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Developmental threshold model challenged by temperature

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ABSTRACT

Question: Does the L-shape of the reaction norm for age and size at ontogenetic transitions arise irrespective of temperature? The developmental threshold model suggests that this shape arises due to a threshold in body size to undertake the transition.

Data studied: Ages and sizes at metamorphosis measured in a common garden experiment in *Rana temporaria* originating from six populations along a latitudinal gradient. Individuals were exposed to three temperature treatments and two levels of food.

Search method: Population-specific age and size at metamorphosis were plotted against each other, and the shape of the reaction norm across populations was further illustrated by an exponential decay function. The observed patterns were further explored via linear mixed-effect models.

Conclusion: The L-shape of the reaction norm depends on temperature, suggesting that at low temperature a developmental threshold in age rather than in size regulates the process of metamorphosis.

Keywords: development, growth, metamorphosis, *Rana temporaria*, temperature, threshold size.

INTRODUCTION

Age and body size at ontogenetic life-history transitions, such as at metamorphosis and maturation, are important determinants of fitness in many organisms and therefore play a fundamental role in the evolution of life histories (Roff, 1992; Stearns, 1992). Consequently, knowledge about how environment influences the age and size at an ontogenetic transition is essential for understanding the patterns of selection and adaptive evolution in natural populations, and a large number of empirical (for reviews, see Berrigan and Charnov, 1994; Nylin and Gotthard, 1998; Morey and Reznick, 2000) and theoretical (e.g. Wilbur and Collins, 1973; Rowe and Ludwig, 1991; Berrigan and Koella, 1994; Abrams *et al.*, 1996; Hentschel, 1999) studies have addressed this issue.

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A common observation in studies of wild populations is that fast-growing individuals make ontogenetic transitions earlier and at larger body sizes than slow-growing individuals, which transit later and at smaller sizes (e.g. Berrigan and Charnov, 1994). The developmental threshold model of Day and Rowe (2002) provides an explanation for this. The model suggests that the observed pattern arises as a consequence of the minimum body size required to make the transition, which can be due to either physical limitations or energetic costs of the transition, and fitness benefits associated with larger body sizes. In practice, slow-growing individuals require more time to reach the minimum size, and no immediate gains in body size would be obtained by further postponing the transition (Day and Rowe, 2002). In contrast, fast-growing individuals can reach the minimum size quickly, but for them it also pays to postpone the transition and gain a larger body size. This leads to an L-shaped reaction norm for age and size at a transition, where the asymptotic lowest body size depicts the developmental threshold size for the transition (Day and Rowe, 2002).

To test the predictions of the developmental threshold model, recent empirical studies have looked at age and size at metamorphosis in the common frog, *Rana temporaria* (Lind *et al.*, 2008), and at maturation in soil mites, *Sancassania berseley* (Plaistow *et al.*, 2004). In both of these studies, the predicted L-shaped reaction norm arose when growth rates were manipulated with food treatments, suggesting that the process of metamorphosis/maturation is indeed regulated by the developmental thresholds (Plaistow *et al.*, 2004; Lind *et al.*, 2008). What is conspicuously lacking, however, is empirical knowledge about the persistence of the L-shaped reaction norm across different temperature regimes. Developmental processes can be affected by temperature not only through somatic growth but also directly (e.g. Van der Have and De Jong, 1996; Dhillon and Fox, 2004), so that even though at certain temperatures the developmental threshold size may regulate the occurrence of metamorphosis/maturation, it is not readily clear that this would hold across a range of temperatures. Here we use a previously collected data set (Laugen *et al.*, 2003, 2005) to address this question by estimating the shape of the reaction norm for the age and size at metamorphosis in *R. temporaria* over a range of temperatures.

