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Genetic and maternal effect influences on viability of common frog tadpoles under different environmental conditions

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The influence of environmental stress on the expression of genetic and maternal effects on the viability traits has seldom been assessed in wild vertebrates. We have estimated genetic and maternal effects on the viability (viz probability of survival, probability of being deformed, and body size and shape) of common frog, *Rana temporaria*, tadpoles under stressful (low pH) and nonstressful (neutral pH) environmental conditions. A Bayesian analysis using generalized linear mixed models was applied to data from a factorial laboratory experiment. The expression of additive genetic variance was independent of pH treatments, and all traits were significantly heritable (survival: $h^2 \approx 0.08$; deformities: $h^2 \approx 0.26$; body size: $h^2 \approx 0.12$; body shape: $h^2 \approx 0.14$). Likewise, nonadditive genetic contributions to variation in all traits were significant, independent of pH treatments and typically of magnitude similar to the additive genetic effects. Maternal effects were large for all traits, especially for viability itself, and their expression was partly dependent on the environment. In the case of body size, the maternal effects were mediated largely through egg size. In general, the results give little evidence for the conjecture that environmental stress created by low pH would impact strongly on the genetic architecture of fitness-related traits in frogs, and hamper adaptation to stress caused by acidification. The low heritabilities and high dominance contributions conform to the pattern typical for traits subject to relatively strong directional selection.
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Introduction

Environmental changes caused by anthropogenic activities have exposed many wild plant and animal populations to increasing levels of environmental stress, and concern about the ability of organisms to adapt to these changes has grown steadily (eg, Bijlsma and Loeschcke, 1997; Hoffmann and Parsons, 1997; Forbes, 1999; Walther *et al*, 2002). Although quantitative genetic studies have revealed that most traits and populations harbor large amounts of additive genetic variation (eg, Mousseau and Roff, 1987; Houle, 1992), two issues of concern have emerged. First, the magnitude of environmental changes may be so large and/or the changes so rapid that genetic variability can become exhausted before adaptation is complete (Lynch and Lande, 1993; Bürger and Lynch, 1997). Second, environmental stress can reduce the expression of additive genetic variance and increase the environmental component of variance in a given trait, thereby constraining the process of adaptation in populations living under environmental stress (Hoffmann and Merilä, 1999). Hence, to make predictions about selection responses and the likelihood of genetic adaptation under stressful conditions, we need to understand not only the properties and dynamics of the fitness landscape (cf, Arnold *et al*, 2001), but also how the expression of genetic variation is modified by ecologically relevant stress situations.

Experimental work on the effects of environmental stress on heritability and the expression of additive genetic variation in metric traits is inconclusive (Hoffmann and Merilä, 1999; Hoffmann and Hercus, 2000). Both decreased and increased heritabilities under stressful environmental conditions have been observed, and the underlying causes have been attributed to changes in both the additive and the environmental components of variance (Hoffmann and Merilä, 1999). One complicating factor hampering generalizations about the effects of environmental stress on the quantitative genetic parameters is the heterogeneous methodologies employed by different studies. Many studies have utilized methods incapable of distinguishing additive genetic from nonadditive and maternal effect contributions, and consequently, the observed changes in heritability estimates cannot be unambiguously attributed to changes in any particular component of the phenotypic variance (Hoffmann and Merilä, 1999). For instance, two recent *Drosophila* studies (Bubliy *et al*, 2000, 2001) using methods capable of estimating pure V_A failed to confirm any environment dependency in the levels of V_A reported by some earlier studies (eg, Sgró and Hoffmann,

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1998), suggesting a role for nonadditive genetic effects in biasing the earlier results. Likewise, although parent–offspring regression estimates of heritability are in principle free from bias because of dominance effects, this method may also give biased estimates of heritability and V_A when the parents and offspring have been grown in different environments (Riska *et al*, 1989; Merilä, 1997; Merilä and Fry, 1998). Hence, excluding studies using methods open to alternative interpretations, the data available on the effects of environmental stress on quantitative genetic parameters are both numerically and taxonomically limited.

