

LETTER

Microgeographical variation in thermal preference by an amphibian

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Abstract

Ectotherms use behaviour to buffer effects of temperature on growth, development and survival. While behavioural thermoregulation is widely reported, localized adaptation of thermal preference is poorly documented. Larval amphibians live in wetlands ranging from entirely open to heavily shaded by vegetation. We hypothesized that populations undergo localized selection leading to countergradient patterns of thermal preference behaviour. Specifically, we predicted that wood frog (*Rana sylvatica*) larvae from closed canopy ponds would be more strongly temperature selective and would prefer higher temperatures than conspecifics from populations found in open canopy ponds. In a study of six breeding ponds in north-eastern Connecticut, USA, these predictions were upheld. The countergradient, microgeographical variation in thermal preference documented here implies that wood frog populations may have diverged rapidly in the face of contrasting selection pressures. Rapid, behaviourally mediated responses to changing thermal environments have important implications for understanding population responses to climate change.

Keywords

Amphibian, behaviour, countergradient variation, divergence, microgeography, thermal preference.

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INTRODUCTION

Among ectotherms, temperature is a critical driver of physiological processes and a potential threat to survival (Angiletta *et al.* 2002). By selecting particular microclimates within a spatially and temporally heterogeneous environment, ectotherms can behaviourally thermoregulate and thus buffer themselves from the negative effects of temperature on physiological performance and ecological interactions (Huey 1991). In recent years, evolutionary ecologists have speculated that behaviour related to temperature preference should be under strong selection (Angiletta *et al.* 2002). There is, however, relatively little evidence to support or dispute this contention. In a few cases, investigators have used geographical clines (latitude or altitude) to evaluate hypotheses related to evolutionary responses of thermal preferences within species (Ellner & Karasov 1993; Diaz 1997).

In the context of thermal preference, countergradient variation predicts that individuals from colder environments should show greater behavioural sensitivity to temperature and should select higher temperatures when presented with

a thermal gradient. Historically, countergradient variation has been considered a macrogeographic phenomenon (Futuyma 1998) in which variation in latitude or altitude drives variation in phenotypes (Berven 1982a,b; Schultz *et al.* 1996; Parsons 1997; Laugen *et al.* 2003). Documentation of countergradient variation at small spatial scales, however, has been scarce (see Skelly 2004 for an exception). Here, we extend the study of thermal preference by asking whether intraspecific variation in behaviour exists on microgeographical scales.

We have chosen to focus on larval amphibians. Tadpoles are typically deposited as embryos in small breeding ponds by their parents and must contend with an environment circumscribed by the pond shoreline. Surrounding terrestrial vegetation can overtop and deeply shade these aquatic environments. As shown below, shading by the vegetation canopy can profoundly alter the thermal environment of amphibian breeding ponds in a predictable manner. A recent body of work indicates that canopy-mediated shading also can significantly impact larval performance (Skelly *et al.* 2002; Halverson *et al.* 2003). Prior work has produced some evidence for localized adaptation of thermal tolerance and

temperature-specific development rate by the wood frog (Skelly & Freidenburg 2000; Skelly 2004).

The goal of this study was to determine if a behavioural trait, thermal preference, varies on a microgeographic scale, and if it does, whether this variation shows a countergradient pattern with respect to canopy-mediated thermal environments. The breeding distribution of the wood frog (*Rana sylvatica* LeConte) spans an enormous range of canopy environments (Skelly *et al.* 1999; Halverson *et al.* 2003), and wood frog tadpoles are known to demonstrate thermal preference (Herreid & Kinney 1967). Whether thermal preference differs at the population level is unknown. Wood frog larvae also are confronted with a time problem; they must grow and develop rapidly in order to metamorphose before pond drying. Given the strong relationship demonstrated between temperature and development and growth in this species (Smith-Gill & Berven 1979), we predicted that larvae from closed canopy ponds should be more selective and choose warmer water temperatures than larvae from open canopy ponds. These predictions were upheld in laboratory thermal preference trials using animals that had been collected from six natural breeding ponds as recently laid embryos and then reared in a common garden setting.

