

# Cryptic divergence: countergradient variation in the wood frog

Nisha F. Ligon<sup>1</sup> and David K. Skelly<sup>1,2</sup>

<sup>1</sup>*Department of Ecology and Evolutionary Biology and* <sup>2</sup>*School of Forestry and Environmental Sciences, Yale University, New Haven, Connecticut, USA*

---

## ABSTRACT

**Questions:** Do wood frog (*Rana sylvatica*) populations show evidence of cryptic variation, wherein inherited variation is masked by environmental effects? Have these populations diverged in larval traits across a gradient of canopy cover? How do trends in larval traits differ when observed in the field versus in a common garden experiment?

**Hypothesis:** Due to strong selection in their cooler environment, wood frogs from heavily shaded wetlands will grow and develop more slowly in the field but more quickly in a common garden experiment, compared with their counterparts from sunnier wetlands.

**Methods:** We monitored growth and development of wood frog larvae from six wetlands of varying canopy cover. We studied populations in the natal wetlands and individuals raised in incubators from embryos through metamorphosis.

**Conclusions:** Observations of natural populations revealed no evidence of strong trends in phenotype. However, when raised in a common environment, larvae from more shaded natal wetlands grew and developed faster, and were larger at any given stage. This is evidence for cryptic divergence wherein the lack of an observed phenotypic trend masks the underlying inherited variation.

*Keywords:* amphibian, countergradient variation, cryptic, divergence, light, microgeography, phenotypic variation, temperature.

## INTRODUCTION

Ecologists are increasingly recognizing that phenotypic variation across space matters for the performance of individuals, the dynamics of populations, and interactions among species (see Endler, 1995; Reznick and Ghalambor, 2001; Doebeli and Dieckmann, 2003; Morrison and Hero, 2003). As studies of the geography of phenotype increase, it is worth considering the conditions under which cryptic inherited variation may underlie a lack of observed phenotypic variation. Countergradient variation, where inherited variation runs counter to environmental effects on variation, can lead to such a situation (Levins, 1969; Conover and Schultz, 1995).

---

Correspondence: N.F. Ligon, Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT 06511, USA. e-mail: nisha.ligon@gmail.com

Consult the copyright statement on the inside front cover for non-commercial copying policies.

---

A number of ectothermic species exhibit countergradient variation for growth and development rates across latitudinal clines: populations from more northerly areas have faster growth and development rates than their southern counterparts when raised in a common environment, but slower rates when observed *in situ* (e.g. Conover and Present, 1990; Arnett and Gotelli, 1999; Laugen *et al.*, 2003; Yamahira and Takeshi, 2008). Researchers have also found cases of countergradient variation in growth or development along gradients of altitude (Berven, 1982; Smith *et al.*, 1994; Marquis and Miaud, 2008), competition (Arendt and Wilson, 1999; Loman, 2003), food availability (Smith *et al.*, 1994), and light environment (Skelly, 2004). Although most known examples of countergradient variation occur across broad geographic clines where populations may have experienced isolation by distance for long periods of time, it may also cause divergence between groups that are not separated by geography. For example, countergradient selection seems to have contributed to the divergence between anadromous and non-anadromous morphs of *Oncorhynchus nerka*, which are sympatric during breeding periods (Craig *et al.*, 2005). Environmental heterogeneity over microgeographic scales can potentially lead to variation and divergence over small temporal and spatial scales (discussed in Hendry and Kinnison, 1999; Kawecki and Ebert, 2004), and this has often been found among amphibian populations and assemblages (Skelly, 2004; Urban, 2004; Ficetola and De Bernardi, 2005).

Wood frogs undergo larval development in temporary wetlands, which generally dry by late summer. Light environment of the wetlands, as determined by canopy cover, mediates important environmental characteristics, including temperature (Halverson *et al.*, 2003) and drying rates (Skelly *et al.*, 1999). Wood frog larvae could face strong selection for efficient growth and development, as well as high stage-specific mass and mass at metamorphosis. In amphibians, faster larval growth rates are often associated with decreased predation risk due to less time spent at small, vulnerable sizes (Caldwell *et al.*, 1980; Travis, 1983). Rapid development can help ensure that larvae reach metamorphosis and leave the water before a wetland dries. Entire cohorts can die in years when wetlands dry prematurely (Berven, 1995; Newman and Squire, 2001). Despite their cooler temperatures, shady wetlands often dry sooner than warm, sunny wetlands (Skelly *et al.*, 1999), thus wood frog larvae in shady wetlands might face strong selection for rapid development due to the combined effects of low temperatures and a short growing season. Although growth rate (the change in mass over time) and development rate (the change in life-history stage over time) in amphibians are often strongly correlated, they are not directly tied (Werner, 1986) and may be affected in different magnitudes by changes in temperature (Smith-Gill and Berven, 1979). Higher stage-specific growth rates lead to greater mass at metamorphosis (Alford and Harris, 1988; Rowe and Ludwig, 1991), which may confer fitness benefits during the adult stage (Smith, 1987; Semlitsch *et al.*, 1988; Berven, 1990).

