Evolutionary Ecology Research, 2003, **5**: 431–437

Thermal environment, survival and local adaptation in the common frog, *Rana temporaria*

Mats Olsson and Tobias Uller*

Department of Zoology, University of Gothenburg, Medicinaregatan 18, Box 463, 405 30 Gothenburg, Sweden

ABSTRACT

Natural selection of organisms results in differences between populations in response to environmental conditions – that is, local adaptation. Understanding heterogeneity at this level requires identification of the different selection pressures that cause this differentiation. Tadpoles of the common frog, *Rana temporaria*, from northern Sweden show superior growth in warmer incubation conditions than tadpoles from the south, and vice versa in cooler conditions. A plausible explanation for this is that the thermal optimum in the northern population has been shifted by selection to correspond better with the relatively warmer developmental conditions experienced in the north compared with the south (northern ponds are warmer because they are shallower and tend to heat more quickly following the melting of snow). To test this hypothesis, we performed an experiment in which frogs from both regions were allowed to develop in water temperatures representative of those in their natural breeding ponds and in those at the other climatic extreme. Tadpoles in the 'warm' (northern) part of the range had markedly reduced survival at low temperatures compared with those from the cooler region in the south, with a non-significant difference at relatively high temperatures. Thus, northern frogs appear to have evolved towards becoming high-temperature 'specialists', with local adaptation being upheld by hard selection on tadpole survival.

Keywords: local adaptation, *Rana temporaria*, survival, temperature.

INTRODUCTION

One of the central questions in evolutionary biology is to what extent natural populations have diverged in response to local environmental conditions – that is, local adaptation. The study of local adaptation is fundamental to modern biology for at least two reasons. First, it depicts an outcome of adaptive evolution. Secondly, it helps to establish whether populations are predominantly homogeneous and close to their evolutionary equilibria, or are small and genetically heterogeneous and relatively far removed from their adaptive optima. Consequently, local adaptation has received much interest from evolutionary biologists, with studies covering a wide range of taxa (e.g. Ayres and Scriber, 1994; Reznick

* Address correspondence to either author. e-mail: mats.olsson@zool.gu.se; tobias.uller@zool.gu.se

and Travis, 1996; Nagy and Rice, 1997; Bronikowski, 2000; Mousseau, 2000; Ståhlberg *et al.*, 2001). A conventional way of demonstrating local adaptation is to perform a common garden experiment in which a trait correlated with fitness is measured in organisms from different environments. Alternatively, reciprocal transplants of organisms between environments can be monitored with respect to the target trait. Unfortunately, comparing two populations (or two species) cannot unequivocally prove local adaptation, as genetic drift could account for the differences between populations (Garland and Adolph, 1994). However, differences between populations cannot be predicted by evolutionary inference from an adaptive perspective (although they may differ to some extent depending on drift).

The common frog, *Rana temporaria*, is one of the most widely distributed amphibians in the world (Gasc *et al.*, 1997). It occurs in a broad range of habitats from sea level to alpine altitudes and is, therefore, well suited for studies of local adaptation. We recently found that tadpoles from the northern and southern parts of Sweden have diverged with respect to optimal thermal conditions for growth, development and performance (Ståhlberg *et al.*, 2001; Olsson and Uller, 2002). This suggests genetic differentiation on a local scale in support of local adaptation to different climatic conditions. However, if these differences in thermal optima from different parts of the range are due to adaptive genetic differentiation, they could have evolved as a corollary to temperature-related survival of tadpoles in their natural breeding ponds. We tested this proposition in a common garden experiment in which tadpoles from both regions were kept at water temperatures paralleling those in the wild. We then monitored how selection modified the relative frequencies of tadpoles from different local environments in three temperature conditions.