No amphibian studies on the effects of environmental stress on quantitative genetic parameters have yet been published, although the early aquatic developmental stages of amphibians are known to be extremely sensitive to various environmental stresses, such as ultraviolet-B radiation (Blaustein *et al*, 1998), chemical pollutants (Hecnar, 1995; Rosenshield *et al*, 1999; Bridges and Semlitsch, 2000) and acidification (Böhmer and Rahman, 1990). In fact, the recent global decline of amphibian populations (Alford and Richards, 1999; Houlahan *et al*, 2000) is believed to be – at least in part – a reflection of worldwide increase in the levels of these stresses (Alford and Richards, 1999; Corn, 2000). To this end, studies on the genetic basis of amphibian stress responses can be useful in elucidating the likelihood of adaptation to increasing levels of environmental stress, as well as in addressing the general question about the possible difference in evolutionary potential under stressful and nonstressful environmental conditions (Hoffmann and Merilä, 1999). An additional reason why amphibians are interesting in this context is that the literature suggests a pervasive role for maternal effects as determinants of amphibian fitness (review in Kaplan, 1998). Given that maternal effects have recently been advocated as a possible pathway for adaptation in a wide number of taxa (Mousseau and Fox, 1998), attempts to understand their potential role in adaptation to environmental stress could be rewarding. In fact, a number of studies have unravelled interactions between maternal effects and environmental conditions (Groeters and Dingle, 1987; Parichy and Kaplan, 1992; Einum and Fleming, 1999), suggesting that differential expression of maternal effects could be an important source of fitness variation under some, but not all, environmental conditions.

The aim of this study was to investigate the relative importance of genetic and maternal effects as determinants of phenotypic variation in viability and viability-related traits in the common frog *Rana temporaria*. In particular, our interest was to assess whether the expression of additive genetic and maternal variances would differ between stressful (low pH) and nonstressful (neutral pH) environmental conditions. In addition, as egg size has been recognized as an important determinant of hatchling performance in amphibians (Kaplan, 1998) and vertebrates in general (reviews in Mousseau and Fox, 1998), we investigated whether the maternal effects could be accounted for by egg size effects alone, or whether they are attributable to factors uncorrelated with egg size (eg, maternal provision of nutrients and/or hormones). To avoid the interpretational caveats characterizing many of the previous experiments on the effects of environmental stress on quantitative genetic parameters, we studied these questions in a factorial laboratory experiment using a half-sib crossing design allowing estimation of heritabilites and additive genetic variances free of bias introduced by dominance and maternal effects, and by subjecting the data to a Bayesian generalized mixed model analysis.

Materials and methods

Study species and crosses

The common frog is a medium-sized anuran which, in our study area in central Sweden, breeds in small ponds and shallow lakeshores shortly after snow melt, usually in mid-April. Although common frogs seem to avoid low pH habitats, they do occur in ponds subject to natural or anthropogenic acidification (eg, Aston *et al*, 1987; Räsänen *et al*, 2002), and populations in northern Scandinavia are subject to 'acid pulses' (Reader and Dempsey, 1989; AMAP, 1998) caused by snow-melt water reaching their breeding ponds. Low pH is known to lead to reduced survival, increased frequency of developmental anomalies, delayed development and decreased growth rate in common frog tadpoles (eg Cummins, 1986; Andrén *et al*, 1988; Tyler-Jones *et al*, 1989; Räsänen *et al*, 2002).

Adult frogs forming the parental generation of this study were collected from two closely situated localities (to avoid creating too much disturbance in a single locality) near Uppsala, Central Sweden (Häggedalen, 59°51′ N, 17°14′ E and Gullsmyra, 60°70′ N, 16°56′ E) 17–19 April 2000. They were maintained in +4°C (ca. 5 days) until crossed artificially (see below) in the laboratory according to a North Carolina I (NC I) design (Kearsey and Pooni, 1996). In brief, we created paternal half-sib families by crossing 30 males each with two different females, producing altogether 60 families. From each male, a sperm suspension was prepared on a Petri dish with 3 ml of 10% Amphibian Ringer solution (Rugh, 1962). The eggs from two different females were stripped on two separate vials, ca. 1 ml of the sperm suspension was added, and the fertilized eggs were gently shaken. After 5 min, more Ringer solution was added to cover the eggs, and after 20 min, the solution was replaced with reconstituted soft water (RSW; APHA, 1985). At 1 h after the fertilizations, a sample of about 30 eggs was photographed for later measurement of the average egg size of each female. Of the remaining eggs, about 250 eggs per cross (ca. 15 000 eggs in total) were used in this experiment. The mean egg size differed significantly among females (ANOVA, $F_{57,1773} = 68.76$, $P < 0.001$), and the differences were related to differences in female body size: larger females tended to have larger eggs (ANCOVA, female identity: $F_{56,1773} = 59.32$, $P < 0.001$; female length: $F_{1,1773} = 30.22$, $P < 0.001$).

