Pool desiccation and developmental thresholds in the common frog, *Rana temporaria*

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The developmental threshold is the minimum size or condition that a developing organism must have reached in order for a life-history transition to occur. Although developmental thresholds have been observed for many organisms, inter-population variation among natural populations has not been examined. Since isolated populations can be subjected to strong divergent selection, population divergence in developmental thresholds can be predicted if environmental conditions favour fast or slow developmental time in different populations. Amphibian metamorphosis is a well-studied life-history transition, and using a common garden approach we compared the development time and the developmental threshold of metamorphosis in four island populations of the common frog *Rana temporaria*: two populations originating from islands with only temporary breeding pools and two from islands with permanent pools. As predicted, tadpoles from time-constrained temporary pools had a genetically shorter development time than those from permanent pools. Furthermore, the variation in development time among females from temporary pools was low, consistent with the action of selection on rapid development in this environment. However, there were no clear differences in the developmental thresholds between the populations, indicating that the main response to life in a temporary pool is to shorten the development time.

Keywords: development time; developmental threshold; local adaptation; pool permanence; *Rana temporaria*; reaction norm

1. INTRODUCTION

Many organisms show ontogenetic niche shifts, e.g. insects and amphibians shift from an aquatic to a terrestrial habitat, and some fish species shift from a littoral to a pelagic habitat (Werner 1988). Making this transition at the right size and time is crucial because these variables are important fitness components (Rowe & Ludwig 1991; Roff 1992; Altwegg & Reyer 2003). There is a considerable variation in size and timing of habitat shifts both within and among species, and the challenge for ecologists is to explain this variation (Reznick *et al.* 1990; Nylin & Gotthard 1998; Morey & Reznick 2004).

A general trend observed in size and time at the transition is that organisms usually shift earlier at a larger size under good food availability or later at a smaller size when food availability is restricted, suggesting a negative relationship between age and size (Berrigan & Charnov 1994; Gotthard & Nylin 1995; Morey & Reznick 2000). As suggested by Wilbur & Collins (1973) and later formalized by Day & Rowe (2002), such a relationship can arise owing to a developmental threshold. A developmental threshold is a minimum size or condition that must be reached before a life-history transition, such as metamorphosis or maturation, can occur. The threshold model predicts that, when resources are abundant, there will be a low variation in age at metamorphosis (or other life-history transitions) but a large variation in size at metamorphosis (Day & Rowe 2002). However, in poor growth conditions, the opposite pattern will be found. These predictions have recently

been supported in a laboratory study of soil mites (Plaistow *et al.* 2004). Under high food conditions, the daily increase in mass is substantial, and delaying maturity only a short time after the developmental threshold has been passed will give a large increase in metamorphic weight. By contrast, under low food conditions, the daily increase in weight is so small that individuals do not gain much in weight by delaying maturity after the developmental threshold has been passed. Owing to the low gain in weight by delaying metamorphosis under these low food conditions, individuals will metamorphose when reaching the minimum size for metamorphosis, i.e. the developmental threshold (Day & Rowe 2002; Plaistow *et al.* 2004). By plotting the size at metamorphosis as a function of the development time under different food conditions, an L-shaped reaction norm (following the terminology of Day & Rowe 2002) is predicted and also found in many systems (Morey & Reznick 2004; Plaistow *et al.* 2004, but see Rudolf & Rödel 2007).

The exact mechanisms underlying thresholds are still unclear and may vary depending upon the threshold type (Day & Rowe 2002), although endocrinological mechanisms have been suggested to regulate the threshold (Wilbur & Collins 1973; Hensley 1993). Morey & Reznick (2000, 2004) found interspecific variation among three species of spadefoot toads with respect to the size at which the developmental threshold of metamorphosis was reached and found that species from the most ephemeral environments had the lowest thresholds when subjected to low food conditions. In one way, this is what would be expected since ephemeral pools are time-constrained environments and a physiologically lower developmental

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threshold is reached more quickly. However, if the threshold represents a physiological constraint in the development, as assumed (e.g. Wilbur & Collins 1973; Day & Rowe 2002), the finding that threshold size can indeed evolve challenges this assumption. If the developmental thresholds can evolve, it is unclear what keeps the developmental threshold high in populations from permanent environments or why thresholds are present at all (Day & Rowe 2002).