MATERIALS AND METHODS

Ideally, we would have tested for differences in water temperature between the two sampling areas throughout a prolonged period of ongoing selection. However, since this was not logistically feasible, we used air temperatures (mean temperature per 24 h) collected by the Swedish Meteorological and Hydrological Institute over a 10-year period less than 14 km from each sampling site and at the corresponding altitudes. These data show large differences in minimum, maximum and mean temperatures between sampling sites during the relevant periods of larval development in natural populations (*c.* 22 March and 7 weeks onward and 22 May and 7 weeks onward in the south and north, respectively; see the legend to Fig. 1 for statistics). Although air temperature is not a perfect estimate of water temperature in each region, it is a conservative measure of the differences in thermal regime between populations because frogs at the northern localities breed in relatively shallow ponds that show a steeper increase in water temperature than ponds in the south (T. Uller, personal observation; J. Elmberg, personal communication; Ståhlberg *et al.*, 2001).

Frog spawn from 47 females from the southern site ('cool' site, Angered, 58°45′N, 12°05′E) and from 47 females from the northern site ('warm' site, Ammarnäs, 65°55′N, 16°15′E) was collected in early April and late May, respectively. Three breeding ponds were sampled at the 'cool' site and two were sampled at the 'warm' site. Eggs had been in their natural ponds for 0–3 days before collection, with a mean temperature at collection of 8.8°C in the southern populations and 13.3°C in the northern populations. The spawn was kept at 4°C for 3 days, including transport at both sampling sites. A pool system was arranged

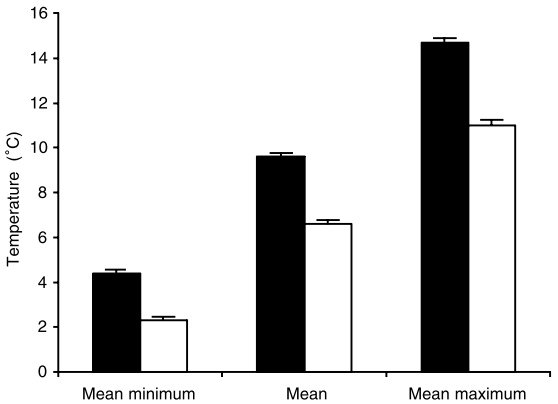

Fig. 1. Mean, mean maximum and mean minimum temperatures (± standard error) for the relevant periods (see Materials and Methods). All differences were highly significant (mean: $t = 12.4$, $P \ll 0.0001$; mean maximum: $t = 11.8$, $P \ll 0.0001$; mean minimum: $t = 8.6$, $P \ll 0.0001$; $n_{north} = 510$, $n_{south} = 464$). ■, north; □, south.

with two replicate pools (A and B; $152 \times 122 \times 25$ cm) for each of three water temperature treatments (10, 15 and 20°C). The temperatures were selected to cover an overlap in temperature regime experienced in natural populations, but with relatively colder temperatures being more common in the southern population and vice versa (Ståhlberg *et al.*, 2001; T. Uller, personal observation; J. Elmberg, personal communication; Fig. 1).

Ninety-four one-litre plastic containers with wire mesh bottoms (to ensure water circulation) were hung from crossbars in all six pools. Each container received 15 frog eggs sampled haphazardly from the original spawn sample; that is, 90 eggs per female were separated into six samples, allocated to two replicates (A and B) for each temperature condition. Water was aerated for a minimum of 4 days before the start of the experiment and Aqutan water conditioner (Sera, Heinsberg, Germany) was added to complex bind any heavy metals and to avoid their potentially detrimental effects. The water was cooled using a custom-made cooling system and heated to the pre-selected temperatures using commercially available, submersible, 300 W aquarium heaters. The water temperatures fluctuated no more than ± 1°C throughout the experiment and water levels were kept constant using a custom-made tap system, fine-tuned to equilibrium flow levels before the onset of the experiment. The room was set to a 14:10 light:dark photoperiod. The tadpoles were fed commercially available fish food (Sera San, manufactured by Sera, Heinsberg, Germany) *ad libitum* with waste food and tadpole excrement being removed continuously by a high-capacity Eheim filter.