Rearing of the eggs

The eggs from each cross were divided into two pH treatments, five replicates per treatment, each replicate with approximately 25 eggs (mean = 24.4 eggs; range = 15–51), and reared in 0.9 l opaque plastic vials in RSW. The pH treatments were selected based on earlier experience and information from the literature to represent stressful (pH 4.6) and nonstressful (pH 7.6) environmental conditions (Andrén *et al*, 1988; Räsänen *et al*, 2002). The water for the low pH treatment was

prepared in 200 l tuns by adding 0.1–1 M H_2SO_4 (adjusting with 0.1–1 M NaOH) to RSW and stabilized and aerated for at least 48 h before use. The water for the neutral pH treatment was prepared in the same way, but without adding any acid.

The vials were placed in a shelf-system divided into five horizontal blocks to control for the effect of varying temperature on embryonic development. Two vials per cross, one from each pH treatment, were placed in each block in a randomized order. Water in the vials was changed every third day to keep the rearing conditions constant. The temperature in the blocks varied slightly between the warmest (uppermost) block (17.9°C ± SD 0.35) and coldest (lowest) block (17.1°C ± SD 0.34). Water pH was monitored daily from several vials with an Orion pH meter (model 250A) equipped with a Ross Sure-flow electrode (model 8172 BN). Slight variations in pH occurred during the course of the experiment, but this variation was small relative to the difference in treatment means.

The embryos were reared until the majority of the individuals in each vial had reached growth stage 25 (absorption of external gills and fully developed operculum; Gosner, 1960). For each replicate, we determined the proportion of surviving and anomalous (coiled or extremely shortened tail, asymmetric body, etc) tadpoles. Two normal individuals from each vial were randomly sampled and stored in 70% ethanol, and the experiment was terminated. Four morphological characters were measured on each sampled tadpole with a stereomicroscope fitted with an ocular micrometer. These were: body length (from the tip of the nose to the end of the body wall), maximum body depth, tail length (from the body terminus to the tip of the tail) and maximum tail depth. All measurements were made by the same person to avoid interobserver variation. To reduce variation in the morphological measurements into fewer uncorrelated variables, the measurements were subjected to a principal component analysis. The first component (PC1) accounted for 79.6% of the variation and was equally highly and postively loaded with all characters (factor loadings: 0.48–0.51), and thus can be interpreted as reflecting tadpole size. The second component (PC2) accounted for 9% of the total variation, and it reflected the contrast between tail length (loading: −0.86) and the other characteristics (loadings: 0.21–0.42), and consequently was a shape component.

Statistical analyses

Number of survivors and anomalous individuals: The number of survivors and anomalous individuals were modelled using a generalized linear mixed model (Breslow and Clayton, 1993) and fitted with a Bayesian approach. The numbers of normal, abnormal and dead individuals were modelled as a nested response (McCullagh and Nelder, 1989). At the first level, individuals are either normal or not, and at the second level those that are not normal are either abnormal or dead. The number of normal tadpoles in batch i, n_i, was assumed to follow a binomial distribution, that is $n_i \sim \mathrm{Bin}(N_i, p_i)$, where p_i is the probability of a single tadpole in batch i being normal and N_i is the total number of individuals. Similarly, if the number of abnormal (but live) tadpoles is m_i, then we assumed $m_i \sim \mathrm{Bin}(N_i\text{-}n_i, q_i)$, where q_i is the probability of a single tadpole being abnormal, given that it is not classed as normal (the probability of it being abnormal is then $q_i/(1\text{-}p_i)$). We modelled p_i and q_i to study whether egg size and genetic and environmental effects on these probabilities would vary across environments. The structures of the two models are similar, and they are presented in terms of the numbers of normal tadpoles. The extension to the abnormal/dead category is obvious.