If the developmental thresholds can evolve, as the intraspecific comparisons suggest (Morey & Reznick 2000, 2004), then adaptive evolution of the threshold level might also be present among populations of the same species. In contrast to discrete species, populations have not evolved mechanisms for reproductive isolation and can therefore be connected by gene flow, although population differentiation is nevertheless common for many traits under divergent selection despite high gene flow (e.g. Reznick *et al.* 1997; McKay & Latta 2002). If the developmental threshold is not considered as a single trait, but rather as a constraint affecting the whole development machinery, only very strong divergent selection pressures would be able to create adaptive divergence in the developmental threshold between populations.

As demonstrated in a number of studies (e.g. Wilbur & Collins 1973; Leips & Travis 1994; Morey & Reznick 2004), amphibian metamorphosis shows potential as a model for exploring the nature of developmental thresholds. The amphibian metamorphosis is a classic example of an ontogenetic niche shift and the amphibians show great variation in size and time at metamorphosis. The general pattern observed is that they, as in most other animal species, metamorphose earlier and at a larger size as food availability improves (Wilbur & Collins 1973; Leips & Travis 1994; Ball & Baker 1996; Weeks & Meffe 1996).

In this study we compare populations of the common frog (*Rana temporaria* Linnaeus 1758) from isolated islands in the same geographical area where breeding pools differ in their drying regimes. These islands do not appear to differ in any other environmental variables than the rate of pool drying, and the populations show adaptive differences in the development time and size at metamorphosis depending upon the risk of pool desiccation (Johansson *et al.* 2005; Lind & Johansson 2007). The island system resembles the one studied by Morey & Reznick (2000), with the exception that we study populations and not different species. Therefore, there is scope for the developmental threshold to diverge among the populations from temporary and permanent environments, as found among different species of spadefoot toads (Morey & Reznick 2000, 2004). There have been attempts to investigate such between-population differences in a plant species (Wesselingh *et al.* 1997); however, the experimental design did not include harsh growth conditions. Consequently, the reported population difference might not have been associated with a difference in the threshold but merely reflect different life-history strategies of the two populations. Comparisons of the size at metamorphosis in spatially widely separated populations of *R. temporaria* have been made (e.g. Merilä *et al.* 2000), although not with the aim of detecting a developmental threshold. However, these studies did not investigate if the food treatments used were low enough to

force the individuals to metamorphose at their developmental threshold, and although no population difference was found this might only reflect the life-history strategies of the populations. In the present study, our lowest food level is 50% lower than the low food level in any comparable study of *R. temporaria* (Merilä *et al.* 2000, 2004a,b). Few studies (see Morey & Reznick 2004; Plaistow *et al.* 2004) have used experimental treatments that control for the life-history strategic choice of the organisms and explicitly address the developmental threshold. To our knowledge, no convincing examples of intraspecific differentiation in the developmental thresholds have been reported, and no comparisons of closely situated populations have ever been made.

First, we examine whether there is a developmental threshold present, and whether the corresponding age and size pattern under different food treatments follows the predictions from the threshold model of Day & Rowe (2002). Second, we investigate the occurrence of population divergence in development time and developmental threshold of metamorphosis. We predict that tadpoles inhabiting temporary islands with temporary pools will have a shorter development time than those from islands with permanent pools. If the developmental threshold is tightly linked to physiological constraints in the developmental process, as assumed (Day & Rowe 2002), we do not predict an evolutionary change of the developmental threshold among the populations from the two environments. However, if the developmental threshold is not tightly linked to physiological constraints, we predict a pattern similar to the interspecific difference found by Morey & Reznick (2000, 2004a,b). That is, an adaptive divergence in development time as well as in the developmental threshold of metamorphosis between the two environments.

2. MATERIAL AND METHODS

The common frog (*R. temporaria*) breeds commonly in temporary and permanent waters across Sweden (Gasc *et al.* 1997). Recent studies on islands in the archipelago within the Gulf of Bothnia, outside Umeå, northern Sweden, found local adaptation in development time and size at metamorphosis (Johansson *et al.* 2005; Lind & Johansson 2007). Populations from pools that dried out faster had smaller sizes at metamorphosis and shorter development times than those from slow drying pools. Based on this pattern, we chose two populations at each end of the drying regime and reared offspring from females under three different growth conditions: low, medium, and high food availability.