Fifteen days after hatching, we counted the number of surviving tadpoles in each of a female's two containers per thermal treatment and used the average of the two containers as her temperature-specific mortality score expressed as the mean absolute number of tadpoles that died in the interval. Because of time constraints, we could not continue the experiment in full beyond this point but kept the 10°C treatment going for another 15 days, when another count of surviving tadpoles was made. Because the number of dead tadpoles per treatment was not normally distributed, we used Pitman's permutation test (Pitman, 1937a,b) to establish differences in treatment-specific survival between the warm and cool regions.

RESULTS

The results of our mortality analysis revealed that tadpoles from the north had the highest mortality in the 10°C condition, whereas southern tadpoles had a somewhat higher mortality in the 15°C condition (Fig. 2). Cool and warm region tadpoles differed significantly on three of four mortality scores (Table 1). After 15 days of development, nearly three times as many warm (north) as cool (south) tadpoles had died in the 10°C treatment (Fig. 2a). This difference was reinforced 15 days later when 10 and 2 tadpoles from the warm and cool regions, respectively, had died on average (Fig. 2b). A similar pattern was apparent at 15°C (Fig. 2c), whereas at 20°C the difference was not statistically significant (Fig. 2d).

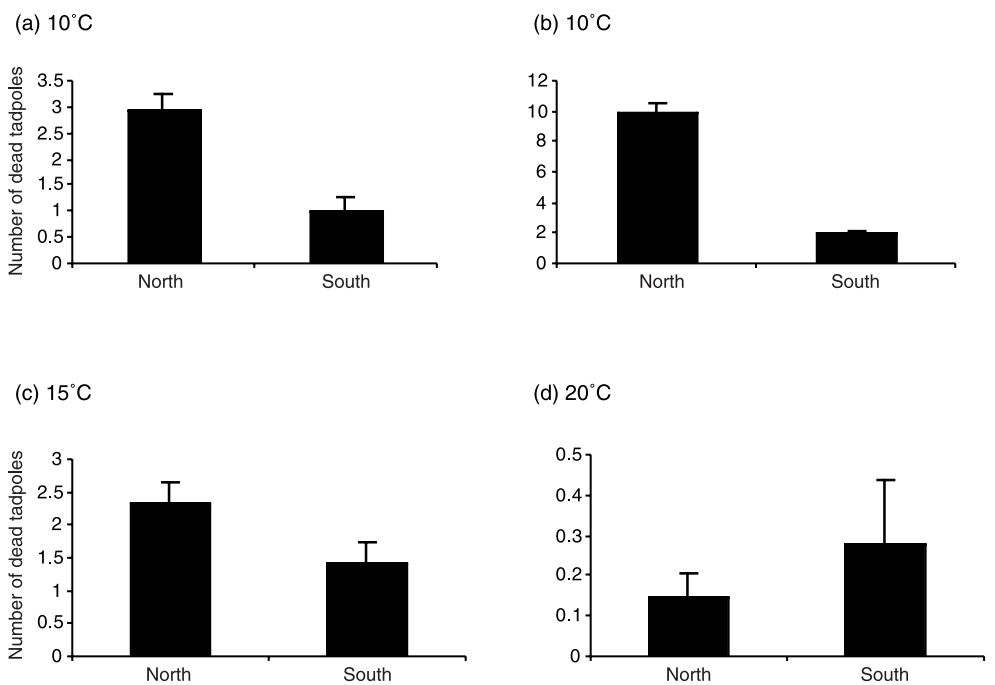

Fig. 2. Mortality (± standard error) expressed as the absolute number of dead tadpoles at a given temperature for each sampling region. (a) 10°C after 15 days; (b) 10°C after 30 days; (c) 15°C after 15 days; (d) 20°C after 15 days. North = 'warm'; south = 'cold'.

Table 1. Results of Pitman's permutation tests

Treatment	Minimum	Maximum	Mean	std	Observed	*P*
10°C[a]	0	164.9	82.4	11.33	54.3	0.0119
10°C[b]	0	463.4	231.7	33.93	28.1	<0.00001
15°C	0	127.3	63.7	10.31	88.5	0.0124
20°C	0	21.0	10.4	4.09	7.0	0.29

Note: Bonferroni-corrected *P*-values were obtained by multiplying by four. [a] After 15 days; [b] after 30 days. std = standard error.