The dependence of p_i on the factors is modelled as follows:

$$\log\left(\frac{p_i}{1-p_i}\right) = \eta_i + \varepsilon_i$$
$$\eta_i = \mu_a + (g_i - \bar{g})\beta_a + \phi_b + \alpha_{f,a} \quad (1)$$

where μ_a is the mean survival at pH a ($a = 1$ for high pH, 2 for low pH), g_i is the mean size of eggs measured from the ith female, $\bar{g}$ is the mean egg size and β_a is the regression coefficient for the egg size at pH a. ϕ_b is the effect of the bth block. Both β_a and ϕ_b are assumed to be constant across females. $\alpha_{f,a}$ is the mean effect of the fth female at pH a, and this contains the genetic variance and is modelled further (below). ε_i captures any residual variance (ie environmental variation).

ϕ_b, α_f and ε_i are modelled as random effects as follows:

$$\phi_b \sim N(0, v_b)$$
$$\alpha_f \sim N(\varphi_m, v_\mathrm{d}) \quad (2)$$
$$\varphi_m \sim N(0, v_\mathrm{s})$$
$$\varepsilon_i \sim N(0, v_\mathrm{e})$$

where $A \sim N(m, v)$ means that A follows a normal distribution with mean m and variance v. Here α_f is nested within φ_m. From this we obtain estimates of three 'statistical' variance components, the variance among sires (v_s), among dams within sires (v_d) and within full sib families (v_e), that can be related to the four 'biological' variance components (Lynch and Walsh, 1998) as:

$$v_\mathrm{s} = \tfrac{1}{4}\sigma^2_\mathrm{A}$$
$$v_\mathrm{d} = \tfrac{1}{4}\sigma^2_\mathrm{A} + \tfrac{1}{4}\sigma^2_\mathrm{D} + \sigma^2_{\mathrm{E_C}} \quad (3)$$
$$v_\mathrm{e} = \tfrac{1}{2}\sigma^2_\mathrm{A} + \tfrac{3}{4}\sigma^2_\mathrm{D} + \sigma^2_{\mathrm{E_S}}$$

where σ^2_A is the additive genetic variance, σ^2_D is the dominance genetic variance, $\sigma^2_{\mathrm{E_C}}$ is the environmental variance because of a common environment (including maternal effects) and $\sigma^2_{\mathrm{E_S}}$ is the environmental variance within a single family (here a single vial).

The model was fitted using a Bayesian approach (Gelman *et al*, 1995). This provides the advantage that we can estimate the full posterior distribution of the estimates of the biological variance components directly, whereas in a classical analysis the likelihood is maximized for v_s, v_d and v_e and then σ^2_A, σ^2_D, $\sigma^2_{\mathrm{E_C}}$ and $\sigma^2_{\mathrm{E_S}}$ are calculated from the estimated values. It should be noted that the estimates of the genetic variance components are not independent but there are four unknown components being estimated from three statistical components. Taking a Bayesian approach allows us to estimate the components, but the estimates are correlated, and therefore the interpretation (especially of joint distributions) must be made with care.

μ_a and β_a were given normally distributed priors with mean 0 and variance of 1.5 and 1, respectively (a variance of 1.5 was used for μ_a because it gives an almost flat prior on the probability scale). σ^2_A, σ^2_D, $\sigma^2_{E_C}$ and $\sigma^2_{E_S}$ were all given inverse gamma-distributed priors, with shape and scale parameters being equal to 1. Missing values (for instance, in cases when all sampled individuals were anomalous) were estimated using multiple imputation, which means that the missing observations were treated as another parameter to be estimated (Gelman *et al*, 1995). As we have more information about egg sizes, the priors were made less vague, and were set as normal distributions with mean 1.8 and variance 0.2. The model was fitted using the WinBugs package (Spiegelhalter *et al*, 1999).

Body size and shape

To break variation in PC1 and PC2 down into genotypic and phenotypic components, a generalized linear mixed model (Breslow and Clayton, 1993) was employed to each component separately. The trait value was assumed to follow a normal distribution with mean η_i, and variance σ^2_ε and η_i, was decomposed in the same way as in equation (2) above. This is the classical quantitative genetic model (Lynch and Walsh, 1998), except that the model was fitted using a Bayesian rather than a frequentist approach. Normal distributions with mean 0 and variance 100 were used as priors for the regression coefficients for the effect of egg size on the principal components. For the variance components, inverse gamma distributions with shape and scale parameters of 1 were used, as above.

