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Variation in heritability of tadpole growth: an experimental analysis

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Heritability characteristically shows large variation between traits, among populations and species, and through time. One of the reasons for this is its dependence on gene frequencies and how these are altered by selection and drift through the evolutionary process. We studied variation in heritability of tadpole growth rate in populations of the Swedish common frog, *Rana temporaria*. In populations evolving under warmer conditions, we have demonstrated elsewhere that tadpoles show better growth and physiological performance at relatively higher temperatures than tadpoles with an evolutionary history in a relatively cooler part of the distribution range. In the current study, we ask whether this process of divergence under natural selection has influenced the genetic architecture as visualised in estimates of heritability of growth rate at different temperature treatments under laboratory conditions. The results suggest that the additive genetic variance varies between treatments and is highest in a treatment that is common to both populations. Our estimates of narrow sense heritability are generally higher in the thermal regime that dominates in the natural environment. The reason for this appears not primarily to be because the component of additive genetic variation is higher in relation to the total phenotypic variation under these conditions, but because the part of the phenotypic variance explained by environmental variation increases at temperatures to which the current populations has been less frequently under selection.
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Introduction

The magnitude of the response to selection is set by the degree to which a trait is determined by parental genes, ie, by its heritability (Falconer and Mackay, 1996). The concept of heritability is, hence, fundamental for our understanding of the evolutionary process and has been defined in two ways, in the broad and narrow sense (Falconer and Mackay, 1996). In the present work, we shall primarily be concerned with narrow sense heritability (heretoforth 'heritability'), defined as the ratio of the additive genetic variance (V_A) to the total phenotypic variance (V_P) (Falconer and Mackay, 1996), ie, $h^2 = V_A/V_P$.

Heritability characteristically shows large variation between traits and taxa, and through time (Mosseau and Roff, 1987; Roff, 1997; Merilä and Sheldon, 1999, and references therein). More importantly in the context of this paper, heritability may also differ drastically among environments within a species. The understanding of this relationship is, however, far from straightforward (reviewed in Hoffman and Merilä, 1999). In the present study, we shall primarily be concerned with two of the most commonly invoked explanations to this phenomenon in the literature, namely that (i) the additive genetic component increases in a benign environment (eg, because trait size can only be realized under good conditions), or, alternatively, (ii) that the environmental variation decreases under good conditions (eg, because selection removes environment-specific poor genotypes in the process of local adaptation) (Hoffman and Merilä, 1999). Evidence in support of both these hypotheses have been reported (eg, Simons and Roff, 1994; Merilä, 1997; Hoffman and Merilä, 1999). Thus, the lack of consistent results across studies suggest that more experimental work is needed to better understand the genetic underpinnings of fluctuations in V_A, V_E, and h^2 among environments.

When the members of a population become better adapted to local environmental conditions, two outcomes of this process may be predicted in terms of the variance components that make up the heritability estimate: (i) additive genetic variation should become depleted (relative to a situation when no additive genetic variation has been 'used up', assuming constant mutation rates in both environments), and (ii) the component of environmental variance should become smaller as a result of directional and stabilizing natural selection resulting in more pronounced trait canalization. Thus, in this scenario, the estimate of heritability is dependent on the relative changes in these variance components due to selection.

In the present study, we exploit the Swedish common frog, *Rana temporaria*, as a model because its populations have become locally adapted to 'warm' sites *vs* 'cold' sites in terms of optimal tadpole development (somewhat contrary to intuition, tadpoles in the northern part of Sweden experience higher temperatures during tadpole develop-

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ment, because of the rapid increase in temperature after snowmelt, Ståhlberg *et al*, 2001). For example, tadpoles from 'warm' sites (in the north) grow almost 30% faster in relatively warmer conditions (20°C) than tadpoles from 'cold' sites (in the south, Ståhlberg *et al*, 2001). Because all metabolic enzyme systems show temperature-sensitivity with optima in species-characteristic windows (eg, Somero 1978), physiological processes in general, and development in particular, are temperature-dependent and best proceed at a specific optimum set by directional and stabilizing selection (eg, Somero, 1978; Bennett *et al*, 1990, 1992; Riehle *et al*, 2001).