METHODS

Data on the age and size at metamorphosis were obtained from *R. temporaria* originating from six populations located along an approximately 1500-km latitudinal gradient (55°40′ to 69°04′N) in Fennoscandia. The full experimental set-up is described in Laugen *et al.* (2003, 2005), but the most important details are given below. Tadpoles were produced by crossing adults collected from the wild in a factorial half-sib mating design in which each male and female was mated with multiple females and males, respectively (for details, see Laugen *et al.*, 2003). Eggs and tadpoles were reared in a common garden experiment in which individuals from each of the 8–16 maternal families (for details, see Laugen *et al.*, 2005) were randomly allocated to one of three temperature treatments (14°C, 18°C, and 22°C) and two food regimes (restricted and *ad libitum*) in a full-factorial design. The temperature treatments are within the range these populations experience in nature (Laugen *et al.*, 2003; A. Laugen, A. Laurila and J. Merilä, unpublished data). Tadpoles were fed a ground mixture of fish flakes and rabbit pellets and received a change of water (dechlorinated tap water) every 7 days. Tadpoles ($N = 4708$) were reared to metamorphosis, at which time their age [time elapsed between Gosner stages 25 and 42 (Gosner, 1960)] and weight (to the nearest 10^{-3} g) were recorded.

To first explore the pattern between age and weight at metamorphosis under restricted and *ad libitum* food conditions, these variables under the two food regimes were plotted against each other separately for each population and each temperature treatment (Fig. 1). To further investigate the general shapes of the reaction norms associated with the temperature and food treatments across the range of genotypes present in the experiment, we pooled the observations over the six populations. For illustrative purposes, we also fitted a negative exponential decay function to the pooled data as done previously (Plaistow *et al.*, 2004; Lind *et al.*, 2008):

$$\text{weight at metamorphosis} = a + b \times e^{-c \times \text{age at metamorphosis}} \tag{1}$$

The function was fitted to the observed ages and sizes at metamorphosis through a non-linear least squares regression.

The effects of temperature and nutrition on the process of metamorphosis were also investigated statistically by accounting for possible differences among populations and correlations arising from family structuring. To this end, log-transformed ages and weights at metamorphosis were analysed with linear mixed-effect models, by treating the temperature treatment, the food regime, and their interaction as well as the latitude as fixed effects, and family nested in population as random effects. Furthermore, we estimated coefficients of variation (i.e. mean/standard deviation) for age and weight at metamorphosis for each population and treatment combination. The coefficients of variation were then analysed with a linear mixed-effect model, with food regime, temperature treatment, and latitude as fixed effects and population as a random effect. All analyses were performed in R 2.10.1 (R Development Core Team, 2009).

RESULTS

In all six study populations, slow-growing tadpoles metamorphosed later and at a lower weight at 18°C and 22°C, whereas fast growth led to an early metamorphosis at a higher weight, yielding the predicted L-shaped reaction norms for age and weight at metamorphosis (Fig. 1). In contrast, at 14°C the food regime appeared to influence only the weight at metamorphosis so that tadpoles fed *ad libitum* metamorphosed at higher weights than those under the restricted diet, yet the age at metamorphosis under the two food treatments was similar (Fig. 1). Notably, at 14°C tadpoles also metamorphosed substantially later and at much higher weights than in the other two temperature treatments (Fig. 1). The same patterns seen within each individual population were seen in the shapes of the reaction norms estimated across the range of genotypes present in the experiment and in the shape of the fitted exponential decay functions (Fig. 2). In the 22°C treatment, estimates for the curve parameters (± S.E.) were $a = 0.21$ (±0.03), $b = 0.25$ (±0.03), and $c = 0.07$ (±0.02), and in the 18°C treatment, $a = 0.27$ (±0.02), $b = 1.25$ (±0.15), and $c = 0.11$ (±0.01). Due to the lack of an L-shaped pattern in the ages and weights at metamorphosis, the exponential decay function could not be fitted to the observations made in the 14°C treatment.