In this study, we examined the effects of wetland light environment on larval growth and development through both field observations of natural breeding aggregations and a common garden laboratory experiment. Our approach was designed to compare phenotypic patterns from these two contexts to determine the relationship between naturally observed and underlying inherited variation in traits critical to performance.

## METHODS

### Study sites

We studied wood frogs from six temporary wetlands located at Yale-Myers Forest in northeastern Connecticut, USA: Blacksmith, Boulder, Centre Pike, Dentist, Shrub, and

Kealoha's, a subset of those used by Skelly (2004). These wetlands are located between 0.79 and 4.7 km of each other (calculated from UTM coordinates). For each wetland, the global site factor (GSF, an index of light environment) was estimated using the Hemiview software package (Delta-T Devices Ltd., Cambridge, UK) to analyse hemispherical photos of the spring forest canopy with leaves on (Halverson *et al.*, 2003). The GSF is represented on a scale from 0.0 (full obstruction and no direct sunlight) to 1.0 (no obstruction and full light). Study ponds ranged in GSF from 0.079 to 0.81.

### Field observations

We monitored larval wood frog growth and development at the six study wetlands. Every 12–16 days between 12 May and 15 July 2008, twenty larvae were collected from each site by dip-netting (except at one wetland, Dentist, where only two individuals could be found on the final collection day). The mass and Gosner stage (Gosner, 1960) of each individual were recorded from the live larvae within 12 h of collection. We estimated wetland-wide growth rate for each site using the slope coefficient from a linear regression of the mean mass for the first three sampling dates: 12 May, 28 May, and 11 June 2009, a period during which all larvae were at Gosner stages <40, beyond which larvae lose mass as they undergo metamorphosis. We estimated wetland-wide development rate using the slope coefficient from a linear regression of the mean Gosner stage for the same three sampling dates, as well as an extra sampling set from 23 to 25 June, which included larvae up through stage 42. We estimated an index of size at stage for each wetland using the slope coefficient from a linear regression of mass versus stage for all individuals of Gosner stage <40. We were unable to collect enough recent metamorphs to analyse mass at metamorphosis for the field study.

### Laboratory experiment

We collected wood frog eggs from the six wetlands from 1 to 3 April 2008. In each pond, approximately 20 eggs were separated from each of eight haphazardly selected egg masses within 24 h of being laid. Egg masses were brought to the campus of Yale University in New Haven, Connecticut, where they were kept in conditioned tap water (aged 2+ days) at 12°C and under ambient light until eggs hatched and larvae developed to Gosner stage 25, when they begin feeding. All individuals used for the experiment were stocked into incubators (Precision Model 818) within 24 h of reaching Gosner stage 25, which occurred over 5 days from 19 to 24 April. We stocked 15 haphazardly selected larvae from each wetland (taking no more than two individuals from any egg mass) in each of two incubator treatments: low temperature (14.2°C, s.e. = 0.0088) and high temperature (17.6°C, s.e. = 0.019). These treatment temperatures were selected to bracket the range experienced by larval wood frogs in their natal wetlands. Both treatments were kept on a 12-h light/12-h dark cycle. Larvae were housed individually, fed a daily ration of 10% of the average body mass (3:1 ratio of ground rabbit chow and fish flakes), and rotated to new positions in the incubator every 3 days. From stages 25 to 30, larvae were housed in 80-ml plastic containers, with water changes every 2–3 days. From stages 30 to 42, they were housed in 940-ml plastic containers, with water changes every 2–4 days.

For each individual, we measured mass and recorded Gosner stage every 4–10 days. As larvae approached metamorphosis, we monitored their condition daily and removed them, making final measurements, when they reached Gosner stage 42. We approximated the

growth rate of each individual using the slope coefficient from a linear regression of mass versus days elapsed (fits were very good with all  $R^2$ -values  $> 0.90$ ). As the larvae approach metamorphosis they lose mass, so growth rate calculations were performed using mass measurements between stages 25 and 39. We approximated development rate using the slope coefficient from a linear regression of Gosner stage versus days elapsed over the entire larval period, from stage 25 to stage 42 (fits were also good with all but one  $R^2$ -value  $> 0.80$ ). We calculated an index of size at stage throughout the period of larval growth using the slope coefficient from a linear regression of mass versus Gosner stage between stages 25 and 39 (fits were very good with all  $R^2$ -values  $> 0.91$ ). Finally, we used mass at metamorphosis as a response variable, which was measured within 24 h of reaching Gosner stage 42 (at least one arm emerged) for each of the individuals that metamorphosed in the laboratory.