METHODS

Wood frogs were collected as recently laid eggs from six ponds (Shrub, Blacksmith, Kealoha's, Centrepik, Dentist, Morse Bog; see Fig. 1 in Skelly 2004) at Yale Myers Forest (3800 ha) in north-eastern Connecticut, USA. Ponds were selected because they were non-permanent, had resident wood frog populations, and because they represented contrasting degrees of tree canopy development above their basins. Canopy cover was indexed by global site factor (GSF) measured using hemispherical photos taken every 5 m along a Cartesian grid within the basin (Halverson *et al.* 2003). For each pond, GSF was based on an average value from two sets of photographs taken while leaves were on, and again while leaves were off trees (see Fig. 3, Skelly 2004, for GSF values). Three ponds had GSF values ≥ 0.75 (hereafter open canopied), and three ponds had GSF values ≤ 0.40 (hereafter closed canopied). A temperature logger placed 10 cm below the water surface and located at the deepest point of each pond recorded water temperature once per hour from 10 to 23 May. For each pond, we have at least 2 years of temperature records between 1998 and 2002.

On 14 April 2001, three recently laid (<36 h) egg masses were selected haphazardly from each pond and placed in plastic containers holding 8 L of water (well water aged >24 h was used throughout this study) and allowed to develop to hatching. Upon hatching, larvae were reared in

150 L plastic wading pools to which rabbit chow and deciduous leaf litter were added such that food was always available. Larvae were subsequently transferred to 1000 L plastic cattle watering tanks (one per pond) provided with rabbit chow and deciduous leaf litter as described above.

In order to provide a uniform environment under which to evaluate thermal behaviour, the thermal preference trials were conducted in a temperature controlled room (mean $^{\circ}\text{C} \pm \text{SE}$: 7.9 ± 0.4) lit by fluorescent fixtures on a 14 : 10 light : dark cycle. Larvae were placed in this room at least 48 h before they were used in a trial and were kept in 8 L plastic containers. Two plastic troughs (130 \times 7 cm, 2 cm water depth) served as experimental arenas. Troughs were divided into 26 sections, each 5 cm in length, by markings on the trough bottom. Placing one end of each trough in an ice bath and the other end on a hotplate 1 h before the trial created a temperature gradient. Temperature in each section was estimated by measuring to the nearest 0.1°C at each end and in the middle section at the onset, and again at the conclusion, of each trial. Practice trials confirmed that linear regression could be used to accurately estimate temperature in all sections based on the average temperatures in the measured sections. Across all trials, the cold end of the troughs averaged $7.9 \pm 0.2^{\circ}\text{C}$ and the warm end of the troughs averaged $29.9 \pm 0.3^{\circ}\text{C}$.

After allowing trough temperatures to equilibrate for 1 h, a trial was initiated by introducing 10 wood frog larvae to the middle section (section 13) of each trough. For each trial, a pair of ponds representing open and closed canopy treatments was used. The same pair of ponds was always used together during the experiment, and we alternated the trough assignment across trials; trial order was randomly determined. An 8 mm video camera (Sony Handycam, Sony Corporation, New York, NY, USA) suspended above the troughs recorded larval positions continuously. Filming began as soon as larvae were added and continued for 80 min. Larvae were then preserved (70% ethanol) and later measured (mm, snout-vent length) and staged (Gosner stage, Gosner 1960). Observations of larvae from each of the six ponds were replicated five times for a total of 15 trials. All trials were run between 08.00 and 17.00 hours.

In reviewing the videotapes of each trial, we set aside the initial 20 min after tadpole introduction for acclimation. During the remaining 60 min, we recorded the position and temperature (estimated by the linear regression for that trough) for each tadpole at 2-min intervals. For each trough, the response variable used in statistical analyses was the average temperature selected (denoted preferred temperature; Floyd 1984). This single number represents the average position of the tadpoles in the trough during the entire observation period. For each canopy type, we evaluated whether distribution of tadpoles was non-random by comparing preferred temperature with the average trough

temperature. Further, we evaluated differences in preferred temperature between open and closed canopy ponds using ANOVA followed by a *post hoc* linear contrast between canopy treatments.