The effect of growth conditions on the age at metamorphosis being provisional on temperature was reflected not only in the main effects of temperature ($F_{2,4515} = 26117.1$, $P < 0.001$) and food regime ($F_{1,4515} = 1153.5$, $P < 0.001$) but also by their interaction ($F_{2,4515} = 346.5$, $P < 0.001$) in the linear mixed model. In addition, latitude was found to be negatively correlated with age at metamorphosis ($F_{1,4} = 63.0$, $P < 0.001$). In general, the model clearly showed that in all six populations age at metamorphosis was reduced by *ad libitum* food at 18°C and 22°C but not at 14°C (Fig. 3a). Population-specific variations in the

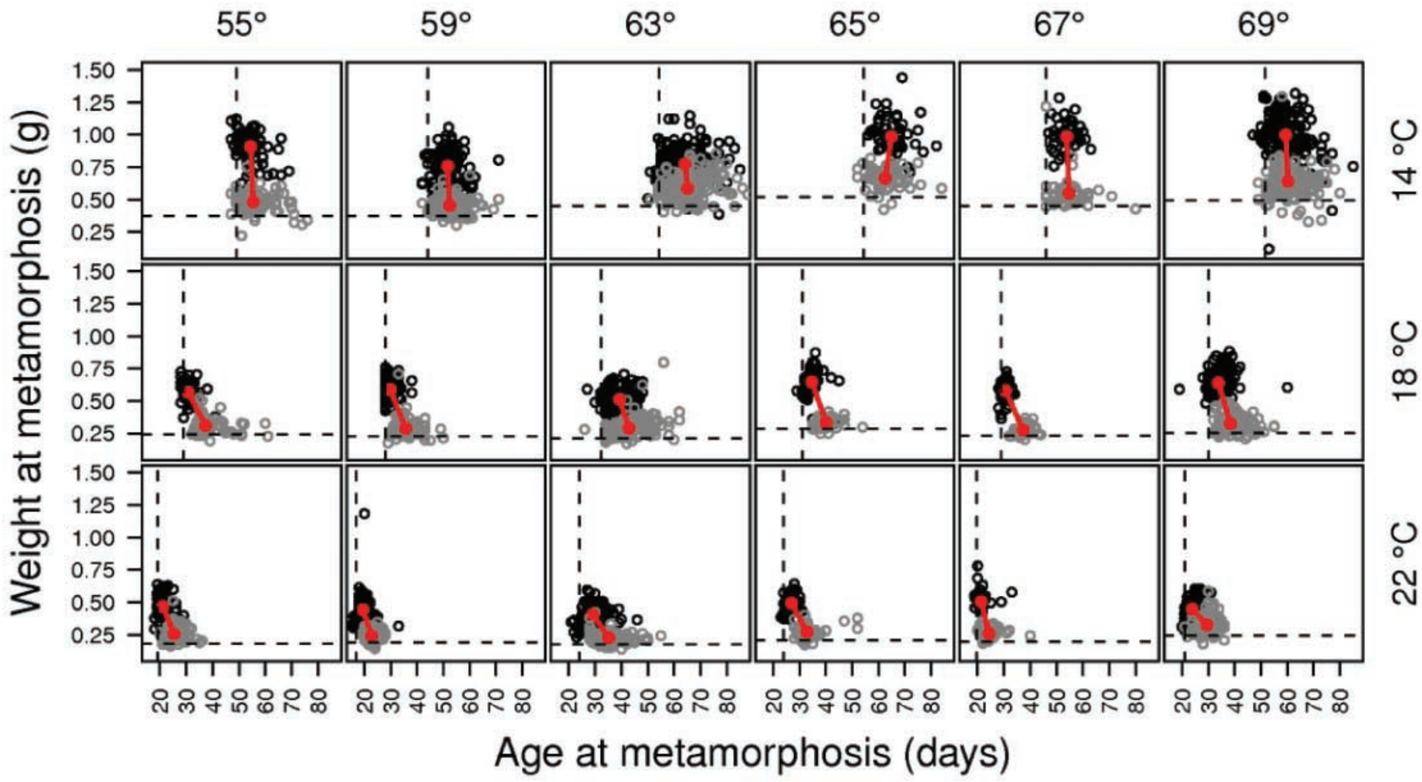

Fig. 1. Age and weight at metamorphosis observed in six *R. temporaria* populations located along a latitudinal cline (panel headers) in temperature treatments of (a) 22°C, (b) 18°C, and (c) 14°C, under restricted (grey symbols) and *ad libitum* (black symbols) diet regimes. The shift in age and weight at metamorphosis associated with a change in growth conditions is illustrated through the treatment-specific averages (red dots) connected with a line. Dashed lines are drawn at 2.5% quantiles of the ages and weights. Each point represents an individual observation.