### Statistical analyses

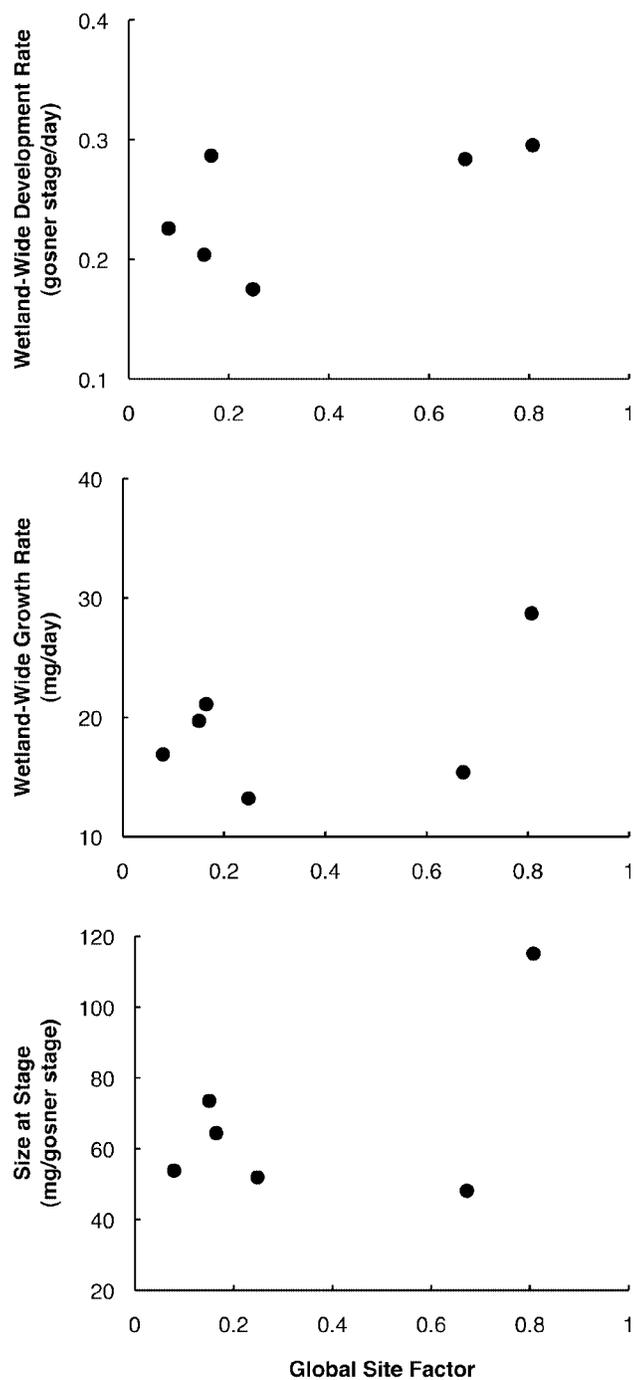
For the field sampling data, we performed linear regressions of the wetland-wide growth and development rates to estimate the effect of light environment [as measured by the global site factor (GSF), a continuous variable] on *in-situ* growth rate, development rate, and size at stage. For the common garden experiment, we confined statistical analyses of estimated growth rates, development rates, and size at stage index to larvae that survived past Gosner stage 38 (140 of 180 individuals). We used analysis of covariance (ANCOVA) to estimate the effects of temperature treatment (high or low, a categorical variable) and light environment of the pond of origin (GSF, a continuous variable) on growth rate. Comparable ANCOVAs were used to estimate effects on development rate, size at stage index, and mass at metamorphosis. For all ANCOVAs, the interaction between temperature and GSF was included in the analysis, but removed if not significant. Analysis of mass at metamorphosis was confined to the 98 individuals that reached metamorphosis in the laboratory.

## RESULTS

Analysis of field-collected larval cohorts revealed no relationship between growth rate and wetland light environment (Regression:  $R^2 = 0.216$ ,  $P = 0.353$ ), between development rate and light environment (Regression:  $R^2 = 0.390$ ,  $P = 0.185$ ), or between size at stage and light environment (Regression:  $R^2 = 0.277$ ,  $P = 0.283$ ) (Fig. 1).