The excellent recent reviews in this field suggest that predicting the outcome of heritability estimates in different environments should be difficult (eg, Hoffman and Merilä, 1999). The present study is based on our recent demonstration that *R. temporaria* has diverged at a temperature-sensitive part of the genome, and that therefore selection history should be an important determinant of the magnitudes of variance components. This information allows us to make the following directional predictions with respect to how the major variance components and their combined influence on the heritability estimate *per se*, will be visualised in tadpoles developing more or less close to their natural thermal optimum:

(i) Additive genetic variance (V_A) will be relatively more eroded at temperatures closer to the local developmental optima, ie, in the temperature to which a population has experienced the strongest selection. Specifically, tadpoles from cold sites will have relatively less additive genetic variance in relatively colder than warmer conditions, whereas the opposite applies for tadpoles from warm sites.

(ii) At optimal developmental temperatures, developmental noise as a component of environmental variance (V_E) should be minimal due to canalization. Thus, tadpoles from cold sites should have their lowest V_E under cold conditions, and lower than tadpoles from warm sites, whereas tadpoles from warm sites should have their lowest V_E in a warmer treatment, and lower than tadpoles from the cold site.

(iii) Under the assumption that local populations have been exposed to selection with a stronger effect on canalization (and hence V_E) than was the eroding effect of selection on V_A, heritability should be highest under optimal developmental conditions.

Materials and methods

Frog spawn was sampled from two regions, one southern, at three sites (Angered, 58°45′ N, 12°05′ E), and one northern, at two sites (Ammarnäs, 65°55′ N, 16°15′ E). The sampling sites were separated by several kilometers in each region and the spawn was collected in early April and late May, in the southern and northern regions, respectively. Forty-seven clutches were sampled from each region, but in the present study we have followed Roff's advice (Roff, 1997, pp 42–43) and dropped families where mortality compromised the balance of the design at the level of family size. A sample of spawn from each clutch was preserved in 5% formalin at collection in order to look for differences in developmental stages between the sites. The clutch sizes and egg masses were estimated by weighing a known number of eggs as well as the whole spawn. The eggs were staged using the standard Gosner table of frog development (Gosner, 1960). Eggs collected at the northern site had developed on average 2.5 more stages at collection than eggs at the southern site (average stage at collection was 8.5 ± 0.5 in the north and 11.0 ± 1.7 in the south, *t*-test, d.f. = 53, $P = 0.0001$), which represents c. 0–3 days at the relevant temperatures (personal observation of development at 10–20°C). This difference in developmental stage at spawn collection resulted in significantly shorter time to hatching in the north than south spawn in all three temperatures. Hatching time differed by 1.7 days on average in 10°C, 1.5 in the 15 degree and 1.0 days in the warmest treatment. Because of this slight difference, we refrained from using pre-hatching data in our analyses. All results in this paper are therefore based on comparison of growth and development of hatched-out tadpoles.

Experimental design

This study follows a full-sib, split-brood analysis outlined by Roff (1997, pp 41–43). The experimental design was made up of two artificial pools (152 × 122 × 25 cm, heretoforth A and B) per three water temperature treatments, 10, 15 and 20°C. The temperatures were chosen to cover an overlap zone for both populations, with lower temperatures being relatively more common at the southern site and *vice versa* (Ståhlberg *et al*, 2001). Thus, all treatments have temperatures within the natural thermal variation (Ståhlberg *et al*, 2001). The north and south regions, however, differ with respect to for how long tadpoles experience a given temperature during normal development in the wild.