RESULTS

Pond origin was related to thermal preference of larval wood frogs. Larvae from closed canopy ponds distributed themselves non-randomly within troughs, tending to select temperatures warmer than those expected by chance (paired *t*-test: d.f. = 14, $P < 0.001$; Fig. 1). By contrast, larvae from open canopy ponds exhibited distributions indistinguishable from those expected by chance (paired *t*-test: d.f. = 14, $P = 0.430$; Fig. 1). Correspondingly, preferred temperature varied among ponds (ANOVA: MS = 20.408; $F_{5,24} = 5.249$; $P = 0.002$) and was higher among closed canopied ponds ($20.4 \pm 0.3^\circ\text{C}$) than open canopied ponds ($17.6 \pm 1.2^\circ\text{C}$; *post hoc* linear contrast: $F_{1,24} = 14.463$, $P = 0.001$).

Open canopied ponds are warmer than closed canopied ponds (temperature logger data; Fig. 2). The preferred temperature of open canopied larvae is within the range of that observed within their natal ponds. The preferred temperature of closed canopied larvae is high relative to

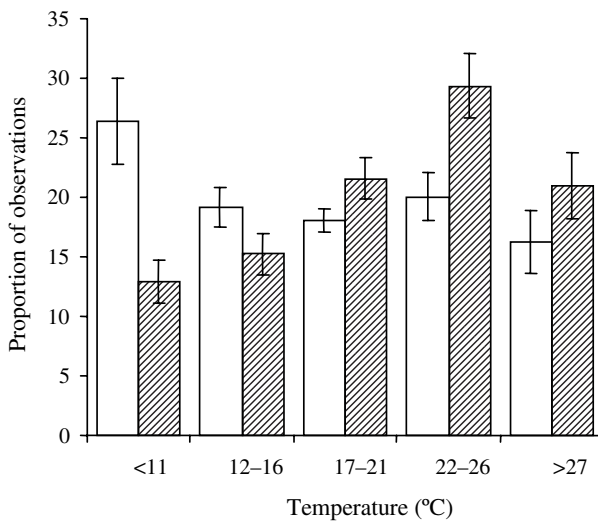


Figure 1 Distribution of preferred temperatures for wood frog tadpoles. Distributions were determined for each trial ($n = 15$) where the preferred temperatures of 10 tadpoles were recorded during 2-min intervals ($n = 30$) for a total of 300 observations per trial. Temperature bins were determined based on the available trough temperatures, and the proportion of tadpoles in each bin was determined for each trial. Open bars represent preferred temperatures for open-canopy tadpoles; shaded bars represent preferred temperatures for closed-canopy tadpoles. Error bars = 1SE and represent the variation in tadpole behaviour within a given pond type.

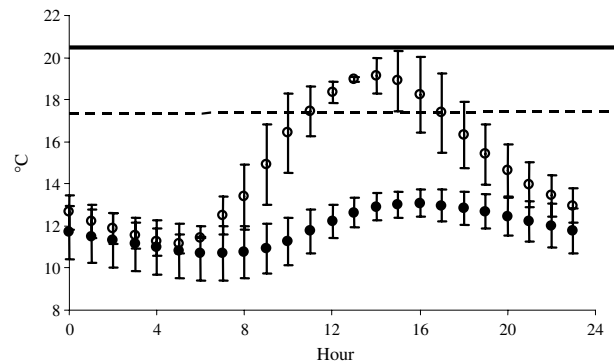


Figure 2 Water temperatures during a 2-week period in May (10 May–23 May). Temperatures were recorded once per hour in six ponds; the data represent 2 years of temperature data from each pond. Temperature profiles during a 24-hour period are shown for open-canopy ponds (○) and closed-canopy ponds (●). Error bars = 1SE. The solid line represents the preferred temperature of closed-canopy tadpoles in the laboratory, and the dashed line is the preferred temperature for open-canopy tadpoles.

water temperatures observed within their natal ponds. Overall, preferred temperature declined with increasing water temperature within the natal pond [linear regression: $n = 6$, preferred temperature = $32.96 - 1.01 \times (\text{field temperature})$; $R^2 = 0.75$, $P = 0.025$].