In the common garden experiment, however, all larval traits studied were significantly influenced by the global site factor (GSF) of the natal wetland, and this influence was in the opposite direction from the non-significant effects seen in the field (Table 1). In the common garden experiment, wood frog larval growth rates were influenced by both incubator temperature (ANCOVA:  $F_1 = 59.69$ ,  $P < 0.001$ ) and light environment of the natal wetland (ANCOVA:  $F_1 = 4.29$ ,  $P = 0.040$ ). There was no significant interaction between temperature and GSF (ANCOVA:  $F_1 = 2.04$ ,  $P = 0.156$ ). Larvae grew 73% faster in the high-temperature treatment than in the low-temperature treatment. Larvae from dark wetlands (GSF  $< 0.3$ ) grew 10% faster than those from light wetlands (GSF  $> 0.6$ ) across both temperature treatments (Fig. 2).

Wood frog larval development rates differed between incubator temperature treatments (ANCOVA:  $F_1 = 154.74$ ,  $P < 0.001$ ) but did not vary with light environment of the natal wetland (ANCOVA:  $F_1 = 1.58$ ,  $P = 0.211$ ). There was, however, an interaction between incubator temperature and light environment of the natal pond (ANCOVA:  $F_1 = 7.47$ ,

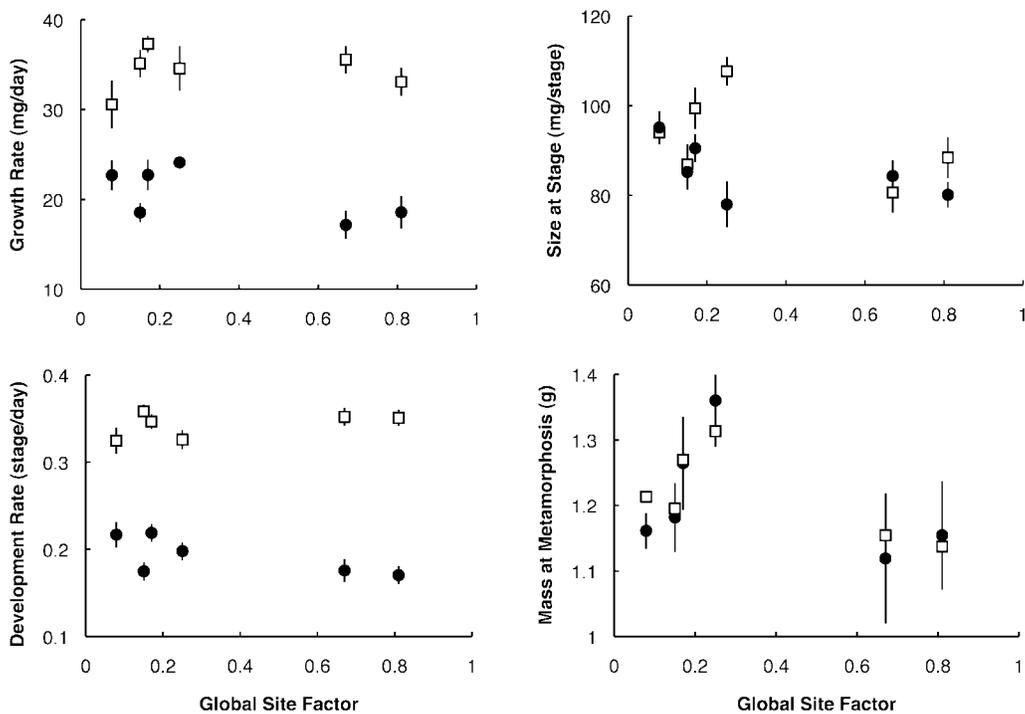


**Fig. 1.** Estimated wetland-wide growth rate, development rate, and size at stage index of *R. sylvatica* larvae sampled from six wetlands varying in light environment (global site factor) due to differences in overhead forest canopy. Estimated using linear regression of means of populations.