Ninety-four plastic containers with wire mesh-fitted bottoms (to ensure water circulation) were hung from crossbars in all six pools, so that each container held one litre of water. The water was cooled using a custom made cooling system, and heated to the pre-selected levels using submersible commercially available 300 W aquarium heaters. The water temperatures fluctuated no more than ±1°C throughout the experiment and the water levels were kept within ±1 cm using a custom made tap-system, fine-tuned to equilibrium flow levels prior to the onset of the experiment. The six pools were set up using tap water (Göteborg, Sweden) with a complex-binder to eliminate any heavy metals (Aqutan, manufactured by Sera, Heinsberg, Germany). The water was aerated for a minimum of 4 days before the experiment and for its duration. The room was set to a 14:10 L:D photo period. Fifteen (± 1) eggs from each female were placed in six containers, one in each pool, ie, c. 90 eggs per female were seperated into six samples, allocated to two replicas (one per A and B pool) in each of the three temperature treatments.

Because water introduced such a major confounding variable on tadpole mass at hatching, and because of the fragility and sensitivity to dehydration, tadpoles could not be accurately weighed at hatching. Instead, an estimation of tadpole mass was made by sampling 20 individuals from each pool that were weighed after excess water was completely soaked up using a paper towel. This mean hatchling mass of each pool was used as a first reading of mass for all tadpoles. This approach overlooked inter-individual differences in mass at hatching, but this variation appeared very small compared to the

large differences in growth and mass at the second reading (see below).

The tadpoles were fed commercially available fish food (Sera San, manufactured by Sera, Heinsberg, Germany) *ad libitum* in each container, to avoid density-dependent growth rate. Fifteen days post-hatching, five tadpoles from each container (ie, 10 tadpoles from each family and treatment) were sampled and weighed to the nearest mg.

Estimates of heritability

A full-sib analysis is particularly sensitive to four sources of variation; dominance variation, within-clutch mixed paternity, maternal effects, and sharing of the same rearing environment. The dominance component included in a clutch of full-sibs may inflate h^2, as will both maternal effects and sharing of the same rearing environment. Although recent research has shown that multiple paternity can occur in the common frog (Laurila and Seppä, 1997), multiple mating is rare (Savage, 1961; Elmberg, 1990). Thus, the risk of overestimating the genetic relatedness of tadpoles from the same spawn is low. To be able to estimate and control for the effect of a common rearing environment, 'families' were split into two rearing containers, following the experimental protocol outlined by Roff (1997, pp 42–43). Although we had no possibility to estimate the contribution to h^2 estimates from dominance variance, there is no reason to believe that across-population, or across-treatment differences (within 'families' and populations) result from differences in dominance variance. We also emphasize that our aim in this work is to contrast estimates of V_A, V_E, and h^2 between treatments and regions with different selection history rather than derive variance components accurate enough for purposes of making inferences about quantitative future change in trait values in response to selection. In this perspective, a full-sib design should be adequate, even if techniques such as parent-offspring regression would yield more robust h^2 scores.

Elsewhere, we have reported on no effects of clutch size (our estimate of maternal effect) on growth rate (Ståhlberg *et al*, 2001). Therefore, we submitted the raw data (without controlling for clutch size) for statistical analysis. First, however, we tested the assumptions of normality (Proc Univariate, SAS). Growth rate in mg was normally distributed at 15 and 20°C. At 10°C, however, the data were skewed but could be normalized by square root transformation ($P > 0.05$, for all variables in tests of deviations from normal expectations using Wilks' Lambda). Heritability was estimated using Proc Nested, SAS, which is designed to model hierarchical analysis of variance of random effects. In our study, both family (female identification number) and pool number (corresponding to 'cage' in Roff's, 1997, outline) were considered random variables. Variance components and the heritability they result in were calculated as given in Table 1. Since our design was balanced, we calculated standard errors following Swiger *et al* (1964) and Roff (1997; equation 2.29, p 42), rather than using the jackknife.