To investigate whether maternal effects were mediated solely through egg size or whether they included other types of effects, we ran the analyses both with and without egg size included into the models as a covariate. Since the variance owing to egg size was always (when not in the model) captured mainly by the maternal effect term, we only present the results of models including the egg size term understanding that variance because of this term would be otherwise absorbed by the maternal effects term. The block effects are included in all models to control for small-scale temperature heterogeneity inherit to our experimental setup, and although these effects do not have any straightforward general biological interpretation outside our experimental setup, they give hints about the potential sensitivity of different traits to small variations in temperature.

In general, we assessed the significance of different effects, as well as differences between different variance component estimates, from the 2.5 and 97.5% percentiles obtained from the analyses. For ease of presentation, however, we have sometimes referred to probability values in comparisons of different effects, and they were obtained by taking the proportion of iterations in which the sample drawn from the posterior was greater (or less than) the test value.

Results

Treatment effects on trait means

Survival from fertilization until the end of the experiment was lower in low pH (70.7%) than in neutral pH (75.3%), but this difference was not significant ($P = 0.097$; Figure 1a). Likewise, developmental anomalies were rare in both pH treatments (neutral pH: 2.1%; low pH: 1.6%), and their frequencies did not differ between the treatments ($P = 0.087$; Figure 1b). Tadpoles raised in neutral pH attained a significantly (5.14% (95% CI = 2.36–7.50%); $P < 0.001$) larger size than their sibs raised in low pH (Figure 1c), but there was no difference in tadpole shape between the two treatments ($P = 0.46$; Figure 1d). Hence, as evidenced by smaller size and tendency towards lower survival, tadpoles experienced the low pH treatment as more stressful environment than the neutral pH treatment.

Variance component analyses

Survival: Both the relative (Figure 2a,b) and absolute (Table 1) magnitudes of different causal components of variance for survival were similar in the two pH treatments. Maternal effects accounted for most (ca. 70%) of the phenotypic variation in probability of survival, whereas additive and nonadditive genetic effects – which were of roughly similar magnitude in their effects – accounted for 7 and 6% of the phenotypic variation in probability of survival, respectively (Figure 1a,b). Egg size had no effect on survival (Figure 2a,b), suggesting that the maternal effects on survival were not mediated through egg size effects. The block effect accounted for 11.6 and 13.4% of the variance in neutral and low pH, respectively.

Developmental anomalies: As in the case of survival, the relative (Figure 1c,d) and absolute (Table 1)

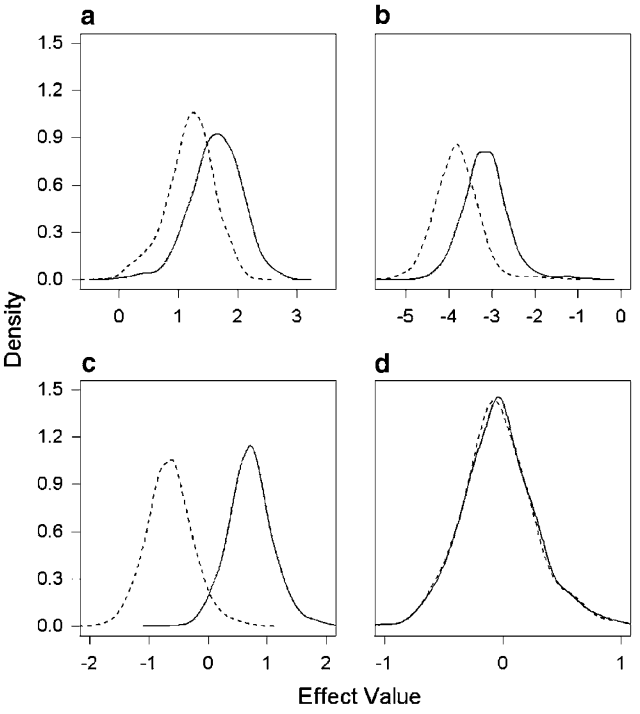

Figure 1 The effect of pH treatment on trait means: (**a**) Tadpole survival, (**b**) probability of being anomalous, (**c**) body size and (**d**) body shape. Graphed are posterior probability distributions from Bayesian models depicting the overall mean effect sizes (effect value; that is log odds of probabilities of survival and abnormality, and body size and shape). Solid line: neutral pH, dashed line: low pH.