**Table 1.** Comparison of field and laboratory results

| Trait         | Study      | <i>n</i> | <i>R</i> <sup>2</sup> | <i>P</i> -value | Dark vs. Light |
|---------------|------------|----------|-----------------------|-----------------|----------------|
| Growth        | Field      | 6        | 0.216                 | 0.353           | -20%           |
|               | Laboratory | 140      | 0.691                 | 0.040           | +10%           |
| Development   | Field      | 6        | 0.390                 | 0.185           | -23%           |
|               | Laboratory | 140      | 0.847                 | 0.211           | +2.8%          |
| Size at stage | Field      | 6        | 0.277                 | 0.283           | -25%           |
|               | Laboratory | 140      | 0.098                 | 0.008           | +10%           |

*Note:* Field sample size (*n* = 6) comes from six wetlands for which wetland-wide traits were calculated, while laboratory sample size (*n* = 140) comes from 140 larvae for which individual traits were calculated. *P*-value refers to significance of global site factor (GSF) as a predictor of traits. Dark vs. Light refers to the percent difference in the means of traits between dark wetlands (GSF < 0.3) and light wetlands (GSF > 0.6).



**Fig. 2.** Larval traits (mean growth rate, development rate, size vs. stage index, and mass at metamorphosis,  $\pm 1$  standard error) of *R. sylvatica* larvae collected as recently laid embryos from six different wetlands of varying light environment (global site factor) and then laboratory-reared in low- and high-temperature treatments. Solid circles represent low-temperature treatment. Open squares represent high-temperature treatment.

$P = 0.007$ ). Larvae developed 83% faster in the high- than in the low-temperature treatment. When temperature treatments were analysed separately, light environment of the natal wetland had no effect on development at high temperature (Regression:  $R^2 = 0.023$ ,  $P = 0.221$ ) but had a significant effect at low temperature (Regression:  $R^2 = 0.089$ ,  $P = 0.017$ ). Low-temperature treatment wood frog larvae from dark wetlands developed on average 15% faster than those from the light wetlands (Fig. 2).

The index of size at stage was influenced by both temperature treatment (ANCOVA:  $F_1 = 5.67$ ,  $P = 0.019$ ) and by GSF (ANCOVA:  $F_1 = 7.33$ ,  $P = 0.008$ ), with no interaction between temperature and GSF (ANCOVA:  $F_1 = 1.45$ ,  $P = 0.230$ ). Larvae in the low-temperature treatment had an estimated 6.9% greater mass per stage than those in the high-temperature treatment. Larvae from dark wetlands had an average of 10% greater mass per stage than those from light wetlands (Fig. 2). Mass at metamorphosis was not significantly influenced by temperature treatment (ANCOVA:  $F_1 = 0.07$ ,  $P = 0.792$ ), although it was influenced by GSF (ANCOVA:  $F_1 = 4.08$ ,  $P = 0.046$ ), and there was no interaction between temperature treatment and GSF (ANCOVA:  $F_1 = 0.21$ ,  $P = 0.908$ ). Across incubator treatments, larvae from dark wetlands were on average 9.2% heavier at metamorphosis than those from light wetlands (Fig. 2).

## DISCUSSION

Field-collected wood frogs showed little evidence of systematic variation in growth rate, development rate or size at stage across a gradient of forest canopy above their natal wetlands. However, when animals from the same wetlands were brought into the laboratory and raised under common garden conditions, they exhibited strong evidence of countergradient variation. Growth rate, size at stage, and mass at metamorphosis each varied corresponding with the light environment of the natal wetland (as measured by the global site factor, GSF). Development rate varied in response to GSF at low but not high temperatures. These findings highlight the potentially cryptic nature of countergradient variation. Ecologists could easily dismiss the likelihood of underlying inherited variation when confronted with a pattern like that shown in Fig. 1. However, in this instance such a conclusion would miss an important underlying pattern in phenotypes directly linked to performance, as shown in Fig. 2.

Previous research on overhead forest canopy has revealed strong influences on larval amphibian populations (Skelly and Freidenburg, 2000; Freidenburg and Skelly, 2004). Canopy can mediate a variety of factors, including water temperature, which can affect performance of ectothermic animals. The effects of thermal variation in habitats on amphibian larval traits are well described (e.g. Smith-Gill and Berven, 1979; Berven, 1982; Harkey and Semlitsch, 1988; Loman, 2003). The results of this study are in line with these previous findings: a temperature increase of approximately 4°C between incubator treatments corresponded to a 73% increase in growth rate and an 83% increase in development rate. These treatments were designed to echo the variation in temperature between the coldest and warmest natural wetlands in our system (Halverson *et al.*, 2003). With such drastic differences in growth and development rates, wood frogs in colder wetlands face conditions that tend to reduce their ability to rapidly reach metamorphosis and complete development before their wetland dries.