Results

General results

Our nested ANOVAs showed consistently high estimates of the variance component for family (Table 2), with significant effects in all treatments ($0.02 < P < 0.0001$), except at 20°C, south ($P = 0.44$). Thus, additive genetic effects were strong and consistent. The corresponding components for environmental variance were significant in all treatments for north tadpoles (Table 2), but was non-significant for south tadpoles in 15°C ($P = 0.18$; Table 2).

Table 1 Analysis of variance for the full-sib design (from Roff (1997))

Source of variation	*d.f.*	*MS*	*Expected MS*
Among families (AF)	N − 1	MS_{AF}	$V_{WC} + kV_{AC} + kcV_{AF}$
Among cages (AC), within families	N(c − 1)	MS_{AC}	$V_{WC} + kV_{AC}$
Within cages (WC)	NC(k − 1)	MS_{WC}	V_{WC}

N is the number of families, k is the number per container and c is the number of cages. Variance components are estimated from $V_{WC} = Ms_{WC}$, $V_{AC} = (MS_{AC} - MS_{WC})/k$, and $V_{AF} = (MS_{AF} - MS_{AC})/kc$. Heritability is calculated as $h^2 = 2V_{AF}/(V_{AF} + V_{AC} + V_{WC})$.

Table 2 Nested random effects ANOVA for growth rate for north and south populations in different temperatures

Temperature/ Population	*Source of variation*	*Variance component*	*F*	*P*	V_A *estimate*	V_E *estimate*
10/N	V_{AF}	0.403	1.97	0.0246	0.81	0.99
	V_{AC}	0.688	5.90	<0.00001		
10/S	V_{AF}	0.769	5.64	<0.00001	1.54	0.00
	V_{AC}	0.222	3.02	<0.00001		
15/N	V_{AF}	2453.9	6.07	<0.00001	4908	−8.10
	V_{AC}	598.9	2.61	<0.00001		
15/S	V_{AF}	1224.4	7.41	<0.00001	2449	407.1
	V_{AC}	69.7	1.22	0.1811		
20/N	V_{AF}	3307.2	3.63	0.0001	6614	3842
	V_{AC}	1352.7	2.17	0.0002		
20/S	V_{AF}	50.9	1.05	0.4437	102	4229
	V_{AC}	1736.4	4.41	<0.00001		

Among-treatment and between-region differences in V_A and V_E

Since the variance components differed between environments due to scale, we calculated the coefficient of variance for both the additive and environmental variance. For CV_A, the pattern of variation was very similar for both north and south tadpoles (Figure 1a), with a peak in CV_A at 15°C, and with lower scores at both tail end temperatures.

The lowest CV_E's (Figure 1b) were found in the temperatures corresponding to the local optima for growth and performance in each population in our previous work (Ståhlberg *et al*, 2001). For south ('cold') tadpoles, which grow and develop more than twice as fast as north ('warm') tadpoles at 10°C (Ståhlberg *et al*, 2001), the lowest CV_E was found in the 10°C treatment with a consistently increasing CV_E score at higher temperatures (Figure 1b). North ('warm') tadpoles, that outgrew and outperformed south tadpoles at 15 and 20°C in our previous study (Ståhlberg *et al*, 2001), showed a corresponding trend in CV_E in the present work. The highest CV_E component was present in the coldest treatment, with a