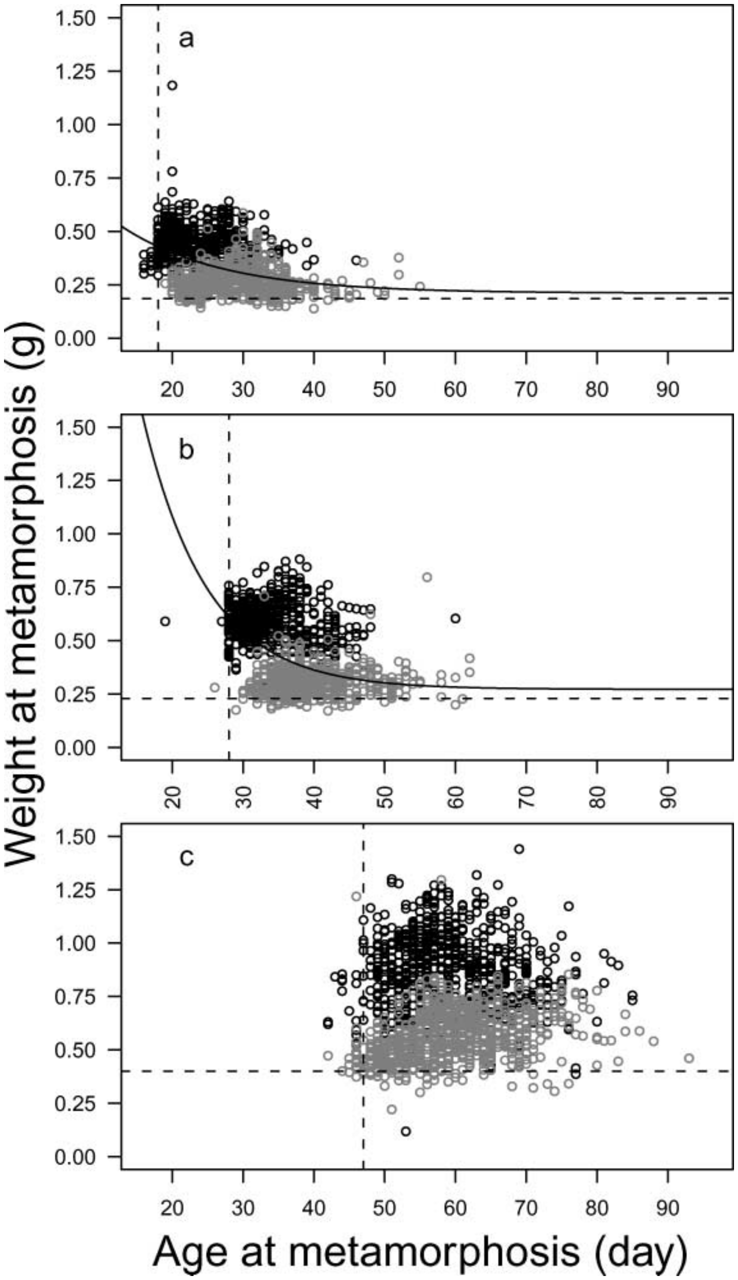

Fig. 2. Reaction norms for age and weight at metamorphosis observed across six *R. temporaria* populations in temperature treatments of (a) 22°C, (b) 18°C, and (c) 14°C, under restricted (grey symbols) and *ad libitum* (black symbols) diet regimes. In panels (a) and (b) shapes of the reaction norms were estimated with non-linear regressions. Dashed lines are drawn at 2.5% quantiles of the ages and weights. Lowest population-specific weights at metamorphosis were 0.14–0.18 g, suggesting that the physical size threshold for metamorphosis was at least this low in *R. temporaria*.