When calculated over the entire larval period, wood frog size at stage index was 6.9% higher in the low-temperature treatment than in the high-temperature treatment, which supports previous work that found that size-specific larval development rates slow at higher

growth rates (Wilbur and Collins, 1973) and increased temperatures (Smith-Gill and Berven, 1979). However, by the time our animals reached metamorphosis, masses appeared to even out and temperature had no significant effect on mass at metamorphosis. Werner (1986) argues that mass at metamorphosis is highly dependent on population-level mortality rates. Because we housed animals individually, we minimized the possibility of plastic responses to density effects and may have seen a threshold for size at metamorphosis in the absence of density, diet, and temperature effects, as postulated by Wilbur and Collins (1973). Despite the lack of variation in mass at metamorphosis between temperature treatments, the GSF of wetland of origin had a strong effect on both size at stage index and mass at metamorphosis. This suggests that mass at metamorphosis is influenced by light environment, although the effect might not be due to temperature.

In addition to mediating wetland water temperature, canopy cover also affects the drying rate of wetlands. Despite their cool temperatures, shady wetlands often dry earlier than sunny wetlands (Skelly *et al.*, 1999). In common garden experiments, *Rana temporaria* from temporary ponds exhibited faster larval development rates than those from permanent ponds (Loman, 2003; Lind *et al.*, 2008). Growing period, as imposed by pond drying time, may also influence larval traits of wood frogs, as rapid drying of shady ponds can magnify the challenge already imposed by cool temperature conditions. As pond drying is hard to monitor and fluctuates greatly between years, we did not analyse drying rates of the ponds, although future research might consider this important factor.

Differences between selective mortality in the laboratory and field could have contributed to some of the differences seen between results. Laboratory conditions were highly controlled, with no competition or predation. Smaller, slower individuals raised to metamorphosis in the laboratory may not have survived in the field. Differences in density, predation, and nutrition between the ponds were not considered and could have contributed significantly to growth and development rates seen in the field. The complex effects and interaction of these environmental characteristics may be the reason why we found no clear relationship between canopy cover and larval traits in the field study, confounding the phenotypic expression of inherited variation.

Our findings provide strong support for the presence of inherited effects on the larval phenotype of wood frogs. These effects may be genetic or they may be non-genetic parental effects. In a previous study we investigated, but found no supporting evidence for, the possibility that egg size, a maternally determined trait, might affect embryonic developmental rate in wood frogs (Skelly, 2004). An independent study of wood frogs (Meier, 2007) found no supporting evidence of parental effects on larval growth and development in an experiment using half-siblings. Although this result might increase the likelihood of genetic differentiation, there is no direct evidence to support this hypothesis. Laugen *et al.* (2002) and Loman (2002) found both genetic and maternal contributions to larval life-history traits of ranids. If the Yale-Myers Forest breeding aggregations are genetically divergent, differentiation is happening at distances documented to be easily traversed by dispersing juvenile wood frogs (Berven and Grudzien, 1990). While dispersal does not necessarily entail gene flow, the high likelihood that wood frog individuals do move between neighbouring ponds (Newman and Squire, 2001) means that divergent selection would be required to maintain differentiation.

We consider the possibility of ecologically dependent reproductive isolation from individuals having higher fitness when breeding in habitats to which they are adapted (Hendry, 2004). It may be that migrants from wetlands with a different canopy environment have lower

fitness and survive or reproduce less than individuals native to that wetland or migrants from wetlands of similar environment. This immigrant inviability (Nosil *et al.*, 2005) could lead to divergence even if migration occurs between the studied wetlands. In fact, habitat selection itself could be an adaptive response to selection against migrants. We consider that dispersing individuals may select ponds with habitats similar to their natal habitat, which would again reinforce the observed divergence.

Cryptic inherited variation is likely to be important to ecologists and conservation scientists. If, as in this study, variation is related to thermal performance, variation in the ability to perform under different climatic conditions may be critical to understand and forecast the influence of changing climate. Such variation may also be important in maintaining threatened and endangered species and in the development of effective strategies for their recovery (Crandall *et al.*, 2000; Ficetola and De Bernardi, 2005). Biologists working to recover salmon populations have learned the fundamental importance of such variation in achieving conservation goals (reviewed in de Leaniz *et al.*, 2007). While relatively well understood in that system, the results of this study highlight the potential ubiquity of variation across environmental gradients, even when it is not readily evident from field sampling. We stress the importance of using experiments to further probe phenotypic patterns observed in the field, or the absence of phenotypic patterns where they might be expected, as cryptic inherited variation may underlie such observed trends.