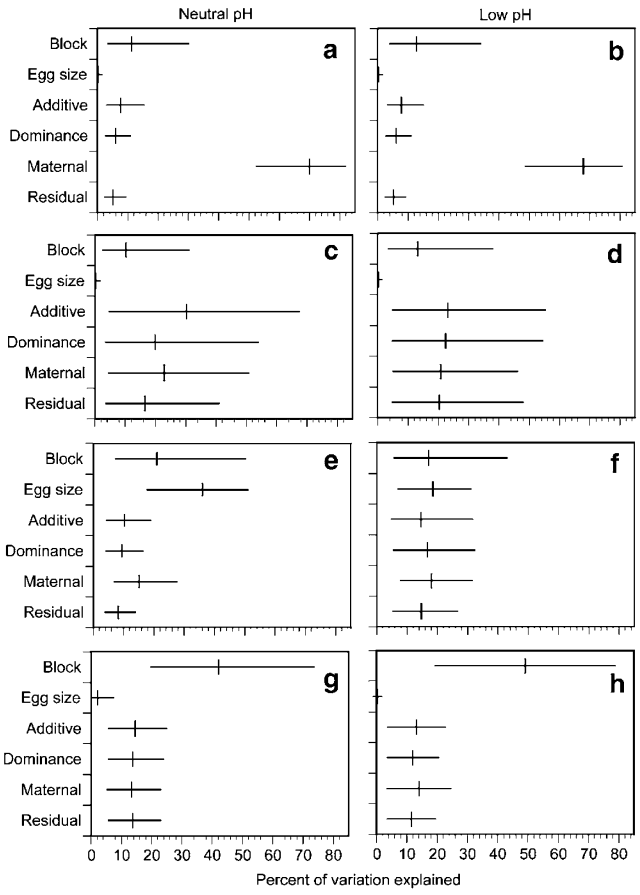

Figure 2 Relative magnitudes of different causal components of variance for different traits in two pH treatments. (**a,b**) Tadpole survival, (**c,d**) probability of being anomalous, (**e,f**) body size and (**g,h**) body shape. The bars indicate 2.5 and 97.5% percentiles.

magnitudes of different causal components of variance in the probability of being abnormal were roughly equal in the two pH treatments. Within a given treatment, maternal, additive and nonadditive effects were all significant and of approximately equal magnitude in their effects (Figure 2c,d). However, the heritabilities (posterior means: $h^2_{\text{Neutral}} = 30.2\%$; $h^2_{\text{Low}} = 23.1\%$), and also other components of variance, were less well estimated for the probability of being abnormal than for the probability of an individual being alive because of the low numbers of abnormal individuals. Egg size had no effect on the probability of being abnormal (Figure 2c,d). The block effect accounted for 10.6 and 13.7% of the total variation in deformation occurrence in neutral and in low pH, respectively.

Body size: Although the mean body size of tadpoles was strongly reduced by the low pH treatment (Figure 1c), the different causal components of variance did not differ significantly among treatments in either an absolute (Table 1) or in a relative scale (Figure 2e,f). The positive effect of egg size on body size observed under both treatments tended to be more pronounced in the neutral than in the low pH treatment (Table 1), but this difference was not significant ($P = 0.16$). Additive ($h^2_{\text{Neutral}} = 10.2\%$; $h^2_{\text{Low}} = 14.6\%$), nonadditive and maternal effect contributions not related to egg size were of similar magnitude within and between the two treatments (Figure 2e,f). Summing up the egg size-related and egg size-independent maternal effects, they accounted for 51.2 and 36.8% of the variation in tadpole size in neutral and low pH, respectively, suggesting an overwhelming role for maternal effects as determinants of body size variation.

Body shape: The variance components owing to additive ($h^2_{\text{Neutral}} = 14.5\%$; $h^2_{\text{Low}} = 13.3\%$), nonadditive and maternal effects on body shape were of roughly similar magnitude in both treatments (Table 1; Figure 2g,h). In contrast to body size, egg size effects were very small, but, surprisingly, almost significantly ($P = 0.05$) different in the two pH treatments (Table 1). In the neutral pH treatment, larger eggs produced tadpoles

Table 1 Mean values of phenotypic variance components together with 2.5 and 97.5% percentiles for tadpole survival, the occurrence of developmental anomalies, size (PC1) and shape (PC2) under different environmental conditions