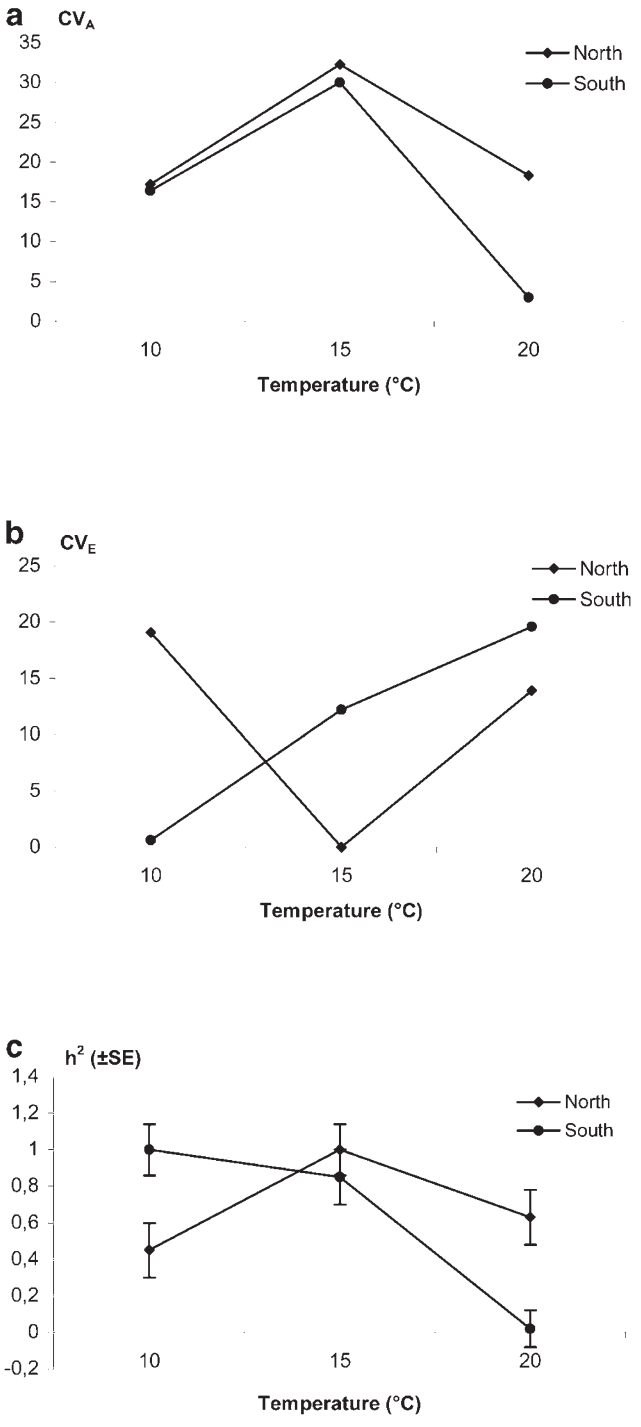

Figure 1 (**a**) additive, and (**b**) environmental variance in different temperatures for north and south populations. (**c**) Heritability estimates (±s.e.) in different temperatures for north and south populations. The differences in heritability are significant in 10 and 20°C (t=2.57 and 3.06; d.f's=77 and 77; $P<0.01$, respectively)

zero score of CV_E in 15°C, that increased again in 20°C (Figure 1b).

Heritability

Our analysis demonstrates that heritability estimates are highest under optimal developmental conditions in both populations (Figure 1c). Although V_A varies by more than 15% up and down between treatments (Figure 1a), this variation is not the main determinant of variation in h^2. Instead, the primary determinant of h^2 is set by variation in V_E. The heritability score reaches 1.0 in the environment we have identified as their local thermal optimum for each population, and this is predominantly an effect of V_E approaching zero under ideal developmental conditions (Figure 1b). Furthermore, that these populations have different levels of genetic variation is strongly suggested by the significant differences in heritability scores between north and south tadpoles at 10°C and 20°C (t = 2.57 and 3.06, respectively: d.f.'s = 77 and 77, respectively; $P < 0.01$, respectively), whereas the corresponding difference at 15°C was not significant (P = 0.25).

Discussion

Our study suggests that there is a relationship between the magnitude of heritability and how benign the conditions for growth and development are for tadpoles from a given region. From a perspective of understanding the link between selection history and heritability estimates, our study offers some strengths compared with single-population studies, since results can be cross-checked between relatively cold and warm treatments for tadpoles from both 'cold'- and 'warm'-selected regions. Thus, we expect cold-selected frogs not only to do better in terms of growth and development in a relatively cold rather than warm treatment, but also to outperform warm-selected frogs in the cold treatment and vice versa. Elsewhere (Ståhlberg *et al*, 2001), we have demonstrated that indeed this is the case, and here we exploit this result to show how their divergence impact on the estimates of the variance components for additive *vs* environmental variance and their combined effects on the heritability estimate.