#### ACKNOWLEDGEMENTS

Thanks to S. Bolden for help and guidance in the field and laboratory, W.A. Ligon for tireless assistance in the laboratory, and H. McMillan, J. Redman, R. Rodriguez, M. Enyeart, and S. Chan for help with fieldwork and tadpole care. Discussions with J. Richardson and S. Brady were helpful in designing the experiment. J. Reuning-Scherer and B. Adrio helped with statistical analyses. J. Richardson and M. Rogalski provided comments on previous versions of the manuscript. This research was supported by the Yale School of Forestry and Environmental Studies, the Trumbull College Richter Fellowship, and the Yale College Dean's Research Fellowship.

#### REFERENCES

- Alford, R.A. and Harris, R.N. 1988. Effects of larval growth history on anuran metamorphosis. *Am. Nat.*, **131**: 91–106.
- Arendt, J.D. and Wilson, D.S. 1999. Countergradient selection for rapid growth in pumpkinseed sunfish: disentangling ecological and evolutionary effects. *Ecology*, **80**: 2793–2798.
- Arnett, A.E. and Gotelli, N.J. 1999. Geographic variation in life-history traits of the Ant Lion, *Myrmeleon immaculatus*: evolutionary implications of Bergmann's rule. *Evolution*, **53**: 1180–1188.
- Berven, K.A. 1982. The genetic basis of altitudinal variation in the wood frog *Rana sylvatica* II. An experimental analysis of larval development. *Oecologia*, **52**: 360–369.
- Berven, K.A. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). *Ecology*, **71**: 1599–1608.
- Berven, K.A. 1995. Population regulation in the wood frog, *Rana sylvatica*, from 3 diverse geographic localities. *Austral. Ecol.*, **20**: 385–392.
- Berven, K.A. and Grudzien, T.A. 1990. Dispersal in the wood frog (*Rana sylvatica*): implications for genetic population structure. *Evolution*, **44**: 2047–2056.
- Caldwell, J.P., Thorp, J.H. and Jervey, T.O. 1980. Predator–prey relationships among larval dragonflies, salamanders, and frogs. *Oecologia*, **46**: 285–289.

- Conover, D.O. and Present, T.M.C. 1990. Countergradient variation in growth rate: compensation for length of the growing season among Atlantic silversides from different latitudes. *Oecologia*, **83**: 316–324.
- Conover, D.O. and Schultz, E.T. 1995. Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends Ecol. Evol.*, **10**: 248–252.
- Craig, K.J., Foote, C.J. and Wood, C.C. 2005. Countergradient variation in carotenoid use between sympatric morphs of sockeye salmon (*Oncorhynchus nerka*) exposes nonanadromous hybrids in the wild by their mismatched spawning colour. *Biol. J. Linn. Soc.*, **84**: 287–305.
- Crandall, K.A., Bininda-Emonds, O.R.P., Mace, G.M. and Wayne, R.K. 2000. Considering evolutionary processes in conservation biology. *Trends Ecol. Evol.*, **15**: 290–295.
- de Leaniz, C.G., Fleming, I.A., Einum, S., Verspoor, E., Jordan, W.C., Consuegra, S. *et al.* 2007. A critical review of adaptive genetic variation in Atlantic salmon: implications for conservation. *Biol. Rev.*, **82**: 173–211.
- Doebeli, M. and Dieckmann, U. 2003. Speciation along environmental gradients. *Nature*, **421**: 259–264.
- Endler, J.A. 1995. Multiple-trait coevolution and environmental gradients in guppies. *Trends Ecol. Evol.*, **10**: 22–29.
- Ficetola, G.F. and De Bernardi, F. 2005. Supplementation or *in situ* conservation? Evidence of local adaptation in the Italian agile frog *Rana latastei* and consequences for the management of populations. *Anim. Conserv.*, **8**: 33–40.
- Freidenburg, L.K. and Skelly, D.K. 2004. Microgeographical variation in thermal preference by an amphibian. *Ecol. Lett.*, **7**: 369–373.
- Gosner, D.J. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, **16**: 183–190.
- Halverson, M.A., Skelly, D.K., Kiesecker, J.M. and Freidenburg, L.K. 2003. Forest mediated light regime linked to amphibian distribution and performance. *Oecologia*, **134**: 360–364.
- Harkey, G.A. and Semlitsch, R.D. 1988. Effects of temperature on growth, development, and color polymorphism in the ornate chorus frog *Pseudacris ornate*. *Copeia*, **4**: 1001–1007.
- Hendry, A.P. 2004. Selection against migrants contributes to the rapid evolution of ecologically dependent reproductive isolation. *Evol. Ecol. Res.*, **6**: 1219–1236.
- Hendry, A.P. and Kinnison, M.T. 1999. The pace of modern life: measuring rates of contemporary microevolution. *Evolution*, **53**: 1637–1653.
- Kawecki, T.J. and Ebert, D. 2004. Conceptual issues in local adaptation. *Ecol. Lett.*, **7**: 1225–1241.
- Laugen, A.T., Laurila, A. and Merilä, J. 2002. Maternal and genetic contributions to geographical variation in *Rana temporaria* larval life-history traits. *Biol. J. Linn. Soc.*, **76**: 61–70.
- Laugen, A.T., Laurila, A., Rasanen, K. and Merilä, J. 2003. Latitudinal countergradient variation in the common frog (*Rana temporaria*) development rates – evidence for local adaptation. *J. Evol. Biol.*, **16**: 996–1005.
- Levins, R. 1969. Thermal acclimation and heat resistance in *Drosophila* species. *Am. Nat.*, **103**: 483–499.
- Lind, M.I., Persbo, F. and Johansson, F. 2008. Pool desiccation and developmental threshold in the common frog, *Rana temporaria*. *Proc. R. Soc. Lond. B*, **275**: 1073–1080.
- Loman, J. 2002. Microevolution and maternal effects on tadpole *Rana temporaria* growth and development. *J. Zool.*, **257**: 93–99.
- Loman, J. 2003. Growth and development of larval *Rana temporaria*: local variation and countergradient selection. *J. Herpetol.*, **37**: 595–602.
- Marquis, O. and Miaud, C. 2008. Variation in UV sensitivity among common frog *Rana temporaria* populations along an altitudinal gradient. *Zoology*, **111**: 309–317.
- Meier, P.T. 2007. Fine spatial scale phenotypic divergence in wood frogs (*Lithobates sylvaticus*). *Can. J. Zool.*, **85**: 873–882.
- Morrison, C. and Hero, J.M. 2003. Geographic variation in life-history characteristics of amphibians: a review. *J. Anim. Ecol.*, **72**: 270–279.