Source	*Neutral*		*Low*		*Neutral*		*Low*	
	Mean	*2.5–97.5%*	*Mean*	*2.5–97.5%*	*Mean*	*2.5–97.5%*	*Mean*	*2.5–97.5%*
	Survival				*Anomalies*			
Total variance	4.399		3.787		6.970		5.407	
Block	0.509	0.142–1.590	0.509	0.142–1.590	0.742	0.185–2.490	0.742	0.185–2.490
Egg size	0.010	<0.001–0.050	0.009	<0.001–0.045	0.018	<0.001–0.090	0.015	<0.001–0.073
Additive	0.326	0.146–0.624	0.284	0.135–0.518	2.110	0.324–5.610	1.24	0.260–3.390
Dominance	0.254	0.128–0.445	0.222	0.114–0.384	1.370	0.255–4.080	1.20	0.256–3.340
Maternal	3.080	1.960–4.690	2.570	1.610–3.900	1.590	0.313–4.030	1.12	0.267–2.920
Within family	0.220	0.115–0.376	0.193	0.105–0.320	1.140	0.256–3.130	1.09	0.260–2.870
	Size				*Shape*			
Total variance	3.040		3.740		1.080		0.931	
Block	0.698	0.191–2.260	0.698	0.191–2.260	0.510	0.143–1.590	0.507	0.143–1.590
Egg size	1.080	0.536–1.640	0.689	0.260–1.290	0.023	<0.001–0.073	0.003	<0.001–0.013
Additive	0.300	0.140–0.532	0.539	0.190–1.150	0.144	0.085–0.222	0.109	0.070–0.160
Dominance	0.276	0.139–0.450	0.612	0.221–1.120	0.137	0.084–0.204	0.100	0.066–0.140
Maternal	0.444	0.230–0.769	0.671	0.299–1.210	0.134	0.078–0.219	0.117	0.071–0.187
Within family	0.242	0.131–0.376	0.535	0.213–0.920	0.137	0.089–0.191	0.095	0.065–0.130

with relatively long tails, but this was not the case in low pH treatment (Table 1). Block effects on body shape were large (Figure 2g,h), suggesting that small variations in developmental temperature had a large effect in determining tadpole body proportions.

Discussion

The most salient features of our results were that all traits – including viability itself – were significantly heritable under the environmental conditions tested, and that the relative magnitudes of additive and nonadditive genetic contributions to variability in a given trait were typically about equal. In general, there was very little evidence for drastic environment dependency in the expression of genetic variability in any of the traits, but there was some evidence for the environment dependency of maternal effects. In what follows, we will discuss the implications of each of these findings in turn, and, in particular, in the context of the current debate on the heritability of different types of traits under different environmental conditions.

A general picture emerging from studies of wild animal populations is that the closer association a trait has with fitness, the lower its heritability is likely to be (Mousseau and Roff, 1987; Houle, 1992; Merilä and Sheldon, 1999, 2000; Kruuk *et al*, 2000; Stirling *et al*, 2002). The results of the present study conform to this general pattern: all four fitness-associated traits were significantly heritable, but the heritabilities were low. However, as in the case of most earlier studies – barring few exceptions (eg Kruuk *et al*, 2000; Merilä and Sheldon, 2000) – we have no objective way of ordering the different traits according to their impact on fitness. Clearly, viability itself must be a very strong correlate of fitness, and the same is probably true in the case of the probability of being abnormal: survival of deformed tadpoles in this species is known to be very low (Beattie *et al*, 1992), which is also suggested by our finding that there was a positive correlation between the posterior probability of a tadpole being alive, and its being abnormal. Also, tadpole body size is likely to be an important component of fitness as it is positively correlated with size at metamorphosis (Kaplan, 1992; Semlitsch and Schmiedehausen, 1994), which in turn correlates positively with further survival probability (Altwegg and Reyer, 2003 and references therein), size at maturity (Smith, 1987) and fecundity (Smith, 1987; Semlitsch *et al*, 1988). The relation between body shape and fitness is less clear, but we note that body shape differences are an important component of antipredator defences in frog tadpoles (Lardner, 2000; Relyea, 2001), and predation is one of the most important sources of mortality in larval amphibians (Newman, 1992). Whatever the relative importance of these traits in their contribution to fitness, our results show that they exhibit heritabilities typical of fitness traits.