Eggs were collected from four islands in the archipelago. Two of these islands have only permanent pools present (Öster Hällskär: 63°48' N, 20°37' E and Stora Fjäderågg: 63°48' N, 21°0' E) and two islands have only temporary pools (Sävar-Tärnögern: 63°45' N, 20°36' E and Ålgrundet: 63°41' N, 20°25' E). From 12 different egg clutches from each island, 40–50 eggs were sampled between 9 and 13 May 2006. The samples were transported to the laboratory where they were kept at 4°C until all eggs were collected. Each egg clutch corresponds to one female. The females of *R. temporaria* in this region breeds only once a year (Elmberg 1991b) and although the female physiologically can lay two clutches, one from each ovary (J. Elmberg 2007, personal communication), experiments where female *R. temporaria* from this area were allowed

to breed in buckets (Elmberg 1991a) showed that they only lay one egg clump, and dissection of the female showed that no eggs were left in her reproductive system.

Prior to hatching, at Gosner stage 10 (Gosner 1960), 10 eggs from each egg clutch were placed in a Petri dish, completely covered with water to reduce distortion of the light caused by the circular gelatinous capsule surrounding the eggs and photographed together with a scale using a vertically placed digital camera (Canon EOS 350D with Tamron SP AF 90 mm F/2.8 Di Macro 1 : 1 lens). Egg sizes were then calculated using the image analysis program IMAGEJ v. 1.36b. This allowed us to calculate the mean egg size for each of the females on the four islands, which allowed us to control for egg-size-mediated maternal effects (Laugen *et al.* 2002). Eggs that had developed beyond Gosner stage 10 (Gosner 1960) were not measured. Use of a half-sib design to control for maternal effects would have been preferable, but applying such a design means that live frogs must be collected and mated artificially in the laboratory before returned to their origin island, which was not logistically possible. However, a previous half-sib experiment indicated that maternal effects were of minor importance, explaining only 5% of the phenotypic variance in our populations (Lind & Johansson 2007).

When all of the eggs were collected, the temperature in the climatically controlled room was raised to 22°C with a light : dark cycle of 18 hours : 6 hours, corresponding to the natural light (space): dark cycle in the area. Experiments were started when tadpoles entered Gosner stage 23 (active swimming). Six tadpoles from each female, two allocated to each food level, were randomly chosen and placed individually into plastic containers (9.5 × 9.5 cm², height 10 cm) filled with 750 ml of tap water previously aged and aerated in a tank together with dried deciduous leaves. The leaves were removed when the water was transferred to the experimental containers.

The tadpoles were fed and the water replaced every fourth day. Three different food levels were used: 26 (high ad libitum); 9 (medium); and 4.5 mg (low) for the first 8 days. During the following 8 days, the food levels were increased to 51, 18 and 9 mg, respectively. From day 16 until the end of the experiment, the tadpoles were given 103, 34 and 18 mg in each treatment, respectively. The high and medium food levels correspond to the ad libitum and restricted food levels used by Merilä *et al.* (2004a,b) in their study of the morphology of *R. temporaria* under different food levels. The low food treatment in our experiment was chosen to be half of the food in the medium treatment, to make sure that the tadpoles were severely food limited in their growth and would metamorphose at the developmental threshold. The food given to the tadpoles was a mixture of finely ground commercial fish flakes and rabbit food in a weight ratio of 1 : 1. The plastic containers were distributed in a pre-decided random pattern in the climatically controlled room.

The experiment ended when tadpoles reached Gosner stage 42 (Gosner 1960). At this stage, the forelimbs are visible and we define this as our estimate of metamorphosis. The tadpoles were weighed and the time from the start of the experiment (at Gosner stage 23) until metamorphosis was recorded as development time.

