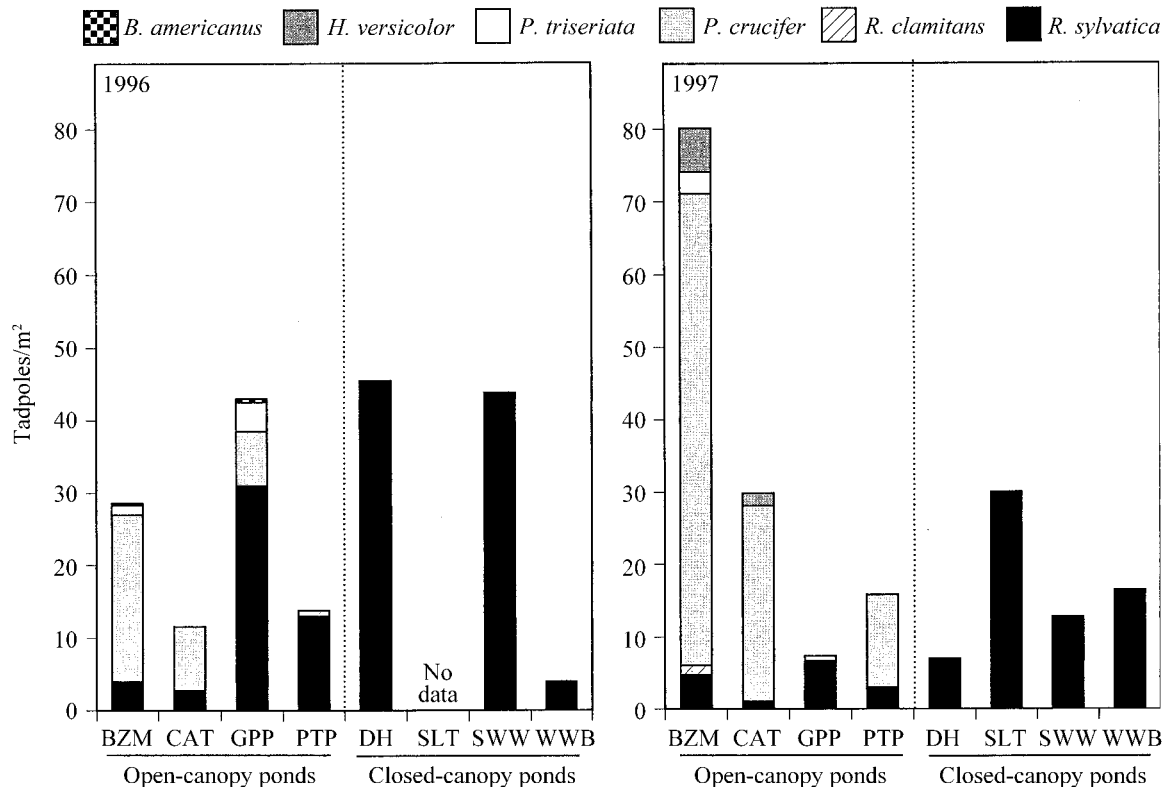


FIG. 8. Tadpole densities in four open-canopy and four closed-canopy ponds in southeast Michigan. Seven of the eight populations were surveyed in early May/late June of 1996 (left panel) and 1997 (right panel). The eighth population (SLT) was only surveyed in 1998 and is presented in the right panel. Population abbreviations are as in Fig. 1.

larval traits and the differences exhibited an intriguing pattern within the two pond canopy types. The four open-canopy populations differed from each other in nearly every response that I examined including behavior, morphology, growth rate, and developmental rate while the four closed-canopy populations did not differ from each other in any of these traits. Given that these population differences were based on only three egg masses per pond population, these population differences must be quite widespread among individuals in each population. The existence of population differences is further supported by the cross-transplant experiment, which employed mixtures of ≥ 10 egg masses from two populations and continued to detect phenotypic differences between populations. Thus, there is strong support for local population differences in wood frogs.

Previous investigators have found genetic differences among amphibian populations, but these studies were conducted over much larger geographic scales. There are several studies in which allozyme differences among populations have been documented (Gorman and Gaines 1987, Reh and Seitz 1990, Green et al. 1996, Hitchings and Beebee 1997). These studies lack information on population phenotypes and whether these genetic differences might represent local adap-

tation or random genetic drift. However, in one of these studies, Gorman and Gaines (1987) found that their subset of eight populations of *Acris crepitans* in eastern Kansas exhibited patterns of protein polymorphisms that could be separated between pond habitats and stream habitats. This suggests that, similar to the present study, different habitats might select for different trait states.