An interesting feature of our results was that the size of the dominance genetic component in all traits was approximately of the same size as the additive genetic component in the same trait. There is a fairly large amount of nonadditive genetic variation corresponding to an average coefficient of dominance variance ($CV_D = V_D/(V_A+V_D)$; Crnokrak and Roff, 1995) of ca. 0.47 (range = 0.39–0.53). Although the data are still scanty, Crnokrak and Roff (1995) found that dominance genetic contributions to fitness traits were larger for traits closely associated with fitness than those less closely associated with fitness. Such a pattern is to be expected under the scenario where directional selection erodes additive genetic variance from traits closely related to fitness, thereby increasing the relative proportion of nonadditive variance to the total genetic variance (Merilä and Sheldon, 1999). The high dominance contributions observed in this study are fully consistent with this reasoning, and hint also about relatively high load of recessive harmful/deleterious mutations segregating in our study population. This genetic load is a potential source of inbreeding depression for which evidence from wild populations has been accumulating rapidly during the past decades (Crnokrak and Roff, 1999; Hedrick and Kalinowski, 2000; Keller and Waller, 2002). However, as our breeding design does not allow dominance variance to be estimated independently of other causal components of variance (cf. equation (3)), some caution is needed in interpretation. Nevertheless, as the size of the dominance contributions were fairly similar in both pH treatments, our results do not suggest that the effects of inbreeding depression for the traits studied would differ depending on the pH to which developing tadpoles are exposed.

Although there is evidence that stress-dependent changes in the expression of genetic variability are common (Hoffmann and Merilä, 1999; Hoffmann and Hercus, 2000), we found little evidence for stress-dependent changes in heritability estimates or underlying causal components of variance. It is unlikely that the environmental stress in our experiment was insufficient: tadpole size was significantly reduced in the low pH treatment as compared to neutral pH treatment, which suggests that tadpoles did experience the low pH treatment as stressful. Hence, varying amounts of environmental stress did not seem to influence the expression of genetic variability in the traits studied. This is consistent with the results of other recent studies that have failed to detect stress-dependent differences in the expression of additive genetic variation (Bubliy *et al*, 2000, 2001). However, it is worth pointing out that our results cannot be generalized to other types of environmental stresses: although different types of stresses can cause the same kind of changes in phenotypic variation (Imasheva *et al*, 1998, 1999), responses to different types of stressors are not necessarily genetically correlated (Dahlgaard and Hoffmann, 2000).

Apart from the small and significant genetic effects, a feature central to our results is the pervasive role of maternal effects as determinants of variation in all traits. In the case of tadpole survival, maternal effects were the most important source of variation accounting for 70% of the variance in the probability of survival. Maternal effects were also important for the probability of being abnormal, but here their estimated effects were of the same magnitude as the genetic effects. In the case of both of these traits, the maternal effects were largely independent of egg size effects, suggesting that viability and developmental stability are not strongly linked to egg size effects *per se*. In contrast, the maternal effects acting on body size and shape were more strongly mediated through egg size effects, and in both cases indications of environmental dependency were obtained. The positive effect of egg size on tadpole size tended to be more

pronounced at neutral pH than at low pH. Evidence for similar environment-dependent expression of maternal/parental effects is also available for other amphibians (Kaplan, 1992; Parichy and Kaplan, 1992) and a wide range of other taxa (eg Einum and Fleming, 1999). Hence, maternal effects are a possible means if improving the fitness of common frog offspring being subjected to acid stress.

In contrast to tadpole size, tadpole shape was not affected by pH treatment. This does not mean that tadpole shape would be more canalised than tadpole size, as the tadpole shape was strongly affected by the block effect, suggesting that small differences in temperature had large effects on tadpole shape. In fact, correlating the mean tadpole shape per block against the block-specific average temperature revealed a tendency for a positive correlation ($r = 0.64$, $n = 5$, $P = 0.087$) between tadpole shape and temperature. This is a well-known phenomenon from amphibians: tadpoles grown under cooler temperatures tend to grow longer tails than those grown under warmer temperatures (Kaplan, 1992).

In conclusion, the results of this study give little evidence to support the conjecture that environmental stress created by low pH would impact strongly on the genetic architecture of fitness-related traits in frogs, and thereby hamper adaptation to stress caused by acidification. On the contrary, our analyses suggest that viability and viability-related traits are heritable under a wide range of pH values, although the heritability of these traits is low, and quite easily over-ridden in importance by maternal and/or environmental effects. The low heritabilities and high dominance contributions in all traits conform to the pattern typical of fitness traits, and suggest that these traits are subject to relatively strong directional selection and inbreeding depression.

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