(a) Statistical analysis

Deviations from the assumption of homogeneous variances in the data were investigated for two reasons. First, the threshold

model predicts that the variances of age and size at metamorphosis will differ depending upon food treatment and proximity to the developmental threshold (Day & Rowe 2002) and second, homogeneity of variances is an assumption for the statistical models used. Deviances from homogeneity of variances were investigated using Bartlett's test and, if the group with the largest variance also had the largest mean, the variances were scaled by the mean to ensure that the result was not a statistical artefact. The Bartlett's tests showed that data for age and weight at metamorphosis deviated from the assumption of variance homogeneity, and therefore both variables were log transformed in all analyses. After log transformation, the variances were not significantly heterogeneous. The effect of the food treatment on age and weight at metamorphosis (defined as Gosner stage 42 in this experiment) was analysed in a factorial ANOVA with food as a fixed factor with three levels (high, medium and low). Egg size was used as a covariate in order to take egg-size-mediated maternal effects into account (Laugen *et al.* 2002). Island was treated as a random factor, nested within environment (temporary or permanent). Since random mortality sometimes reduced the replicate in some female × treatment combinations to one, we randomly removed one replicate from every female × treatment combination so that the same experimental unit was used in all comparisons. Model simplification was performed when necessary by removing non-significant terms from the maximal model in order to arrive at the minimal adequate model, following Crawley (2002). Egg-size differences between the populations were investigated in an ANOVA with island populations nested within environments.

To compare the shape of the relationship between age and size among the populations, a nonlinear regression of the form: size at metamorphosis = $a + b \times \exp(-c \times \text{age at metamorphosis})$ (Plaistow *et al.* 2004) was fitted to the data. To avoid the parameter b (estimated y -axis intercept) reaching infinity, 22.5 days, which is the metamorphic age of the fastest developing tadpole, were subtracted from the age at metamorphosis before running the model. Unfortunately, a nonlinear regression analysis was possible for only three of the four populations, due to lack of fit of the model in one population.

The gain in weight by delaying metamorphosis was investigated in an analysis of covariance, using weight at metamorphosis as a response variable, age at metamorphosis as a covariate, food treatment as fixed factors and island as a random factor. Model simplification was performed when necessary, by removing non-significant higher-order interaction terms.

Under low food conditions, individuals will not gain much weight by delaying metamorphosis, and will therefore metamorphose at the developmental threshold (Plaistow *et al.* 2004). Therefore, the difference in weight at Gosner stage 42 between the tadpoles from the two environments (islands with temporary or permanent pools) under the low food treatment was used to investigate the existence of an environment-mediated difference in the developmental threshold of metamorphosis. The test was performed using a nested ANOVA with the islands nested within the two environments. Egg size was used as a covariate to estimate egg-size-mediated maternal effects, and model simplification was performed when necessary.

All analyses were performed using the R statistical package (R Development Core Team 2005), using the libraries BASE, NLME and LME4.

Figure 1. Age and size at metamorphosis for the four populations originating from islands with temporary (*a*, Ålgrundet; *c*, Sävar-Tärnögern) or permanent (*b*, Öster Hällskär; *d*, Stora Fjärderägg) pools. Closed circles correspond to the high food level, crosses to the medium food level and plus signs to the low food level.

3. RESULTS

(a) Hatching survival

Of the 288 tadpoles in the experiment, 277 survived until metamorphosis. For one female from Stora Fjärderägg, both the tadpoles in one treatment died, so this female was therefore excluded from the analysis. On the other three islands, tadpoles from all 12 females were used.

(b) Population responses to different food levels

The variances in weight at metamorphosis were larger in the high food treatment than the low food treatment (Bartlett's $K^2_2=43.9$, $p<0.001$). Since the largest variances were present in the food treatment with the largest mean, the result could be a statistical artefact as variances often increases with the mean. Therefore, an ANOVA was performed using variance to mean ratios (VMRs). Nevertheless, the VMRs also differed significantly between the food treatments ($F_{2,6}=0.03384$). For age at metamorphosis, the variances were not significantly different among the food treatments (Bartlett's $K^2_2=3.85$, $p=0.15$). The variances for age at metamorphosis also differed among the four islands under all the three food conditions (high food: $K^2_3=29.92$, $p<0.001$; medium food: $K^2_3=14.54$, $p=0.002$; low food: $K^2_3=220.11$, $p<0.001$), since the variances in age at metamorphosis were smaller for the two populations originating from islands with temporary pools (figure 2*a*).