Other investigators have focused on phenotypic differences among amphibian populations and the relationships with temperature, food availability, and pond permanence. Looking at populations from different altitudes, several researchers have found differences in growth and development that appear to be adaptations to differences in temperature (Berven 1982a, b, 1987, Berven and Gill 1983, Riha and Berven 1991), food availability (Bernardo 1994), or pond permanence (Semlitsch et al. 1990). In all of the above studies, interpopulation differences varied from 20 to 1000 km, allowing little or no dispersal between populations. Such large interpopulation distances should encourage independent evolution of phenotypes among populations. In contrast, the populations in the present study are much closer together (0.3–8.0 km), well within known dispersal distances for wood frogs (Berven and Grudzien 1990).

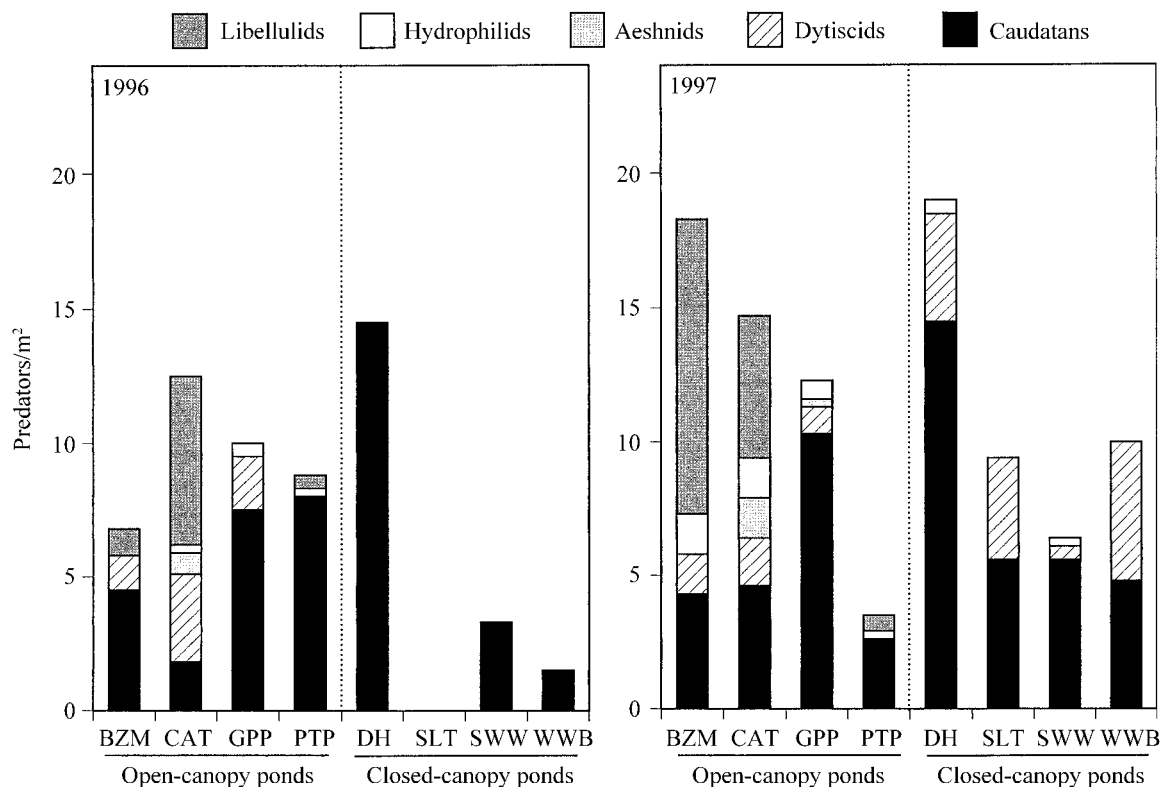


FIG. 9. Predator densities in four open-canopy and four closed-canopy ponds in southeast Michigan. Seven of the eight populations were surveyed in early May/late June of 1996 (left panel) and 1997 (right panel). The eighth population (SLT) was only surveyed in 1998 and is presented in the right panel. Population abbreviations are as in Fig. 1.