DISCUSSION

Previous work on the Swedish common frog, *Rana temporaria*, has shown that populations have diverged with respect to temperature optima for growth and performance. Frogs from southern populations have tadpoles that grow faster, and with less variation in growth rate, at relatively lower temperatures, whereas warm-selected frogs show corresponding superior growth at higher temperatures (Ståhlberg *et al.*, 2001; Olsson and Uller, 2002; Uller *et al.*, 2002).

Although local adaptation may appear a well-established phenomenon, evidence in its favour is most often restricted to demonstrating physiological outcomes of a history of selection, such as environment-dependent growth rate (e.g. Bronikowski, 2000; Kjartansson *et al.*, 2001; Ståhlberg *et al.*, 2001). However, authorities in this field have recently stressed that much might be gained from studying the selection process *per se* to determine which selection pressures result in the observed variation in phenotypic traits (Badyaev *et al.*, 2000; Hoffmann *et al.*, 2001). Unfortunately, such work is exceedingly rare, in particular on vertebrates. Our previous work, for example, suggests that in *R. temporaria*, temperature-dependent selection acts primarily via growth and its correlated fitness effects later in life (such as increased fecundity from a larger body size). However, in the present study, we also demonstrated that tadpoles are likely to be under hard selection (*sensu* Haldane, 1957) from temperature-dependent survival, and hence physiological processes such as growth rate may not necessarily be the direct target of selection.

All physiological processes are temperature-dependent, with development best proceeding at a species- and/or population-specific optimum, with the organism being progressively constrained from optimal development with increasing deviation from this optimal temperature (e.g. Somero, 1978; Huey and Kingsolver, 1989). Thus, if the shape of the reaction norm is constrained, when selection pushes a thermal developmental optimum to a higher temperature, the corresponding effect is an elevation of the minimum temperature at which normal development proceeds. Although there is evidence of trade-offs between performance at different temperatures, the results are ambiguous. For example, lineages of *Escherichia coli* cultured at different temperatures showed improved fitness at their own temperature after 2000 generations, but only some lineages showed a reduction in fitness at other temperatures (Bennett *et al.*, 1992; Bennett and Lenski, 1993). Furthermore, the association between trade-offs at other temperatures was asymmetrical with respect to the temperature of adaptation, with lineages cultured at relatively low temperatures showing more pronounced fitness loss at higher temperatures than vice versa (Bennett and Lenski, 1993; Mongold *et al.*, 1996).

In accordance with evolutionary predictions, northern (warm-region) tadpoles had significantly higher mortality than populations from the relatively colder (southern) region (Table 1, Fig. 2). Although the corresponding difference at relatively high temperatures was non-significant, two lines of evidence suggest that warm-selected frogs not only have a lower ability to develop in cool conditions compared with cold-selected tadpoles, but perhaps also have an increased ability to develop under warmer conditions. First, the ratios in mortality between the regions in the 10, 15 and 20°C treatments at 15 days were 1:2.9, 1:1.6 and 1.9:1, respectively – that is, almost a complete reversal of the relative mortality rates with increasing temperature. Second, prolonged exposure to 10°C strongly exaggerated the difference in mortality between warm- and cold-selected frogs at 10°C, suggesting that prolonged exposure to 20°C is likely to have highlighted a significant difference in heat

tolerance, with warm-region tadpoles demonstrating not only inferior cold tolerance, but also improved heat tolerance.

In conclusion, our results show that *R. temporaria* tadpoles from different parts of the distribution range show patterns in mortality rate that covary with their thermal selection history. Thus, local adaptation may be achieved in part by selection on survival.

ACKNOWLEDGEMENTS

We are grateful to Johan Elmberg for providing us with information on the northern *R. temporaria* populations and to Raymond Huey for constructive comments on the manuscript.

REFERENCES

Ayres, M.P. and Scriber, J.M. 1994. Local adaptation to regional climates in *Papilio canadensis* (Lepidoptera: Papilionidae). *Ecol. Monogr.*, **64**: 465–482.