Egg size was used as a covariate in all ANOVA and regression analyses, but was not significant in any analysis.

We therefore excluded the term from the minimum adequate models in the model simplification process. Moreover, size of the eggs did not differ between the environments ($F_{1,2}=0.0508$, $p=0.84$).

The reaction norm of age and size at metamorphosis followed the same general pattern in all four populations (figure 1). Tadpoles in the high food treatment had the shortest development times and metamorphosed at the largest weights, while tadpoles from the low food treatment delayed metamorphosis and when metamorphosing did so at smaller sizes (tables 1 and 2; figure 2*a,b*). The pattern from the medium food treatment was intermediate to that of the two extreme treatments. As repeatedly shown in this system (Johansson *et al.* 2005; Lind & Johansson 2007), tadpoles from the two islands with temporary pools had shorter development times than those from islands with permanent pools, and the strong interaction between food treatment and environment reveals that the differentiation in development time between the environments is larger, the higher the food availability. (table 1; figure 2*a*).

For weight at metamorphosis, there were no differences among the populations (table 2). The overall weight at metamorphosis for the populations differed among food treatments in a similar pattern as did development time (table 2; figure 2*b*).

The age and size at metamorphosis of all populations pooled together showed an L-shaped reaction norm

Table 1. ANOVA table of the minimal adequate model of factors influencing development time. (Environment corresponds to islands with permanent or temporary pools.)

	d.f.	MS	<i>F</i>	<i>p</i>
(intercept)	1	789.7	165 116	<0.001
food treatment	2	0.2976	62.23	<0.001
environment	1	0.0075	15.60	0.0585
food treatment × environment	2	0.0494	5.16	0.0070
residuals	124	0.00478		

Table 2. ANOVA table of the minimal adequate model of factors influencing weight at metamorphosis. (Environment corresponds to islands with permanent or temporary pools.)

	d.f.	MS	<i>F</i>	<i>p</i>
(intercept)	1	27.64	2271.3	<0.001
food treatment	2	11.223	922.05	<0.001
environment	1	0.0284	2.3303	0.2664
food treatment × environment	2	0.0146	1.2024	0.3040
residuals	124	0.0122		

(figure 3) The data could be fitted by a nonlinear regression following the model: size at metamorphosis = $a + b \times \exp(-c \times \text{age at metamorphosis})$ with the coefficients $a = 0.208 \pm 0.054$ ($t_{138} = 3.87$, $p < 0.001$), $b = 0.382 \pm 0.047$ ($t_{138} = 8.22$, $p < 0.001$) and $c = 0.266 \pm 0.102$ ($t_{138} = 2.61$, $p = 0.01$). However, when analysed separately, nonlinear regression lines could be fitted only for three of the four populations and hence no statistical comparisons of the shapes of the age and size at metamorphosis patterns were made between the temporary and permanent pool environments.

The gain in weight by delaying metamorphosis was investigated in an ANCOVA. However, the result of the analysis was heavily dependent upon two very influential points in the high food level treatments (figure 3, top right corner). With these data points included, the slope of the change in weight did not differ between the three food levels ($F_{2,131} = 0.11$, $p = 0.89$). As the two data points were very influential for the result of the analysis, they were excluded from the model presented below. These two data points were also identified in residual plots against both fitted values and quantiles of standard normal, further suggesting a removal. The gain in weight by delaying metamorphosis differed between the treatments, as indicated by the treatment × age at metamorphosis interaction in the ANCOVA (table 3). Investigation of treatment contrasts revealed that the increase in weight by delaying metamorphosis was significantly higher in the high food treatment than the two lower food treatments, as predicted, if tadpoles metamorphose close to their developmental threshold in the low food treatment ($t_{130} = -3.41$, $p < 0.001$ for the comparison between high and low food levels).