At the time of the preference trials, developmental stage and size of larvae differed among larvae with different pond origins (ANOVA: stage: $F_{5,24} = 6.173$, $P = 0.001$; size: $F_{5,24} = 11.418$, $P < 0.001$) and *post hoc* linear contrasts indicated that larvae collected as eggs from closed canopy ponds were slightly (*c.* 4%) larger and more developed than larvae collected as eggs from open canopy ponds (stage: $F_{1,24} = 7.245$, $P = 0.013$; size: $F_{1,24} = 7.418$, $P = 0.012$). As developmental stage can influence thermal preference of larval anurans (Sherman 1980; Floyd 1984; Dupré & Petranks 1985), we used linear regression analysis to determine if stage or size was related to preferred temperatures. Neither factor was significant (stage: $F_{1,28} = 0.749$, $r^2 = 0.03$, $P = 0.394$; size: $F_{1,28} = 1.056$, $r^2 = 0.04$, $P = 0.313$).

DISCUSSION

Larval amphibians inhabiting temporary ponds must grow and develop rapidly to reach metamorphosis before pond drying. The linkage between amphibian development and temperature is well known (Smith-Gill & Berven 1979), as is the tendency for amphibian larvae to prefer warmer water. Thus, the observation in this study that larval wood frogs are capable of detecting a thermal gradient and responding by selecting warmer water is not unexpected (Herreid & Kinney 1967). However, the fact that this behavioural

pattern is present in some populations and absent in others is surprising. More specifically, we found that wood frogs from heavily shaded, cooler ponds actively select warmer water while their conspecifics from open, warmer ponds did not discernibly select water temperature.

Collectively, our results show that thermal preference behaviour of larval wood frogs varies on small geographic scales in a countergradient manner. These findings are significant because they suggest that wood frogs may undergo rapid, localized behavioural adaptation to pond-scale thermal regimes. Distances among the ponds used in this study are relatively small (minimum = 213 m, median = 1576 m, maximum = 4216 m). Although adult wood frogs are known to traverse the interpond distances used in this study (Berven & Grudzien 1990), we have evidence that populations have diverged among the wetlands used in this study (Skelly 2004). Additionally, the canopy environments of the wetlands we study are known to have changed markedly during the last 40 years; afforestation has increased canopy cover in some ponds, and beaver re-introduction has dramatically reduced canopy cover in other ponds (Skelly & Freidenburg 2000).

While it is not difficult to imagine the advantages of thermal preference behaviour to larvae in a shaded pond dappled with sunflecks, what explains the absence of thermal preference among larvae in open canopied ponds? One possibility is that the thermal environment in sunlit ponds negates the advantage of seeking warmer microhabitats. In the laboratory, rates of growth and development of wood frogs and other amphibians saturate and then decline as temperature increases (Smith-Gill & Berven 1979). In some cases, high temperature regions of a sunny pond can even be dangerous to tadpoles, exceeding their critical thermal maximum (Skelly & Freidenburg 2000). In addition, open canopy ponds have much higher density and diversity of predators (Freidenburg 2003); in these environments, moving may be dangerous (Skelly 1994). It is possible that a lack of thermal selectivity facilitates avoidance of predation risk for tadpoles in open canopy ponds.

The pattern we uncovered suggests that animals can undergo rapid behavioural evolution in response to changing thermal environments over very small scales of space. Such responses have been the subject of speculation among scientists studying changes in global climate (Clarke 2003). However, researchers modelling responses of animals to climate change generally have not accounted for the possibility that natural selection may mitigate the effect of rising temperatures by altering thermal preference behaviour (Thomas *et al.* 2004). Localized adaptation to thermal environments may be more common than is generally realized. If this is true, then projections of the biotic impacts of climate change will need to be revised.

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