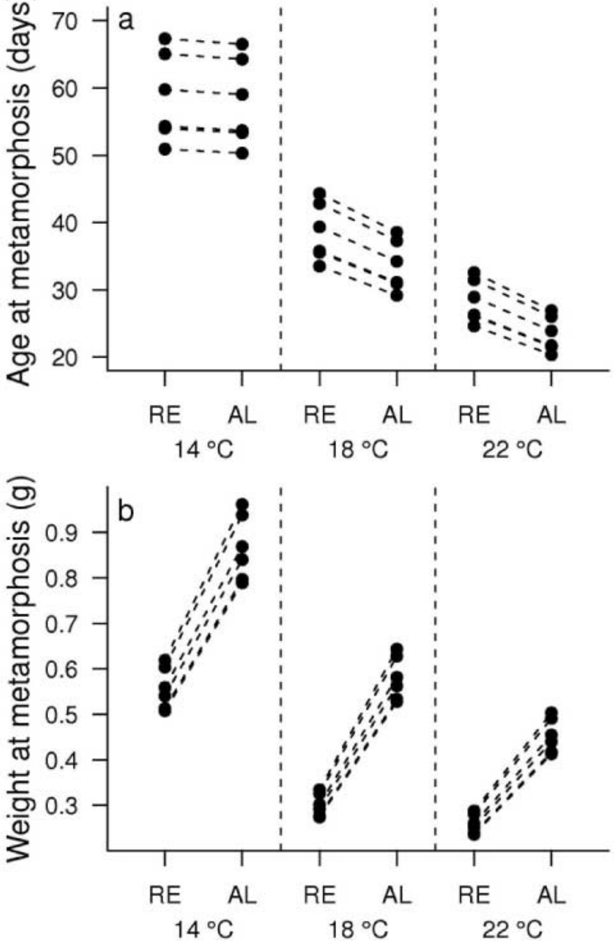

Fig. 3. Population-specific (a) age and (b) weight at metamorphosis predicted by the linear mixed-effect model under each temperature treatment (14°C, 18°C, 22°C) and food regime (RE = restricted, AL = *ad libitum*). Predictions for the same population under restricted and *ad libitum* feeding conditions are connected with a dashed line to illustrate the shift induced by good versus poor growth conditions.

development rate (Fig. 3a) were largely but not entirely explained by latitude, so that variance component estimates for the population and the family effects accounted for 7.9% and 12.3% of phenotypic variance, respectively. Similarly, weight at metamorphosis was significantly affected by temperature ($F_{2,4515} = 7563.3$, $P < 0.001$), food regime ($F_{1,4515} = 13147.7$, $P < 0.001$), and their interaction ($F_{2,4515} = 165.7$, $P < 0.001$), such that weight at metamorphosis was increased by *ad libitum* food and by lower temperatures in all populations (Fig. 3b). Latitude had no effect on weight at metamorphosis ($F_{1,4} = 0.08$, $P = 0.798$). Variance components for the population and family accounted for 22.2% and 5.3% of the total variance, respectively.

The coefficient of variation for weight at metamorphosis was not affected by food regime ($F_{1,27} = 1.14$, $P = 0.296$), temperature ($F_{2,27} = 1.68$, $P = 0.206$) or latitude ($F_{1,4} = 0.0004$,

$P = 0.937$), and only 1.2% of the variation was associated with population. In turn, the coefficient of variation for age at metamorphosis was negatively correlated with latitude ($F_{1,4} = 9.06$, $P = 0.040$) and was lower in the restricted than in the *ad libitum* food treatment ($F_{1,27} = 4.69$, $P = 0.039$), whereas no significant effect of temperature was observed ($F_{2,27} = 1.75$, $P = 0.93$). Virtually no variance in the coefficient of variation for age at metamorphosis was associated with population (variance component 0.0%).

DISCUSSION

Our analyses suggest that an L-shaped reaction norm between the age and size at metamorphosis in *R. temporaria* depends on the rearing temperature. The expected L-shaped pattern (i.e. slow-growing individuals metamorphose at an older age and lower weight than fast-growing individuals) arises at higher temperatures, but as temperature decreases the age at metamorphosis appears to become independent of growth conditions (Figs. 1 and 2). As the L-shaped pattern is considered a consequence of body-size-related developmental thresholds regulating the timing of ontogenetic life-history transitions (Day and Rowe, 2002), our findings suggest that in *R. temporaria* the process of metamorphosis is at low temperatures no longer regulated by a threshold body size but some other regulatory mechanism, such as the effect of temperature on the developmental rate. This is highlighted by our observation that at 14°C even the slowest growing tadpoles metamorphosed at much larger sizes than the tadpoles at higher temperatures. Specifically, at 14°C tadpoles had passed the sizes at which individuals metamorphosed at higher temperatures long before they started to metamorphose, suggesting that age rather than size might have controlled metamorphosis and led to a vertical reaction norm (Fig. 2).