(c) Developmental thresholds

The developmental threshold was defined as the weight at Gosner stage 42 (Gosner 1960) of the tadpoles under the

Figure 2. (a) Development time and (b) weight at metamorphosis for each population in the different food levels (mean $\pm$ s.e.). Broken lines and open symbols illustrate islands with temporary pools, unbroken lines and closed symbols correspond to populations originating from islands with permanent pools.

low food treatment (Morey & Reznick 2004; Plaistow *et al.* 2004). The developmental threshold was not significantly lower for the two populations from islands with only temporary pools ($0.2156 \text{ g} \pm 0.0004$), compared with the tadpoles from islands containing only permanent pools ($0.2185 \text{ g} \pm 0.0013$, $F_{1,2} = 11.95$, $p = 0.0744$, figure 2b).

4. DISCUSSION

As previously shown in other systems (Morey & Reznick 2004; Plaistow *et al.* 2004), we found a negative relationship between age and size at metamorphosis under different food availabilities. This relationship is predicted by the threshold model (Day & Rowe 2002) as a general consequence of incorporating a developmental threshold, and follows the L-shaped reaction norm predicted by the model.

Figure 3. The L-shaped reaction norms of age and size at metamorphosis. The line is fitted to the pooled dataset by nonlinear regression using the following model; size at metamorphosis = $a + b \times \exp(-c \times \text{age at metamorphosis})$ with the coefficients $a = 0.208 \pm 0.054$, $b = 0.382 \pm 0.047$ and $c = 0.266 \pm 0.102$. Closed circles correspond to the high food level, crosses to the medium food level and plus signs to the low food level.

Table 3. ANCOVA table of the minimal adequate model of how weight at metamorphosis is influenced by development time, taking population and food treatment into account. (Island population is included in the model as a random factor.)

	d.f.	MS	F	p
(intercept)	1	4.99	5714.11	<0.001
food treatment	2	1.54	1760.33	<0.001
development time	1	0.055	63.17	<0.001
food treatment $\times$ development time	2	0.0061	6.69	0.0015
residuals	130	0.0009		

(a) Developmental thresholds

When comparing three species of spadefoot toads specialized towards pools with different risks of desiccation, Morey & Reznick (2000, 2004a,b) found that the genetically determined development time, as well as the developmental threshold, had evolved adaptively, so that species living in the most ephemeral environment had the shortest development times as well as the lowest developmental thresholds. In accordance with the between-species adaptive differentiation in development time, we found that island populations of *R. temporaria* had shorter development times when originating from islands with more ephemeral pools, as has been shown repeatedly in this system (Johansson *et al.* 2005; Lind & Johansson 2007). Furthermore, the low variation in development time among families from the same temporary pool suggests a strong directional selection pressure on rapid development time, reducing variation. However, in contrast to the between-species comparison, the developmental threshold of the rapidly developing tadpoles of

R. temporaria populations originating from islands with temporary pools was not significantly lower than that of more slowly developing tadpoles originating from island populations with permanent pools. Although not far from statistical significance, the difference in weight between the two environments is only 2.9 mg, which is only 1.3% of the mean weight under low food conditions, and this difference in the developmental threshold of metamorphosis is probably too small to be of biological importance.

The reason for the absence of differentiation in the developmental threshold is probably explained by the very nature of the developmental thresholds. Development time and size at metamorphosis can easily be adjusted in many populations by phenotypic plasticity, and these two variables also show genetic differentiation among populations of *R. temporaria* (e.g. Loman 1999; Merilä *et al.* 2004a,b; Van Buskirk & Arioli 2005; Lind & Johansson 2007). Therefore, populations can adapt to express different development times over the continuum from fast to slow growth rate. The flexibility of development time is also demonstrated by its sensitivity to many other environmental conditions, such as food availability (Leips & Travis 1994; Laugen *et al.* 2003; Morey & Reznick 2004; this study) and temperature (Ståhlberg *et al.* 2001; Laugen *et al.* 2003). By contrast, the developmental threshold is defined as the minimum size that must have been reached before the life-history transition can occur (Day & Rowe 2002), and individuals smaller than the threshold size cannot pay the cost of metamorphosis. Hence, the developmental threshold defines an endpoint: individuals smaller than the threshold size will not metamorphose, but other life-history trade-offs determine whether metamorphosis will occur at the threshold or at a larger size. Therefore, the threshold is probably determined by strong constraints in the physiology of the individual (as suggested by Wilbur & Collins 1973) and inter-population differentiation in these physiological constraints to metamorphosis is likely to require changes in key aspects of the whole developmental machinery. The developmental threshold as strongly constrained by physiological processes in *R. temporaria* is further supported by the comparison of the weight at metamorphosis of two *R. temporaria* populations separated by 1500 km by Merilä *et al.* (2000; figure 2b). Although the low food level used was corresponding to the medium food in our experiment, and thus not measuring the tadpole size at the developmental threshold, the lack of population differentiation in weight at metamorphosis in the experiment of Merilä *et al.* (2000) is consistent with the view of the developmental thresholds as strong physiological constraints in the development. The physiological processes that lie behind the threshold are still unknown (Day & Rowe 2002), although endocrinological mechanisms are likely to be involved (Wilbur & Collins 1973; Hensley 1993), at least as regulators. Our populations are probably less than 300 generations old (Johansson *et al.* 2005) and population differentiation is therefore much more likely to be found in the development time than the developmental threshold. Although the populations in this study have not diverged in the developmental threshold, there are adaptive differentiations in the life-history strategies they express once they have passed the developmental threshold. The populations from temporary environments have a rapid