Badyaev, A.V., Hill, G.E., Stoehr, A.M., Nolan, P.M. and McGraw, K.J. 2000. The evolution of sexual size dimorphism in the house finch. II. Population divergence in relation to local selection. *Evolution*, **54**: 2134–2144.

Bennett, A.F. and Lenski, R.E. 1993. Evolutionary adaptation to temperature II. Thermal niches of experimental lines of *Escherichia coli. Evolution*, **47**: 1–12.

Bennett, A.F., Lenski, R.E. and Mittler, J.E. 1992. Evolutionary adaptation to temperature. I. Fitness responses of *Escherichia coli* to changes in its thermal environment. *Evolution*, **46**: 16–30.

Bronikowski, A.M. 2000. Experimental evidence for the adaptive evolution of growth rate in the garter snake *Thamnophis elegans. Evolution*, **54**: 1760–1767.

Garland, T., Jr. and Adolph, S.C. 1994. Why not to do two-species comparative studies: limitations on inferring adaptation. *Physiol. Zool.*, **67**: 797–828.

Gasc, J.P., Cabela, A., Crnobrnja-Isailovic, J. *et al.* 1997. *Atlas of Amphibians and Reptiles in Europe*. Paris: Societas Europaea Herpetological and Muséum National d'Histoire Naturelle.

Haldane, J.B.S. 1957. The cost of natural selection. *J. Genet.*, **55:** 511–524.

Hoffmann, A.A., Hallas, R., Sinclair, C. and Mitrovski, P. 2001. Levels of variation in stress resistance in *Drosophila* among strains, local populations, and geographic regions: patterns for desiccation, starvation, cold resistance, and associated traits. *Evolution*, **55**: 1621–1639.

Huey, R.B. and Kingsolver, J.G. 1989. Evolution of thermal sensitivity of ectotherm performance. *Trends. Ecol. Evol.*, **4**: 131–135.

Kjartansson, I.A., Foss, A., Naevdal, G. and Stefansson, S.O. 2001. Selection or adaptation: differences in growth performance of juvenile turbot (*Scophthalmus maximus* Rafinesque) from two close-by localities off Norway. *Sarsia*, **86**: 43–51.

Mongold, J.A., Bennett, A.F. and Lenski, R.E. 1996. Evolutionary adaptation to temperature IV. Selection at a niche boundary. *Evolution*, **50**: 35–43.

Mousseau, T.A. 2000. Intra- and interpopulation genetic divergence: explaining the past and predicting the future. In *Adaptive Genetic Variation in the Wild* (T.B. Mousseau, B. Sinervo and J. Endler, eds), pp. 219–250. New York: Oxford University Press.

Nagy, E.S. and Rice, K.J. 1997. Local adaptation in two subspecies of an annual plant: implications for migration and gene flow. *Evolution*, **51**: 1079–1089.

Olsson, M. and Uller, T. 2002. Developmental stability and genetic architecture: a comparison within and across thermal regimes in tadpoles. *J. Evol. Biol.*, **15**: 625–633.

Pitman, E.J.G. 1937a. Significance tests which may be applied to samples from any population. *J.R. Statist. Soc. Suppl.*, **4**: 119–130.

Pitman, E.J.G. 1937b. Significance tests which may be applied to samples from any populations. II. The correlation test. *J.R. Statist. Soc. Suppl.*, **4**: 225–232.

Reznick, D.N. and Travis, J. 1996. The empirical study of adaptation in natural populations. In *Adaptation* (M.R. Rose and G.V. Lauder, eds), pp. 243–290. San Diego, CA: Academic Press.

Somero, G.N. 1978. Temperature adaptation of enzymes: biological optimization through structure–function compromises. *Annu. Rev. Ecol. Syst.*, **9**: 1–20.

Ståhlberg, F., Olsson, M. and Uller, T. 2001. Populational divergence of developmental optima in Swedish common frogs, *Rana temporaria. J. Evol. Biol.*, **14**: 755–762.

Uller, T., Olsson, M. and Ståhlberg, F. 2002. Variation in heritability of tadpole growth: an experimental analysis. *Heredity*, **88**: 480–484.