Such a comparison shows one strong, straightforward result: the major determinant of variation in heritability is V_E. In our previous study, we identified the cold treatment as the temperature at which cold-adapted frogs grew relatively better than did the warm-selected frogs, whereas warm-selected frogs outperformed the cold-selected frogs by the same magnitude at both 15 and 20°C (Ståhlberg *et al*, 2001). Since cold-adapted frogs do better at 15 than 20°C we concluded that the warm selected frogs are likely to have an optimal temperature closer to 15 than 20°C. This corresponds with the result of the variation in V_E for warm-selected frogs (Figure 1b). Similar relationships have been described elsewhere (eg, Simons and Roff, 1994; Merilä, 1997; Hoffmann and Schiffer, 1998; Hoffmann and Merilä, 1999). Strong corresponding shifts were seen in h^2, demonstrating that in our study variation in V_E is a stronger determinant of h^2 than is variation in V_A.

For additive genetic variance, V_A, the trend is less congruent between our theoretical prediction and our empirical results. If selection erodes additive genetic variance, it should have done so more in the environment at which the population has been under the strongest selection. This runs counter to the result found in the warm-selected frogs, which showed the highest rather than lowest V_A estimates at their optimum, ie, 15°C. Furthermore, the same trend was observed in cold-selected tadpoles, suggesting that the corresponding additive genes are in

fact present in both populations. How, then, can two populations/regions have similar patterns in V_A between environments, but different trends in V_E? We do not know, but since the estimates are based on a full-sib design, both dominance and epistatic interactions could be part of the explanation and we had no way of estimating additive × dominance interactions. Furthermore, the change in the genetic variance components across treatments and between populations may be a result of low genetic correlation between the environments, which can be seen as evidence that the trait is influenced by different genes in different environments (Falconer and Mackay, 1996). Holloway *et al* (1990) suggested that the additive variance should increase when the organism was presented a novel environment. This increase should be due to the expression of genes that have not yet been exposed to natural selection. However, in our case, the temperatures experienced by the tadpoles are within the natural range, but the time they are exposed to selection at these temperatures are likely to differ. Thus, the environmental 'novelty' is likely to vary even if tadpoles from both cold and warm sampling regions are likely to have encountered all treatments through their history of selection. Our results therefore seem contrary to Holloway *et al*'s prediction, since both populations showed the same pattern with the highest V_A in a relatively common environment, and for one population this peak in V_A coincides with its temperature optimum (Figure 1a).

Our work demonstrates that additive and environmental variances and heritabilities may vary considerably even within the laboratory but between conditions to which the organism is more or less often exposed in the wild. In a recent review, Weigensberg and Roff (1996) conclude that estimates of heritability in the laboratory are often likely to agree well with those in the wild. However, this review was based primarily on homeotherms, that show relatively little environmentally induced trait variation. Thus, ectothermic models may provide higher resolution in tests of Weigensberg's and Roff's (1996) proposition, and in the analysis of environmental *vs* genetic determinants of trait expression. In our study, several heritability estimates seem unrealistic (eg, being 1.0 under optimal conditions). However, we again emphasize that we do not claim to have estimated natural heritabilities but rather attempt to contrast h^2, V_A, and V_E estimates between environments that we have good reasons to believe are better or worse for tadpole development, and in further support of such a comparison contrast the results from populations which will experience these environments differently in terms of being benign. In this respect, we believe that across-treatment, and between-population comparisons should serve as a useful guide for understanding the influence of selection history on components of heritability.

To conclude, our results indicate that environmental variance compared to additive genetic variance is the strongest determinant of variation in heritability. Under ideal developmental conditions, V_E approaches zero and heritability one in tadpoles from both our sampling regions.

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