development and begin metamorphosis earlier, while those from permanent environments do not initiate metamorphosis until later and remain longer in the aquatic phase (Lind & Johansson 2007).

Although the asymptote of the nonlinear regression, which represents the developmental threshold, levels off at the minimum size found in our experiment, it could be argued that we cannot determine that the metamorphic size at the low food treatment is the actual developmental threshold, since an even lower food treatment was not used. However, the populations converge to the same size in the low food treatment (figure 2b) and, in addition, tadpoles consumed all food in both the medium and low food treatments before the next feeding, indicating that we had two levels of food limitation. Furthermore, the gain in weight by delaying metamorphosis was significantly lower for tadpoles under the low food treatment than the high food treatment, as predicted, when tadpoles metamorphose close to their developmental threshold (Day & Rowe 2002). We therefore consider our conclusion that there is no difference in the developmental threshold between different environments to be robust.

(b) Reaction norms

One prediction of the developmental threshold model is that the size at metamorphosis increases with improved food availability (Day & Rowe 2002) and our study adds to the growing number of examples of this seemingly general phenomenon (Roff 1992; Berrigan & Charnov 1994; Gotthard & Nylin 1995; Morey & Reznick 2000, 2004; Plaistow *et al.* 2004). Under restricted food conditions, the daily increase in weight is so small that individuals do not gain much in weight by delaying metamorphosis after the developmental threshold has been passed (Day & Rowe 2002; Plaistow *et al.* 2004). By contrast, under high food conditions, the daily increase in mass is substantial, and delaying metamorphosis only a short time after the developmental threshold has been passed will give a large increase in metamorphic weight, known to be important for fitness in amphibians (Smith 1987; Berven 1990; Altwegg & Reyer 2003). This was also seen in the high food treatment in our populations, where the weight at metamorphosis increased when metamorphosis was delayed. Following the reasoning above, the variation in age at metamorphosis should be large when individuals are grown in low food conditions, while at high food conditions all individuals will metamorphose at a similar time but with a large variation in size. However, we found only partial support for this second prediction. Although there was a larger variation in size at metamorphosis for individuals grown under high food conditions compared with poor food conditions, there were no differences in the variation in age at metamorphosis among the growth conditions. This result is not consistent with the findings of Plaistow *et al.* (2004), who found support for both predictions in their study of soil mites. However, development time is strongly linked to fitness in *R. temporaria*, since the tadpoles must complete development and metamorphose before the pool dries. Hence the time constraint imposed by pool drying might explain the difference between our study and that of Plaistow *et al.* (2004) with regard to the variation in age at metamorphosis. This interpretation is further supported by the lower variation in development time among families from

temporary environments compared with those from permanent pools, consistent with the action of selection for a fixed rapid development time in temporary environments.

In contrast to development time, the weight at metamorphosis did not differ between the populations from the two environments under any food conditions. Previous studies on the effect of pool permanence on the metamorphic weight in this system have provided mixed support (Johansson *et al.* 2005; Lind & Johansson 2007). The results nevertheless indicate that the main response to life in temporary pools is to shorten the development time, and that the strong selection pressure favouring rapid development in temporary pools leads to a decreased variation in development time between families.

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