Notably, the restricted food treatment of the experiment was not low enough to push weights at metamorphosis down to the developmental threshold. This is because if growth conditions were so poor that individuals had to metamorphose immediately after reaching the threshold size, then this would be reflected in declining variation of the weight at metamorphosis with an increasing age at metamorphosis (cf. Lind *et al.*, 2008), but such a pattern was not observed in our data. Therefore, the observed minimum weights at metamorphosis only provide an upper boundary for the species-specific developmental threshold size. However, this still does not affect our main finding that the same feeding regime can lead to a different shape and elevation of the reaction norm depending on the prevailing temperature (Fig. 1), thus affecting the conclusions about the processes controlling metamorphosis. Moreover, the observation that age rather than size might control metamorphosis at low temperatures raises the question of whether the developmental threshold size could be reached at all in such conditions, no matter how restricted the food was.

The effect of temperature on the rate of growth and development is well known in many organisms, particularly in ectotherms (Angilletta, 2009). The temperature–size rule, which predicts that ectotherms metamorphose/mature later and at a larger body size with decreasing temperature, applies to most empirical case studies (Atkinson, 1994; Berrigan and Charnov, 1994; Atkinson and Sibly, 1997). This pattern can also be seen in our experiment by comparing the food regime specific ages and weights at metamorphosis over the three temperature treatments (Figs. 1, 2). However, in the light of the developmental threshold model, it is notable that temperature can profoundly alter the shape of the pattern between age and weight at metamorphosis. Independent of whether a developmental threshold size would be species, population or population × environment specific, the absence of an L-shape at low temperatures suggests that mechanisms regulating metamorphosis are different in differing environmental conditions, so that at low temperatures the process is no longer regulated by body size but some other mechanism, such as the effect of temperature on the developmental rate (i.e. the age at metamorphosis). This suggests that in addition to body-size-related developmental thresholds, a threshold age should also be accounted for to predict the age and size at an ontogenetic life-history transition, particularly at temperatures at which age, in addition to body size, can regulate the occurrence of a transition.

The decreased age at metamorphosis in northern populations is likely a result of the need to metamorphose before the onset of winter, which tadpoles cannot survive (Laugen *et al.*, 2003). This adaptive explanation is reinforced by the fact that, in the present study, the variance in age at metamorphosis decreased with latitude. However, the lack of variation in the age at metamorphosis between the feeding regimes at the lowest temperature can be found across all populations (Fig. 3a) and therefore needs further clarification. A proximate explanation for an age threshold is that enzyme kinetics underlying ontogenetic processes depend highly on temperature and thus the minimum age required to complete all necessary ontogenetic steps will vary with the temperature regime in ectotherms (Van der Have and De Jong, 1996). While the adaptive role of this variation is unclear, it may reflect a trade-off in energy acquisition and allocation under sub-optimal temperature regimes as tadpoles allocate the extra energy to increase their body size rather than to increase the developmental rate with increasing food availability. As noted by Day and Rowe (2002), even though the effect of temperature on developmental time was not accounted for in the original formulation of the developmental threshold model, the framework should easily allow for such extensions and, as further stressed by our results, is a topic that warrants future research.

A practical implication to emerge from our study is that when searching for the developmental threshold size for a particular species or a population by experimentally manipulating growth conditions (e.g. Plaistow *et al.*, 2004; Lind *et al.*, 2008), the role of temperature on development must be considered. Specifically, the temperature must be sufficiently high so as not to limit the occurrence of a transition, so that any limitation will stem from the threshold with respect to body size. Otherwise, the physical features limiting the processes of metamorphosis and maturation may be obscured.

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