Population differences in predator-induced behavior have been documented in a variety of taxa but investigations of morphology appear to be rare. Most frequently, investigators have compared prey populations that are sympatric and allopatric with a given predator; the sympatric population typically exhibits more appropriate and more extreme behavioral responses to the predator than the allopatric population (zooplankton [Dodson 1988, Neill 1992, De Meester 1993]; fish [Giles and Huntingford 1984, Mathis et al. 1993]; insects [Juliano and Reminger 1992]; arachnids [Riechert and Hedrick 1990]; reptiles [Blazquez et al. 1997]; amphibians [Kiesecker and Blaustein 1997]; birds [Maloney and McLean 1995]). In contrast, there appear to be few studies documenting population (or clonal) differences in predator-induced morphology; most of these studies involve zooplankton (Barry and Bayly 1985, Spitze 1992). For example, Barry and Bayly (1985) found that *Daphnia* populations from a permanent pond (with presumably more consistent predator presence) possessed a nonplastic and well-defended morphology whereas populations from a temporary pond (with presumably more variable predator presence) possessed a highly plastic, defensive morphology. In what appears to be the only other study of population differences in predator-induced morphology

in tadpoles, Lardner (1998) found no population differences. However, the study calculated relative tail length as tail length/snout-vent length and calculated relative tail depth as tail depth/tail length. Because both the numerators and divisors can be affected by predator environments and by population differences, using this method can result in a conclusion that there are no differences in morphology when differences actually exist (e.g., when both tail length and snout-vent length increase in size). Therefore, it is unclear whether the results of this study are robust. An important difference between many of these studies and the current study is that the current study is not examining populations that are either sympatric or allopatric with predators. All of the populations in my study have predators, but the composition of predator species varies. Thus, all of the populations should respond to predator cues by altering their phenotypes but the precise phenotypes expressed by each population should be the evolved product of past selection within each population.

One of the difficulties in examining population differences using common-garden and cross-transplant experiments is that population differences could be due to either genetic differences or maternal effects. Maternal effects can have dramatic effects on an individual's phenotype (Mousseau and Dingle 1991, Mousseau

and Fox 1998); thus, one must address the possibility that maternal effects are causing the observed patterns. One way in which maternal effects could influence a population's phenotype is through differential addition of energy resources to the eggs. Populations that receive more energy as eggs should grow faster and have a growth advantage that can be maintained throughout the larval period through a positive feedback on large initial size. In this study, the initial mass of the larvae going into the experiment did not differ, suggesting that any differences in maternal investment in egg yolk resources had a negligible effect on subsequent tadpole mass. A second way in which maternal investment might affect the tadpole's phenotype is by altering the initial tadpole morphology and causing subsequent effects on tadpole morphology throughout the larval period. The analysis of initial tadpole morphology indicated that the hatchlings only differed in one of the seven morphological traits (muscle width). It seems unlikely that this single difference could account for the subsequent population differences in tail depth, muscle depth, and body length. This conclusion is supported by the work of Travis et al. (1987) who found that maternal effects in *Pseudacris crucifer* were only detected in hatchling tadpoles and not later in ontogeny. Thus, while the impact of maternal effects cannot be directly assessed, the existing data suggest that genetic effects, rather than maternal effects, underlie the observed phenotypic patterns among the wood frog populations. Multigenerational common-garden experiments would be necessary to confirm this conclusion.

If the population differences observed in my study are primarily genetically based, they could be caused by a number of mechanisms including random genetic drift, random mutation, and natural selection. If random drift or mutation were responsible, then we would be unlikely to observe the patterning of population differences in which open-canopy ponds differed in their phenotypes but closed-canopy populations did not. If natural selection were responsible, then selection must differ among the open-canopy ponds and be similar among the closed-canopy ponds. Further, breeding frogs must have a high fidelity to their natal ponds (i.e., interpond dispersal must be low) to prevent genetic mixing. Berven and Grudzien (1990) have examined interpond dispersal of wood frogs in Virginia and found that adults exhibit 82% fidelity to their natal pond and 100% fidelity to the pond that they first breed in. In fact, two of Berven and Grudzien's (1990) ponds were separated by only 50 m but 81% of returning frogs bred in their natal pond. Because maximal dispersal distance of newly metamorphic wood frogs was 1.2 km in their study (which they felt might be an overestimate; Berven and Grudzien 1990), they expected populations that were <1 km apart to be genetically similar due to genetic mixing, yet populations in my study were as little as 0.3 km away. This suggests that wood frogs in Michigan may have higher philopatry resulting in even less

genetic mixing among populations than in Virginia. Alternatively, natural selection for phenotypic differences within each pond is strong enough to select against larvae from other populations and maintain the population differences. An examination of population genetics is needed to confirm these hypotheses.

Possible causes of selection in the open- and closed-canopy ponds are differences in abiotic and biotic conditions. For example, open-canopy ponds tend to have higher incidence of sunlight, higher productivity, higher oxygen levels, and warmer temperatures than closed-canopy ponds (Werner and Glennemeier 1999). However, within each habitat type, there is not a pattern of closed-canopy ponds possessing more similar abiotic conditions and open-canopy ponds possessing dissimilar abiotic conditions (Werner and Glennemeier 1999; E. E. Werner, R. A. Relyea, D. K. Skelly, K. L. Yuriewicz, unpublished data).