- Newman, R.A. and Squire, T. 2001. Microsatellite variation and fine-scale population structure in the wood frog (*Rana sylvatica*). *Mol. Ecol.*, **10**: 1087–1100.
- Nosil, T., Vines, T.H. and Funk, D.J. 2005. Perspective: Reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution*, **59**: 705–719.
- Reznick, D.N. and Ghalambor, C.K. 2001. The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. *Genetica*, **112/113**: 183–198.
- Rowe, L. and Ludwig, D. 1991. Size and timing of metamorphosis in complex life cycles: time constraints and variation. *Ecology*, **72**: 413–427.
- Semlitsch, R.D., Scott, D.E. and Pechmann, J.H. 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. *Ecology*, **69**: 184–192.
- Skelly, D.K. 2004. Microgeographic countergradient variation in the wood frog, *Rana sylvatica*. *Evolution*, **58**: 160–165.
- Skelly, D.K. and Freidenburg, L.K. 2000. Effect of beaver on the thermal biology of an amphibian. *Ecol. Lett.*, **3**: 482–486.
- Skelly, D.K., Werner, E.E. and Cortwright, S.A. 1999. Long-term distributional dynamics of a Michigan amphibian assemblage. *Ecology*, **80**: 2236–2337.
- Smith, D.C. 1987. Adult recruitment in chorus frogs: effects of size and date at metamorphosis. *Ecology*, **68**: 344–350.
- Smith, G.R., Ballinger, R.E. and Nietfeldt, J.W. 1994. Elevational variation of growth rates in neonate *Sceloporus jarrovi*: an experimental evaluation. *Funct. Ecol.*, **8**: 215–218.
- Smith-Gill, S.J. and Berven, K.A. 1979. Predicting amphibian metamorphosis. *Am. Nat.*, **4**: 563–585.
- Travis, J. 1983. Variation in growth and survival of *Hyla gratiosa* larvae in experimental enclosures. *Copeia*, **1983**: 232–237.
- Urban, M. 2004. Disturbance and heterogeneity determines freshwater metacommunity structure. *Ecology*, **85**: 2971–2978.
- Werner, E.E. 1986. Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. *Am. Nat.*, **128**: 319–341.
- Wilbur, H.M. and Collins, J.P. 1973. Ecological aspects of amphibian metamorphosis. *Science*, **182**: 1305–1314.
- Yamahira, K. and Takeshi, K. 2008. Variation in juvenile growth rates among and within latitudinal populations of the medaka. *Popul. Ecol.*, **50**: 3–8.