Alternatively, the populations may be adapted to the local competitors and predators found in each pond. The pond survey data indicated that closed-canopy populations contained exclusively wood frog tadpoles; many anuran species cannot survive in closed-canopy ponds due to a lack of suitable food (Werner and Glennemeier 1999). In contrast, open-canopy populations have a large and variable assemblage of generalist anuran species that scrape periphyton, bacteria, and fungi from surfaces and, as a result, may compete with larval wood frogs (DeBenedictis 1974, Werner 1992, Relyea 2000). Recent experiments have demonstrated that differences in wood frog morphology can play a major role in the competitive ability of wood frog larvae (Relyea 2000, 2002). Wood frog tadpoles can alter their behavior and morphology in a competitor-specific fashion and these changes provide a competitive advantage to the tadpoles. Thus, historic exposure to different assemblages of competitors should favor different morphological trait states.

There also were differences among ponds in predator composition. Closed-canopy ponds typically had only two dominant predators (salamanders and predaceous diving beetles) whereas open-canopy ponds contain a large and variable assemblage of predators including salamanders, predaceous diving beetles, hydrophilid beetles, and dragonfly larvae. This is not to say that wood frogs from closed-canopy ponds should not respond to other species of predators (as in the common-garden experiment); we simply know too little about chemical cues produced during predation events to draw such conclusions. What we do know is that tadpoles can exhibit predator-specific phenotypes (both behavioral and morphological). We have documented the adaptiveness of these responses in many cases (Van Buskirk et al. 1997, Van Buskirk and Relyea 1998), suggesting that different predators are causing selection for different prey phenotypes. If so, then historic exposure to different assemblages of predators should favor different behavioral and morphological trait

states. Thus, there exists a pattern of closed-canopy ponds being similar for competitor and predator assemblages, which should favor the evolution of similar wood frog phenotypes among closed-canopy populations. In contrast, there exists a pattern of open-canopy ponds being different for competitor and predator assemblages, which should favor the evolution of different wood frog phenotypes among open-canopy populations.

The cross-transplant experiment tested whether the population differences might be adaptations to local pond conditions. The results from the pens placed into Buffer Zone Marsh supported the hypothesis; tadpoles from Buffer Zone survived better than tadpoles from Southwest Woods. This result was not observed when predators were present, but predation had reduced survivorship to very low levels, leaving little room for any scope of response. This result suggests that the Buffer Zone Marsh wood frogs are locally adapted to their native pond. In contrast, when the same experiment was conducted in Southwest Woods Pond, I observed no differences in growth or survival between the two populations, suggesting that the Southwest Woods wood frogs were not locally adapted to their native pond. Clearly, we need more extensive studies using a higher number of combinations of populations and ponds to determine whether adaptation to local pond conditions is a general phenomenon.

If selection is responsible for the observed population patterns, selection must be relatively strong because this pattern likely developed in a relatively short period of time. Based on aerial photographs of the ESGR, the six ponds located on the ESGR (currently three open-canopy and three closed-canopy ponds) were all open-canopy ponds in 1970. Thus, if the local pond environments are selecting for different phenotypes, then the hypothesized convergence of the closed-canopy pond populations would have taken place within the past 26 yr, or within ~13 generations of wood frogs. It is important to note that similarity in phenotypes of the four closed-canopy populations does not imply similarity of genotypes of these four populations. In fact, when I examined the morphology of the metamorphs that emerged from this experiment (Relyea 2001b), I found morphological differences among the four closed-canopy populations in hindleg length, body length, and body width. This implies that while larval, closed-canopy environments may be similar and selecting for similar traits, the surrounding terrestrial environment may be quite different and be selecting for different traits among populations that are uncoupled from the larval traits.

Conclusions

Larval wood frogs appear to be a taxon with a natural history that meets the assumptions of models for the evolution of population-specific plasticity. Populations are highly philopatric and experience spatial

heterogeneity in the biotic and abiotic conditions. In this study, closed-canopy populations appear to have very similar competitor and predator assemblage conditions and this seems to have favored the evolution of similar behavioral, morphological, and life historical phenotypes. In contrast, open-canopy populations appear to have different competitor and predator assemblage conditions and this may have favored the evolution of different phenotypes. Such population differences should have widespread implications, including the importance of localized selection, the maintenance of genetic structure and diversity at local and regional scales, and the impact of local adaptation on metapopulation dynamics. Further studies that combine a quantitative genetics approach with the current phenotypic approach should go a long way in addressing these issues. Collectively, these data demonstrate that spatially structured populations can evolve population-specific phenotypic plasticity on a very local geographic scale.

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