Quantitative genetics of larval life-history traits in *Rana temporaria* in different environmental conditions

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Summary

The degree to which genetic variation in a given trait varies among different populations of the same species and across different environments has seldom been quantified in wild vertebrate species. We investigated the expression of genetic variability and maternal effects in three larval life-history traits of the amphibian *Rana temporaria*. In a factorial laboratory experiment, five widely separated populations (max. 1600 km) were subjected to two different environmental treatments. Animal model analyses revealed that all traits were heritable ($h^2 \approx 0.20$) in all populations and under most treatment combinations. Although the cross-food treatment genetic correlations were close to unity, heritabilities under a restricted food regime tended to be lower than those under an *ad libitum* food regime. Likewise, maternal effects ($m^2 \approx 0.05$) were detected in most traits, and they tended to be most pronounced under restricted food conditions. We detected several cross-temperature genetic and maternal effects correlations that were lower than unity, suggesting that genotype–environment interactions and maternal effect–environment interactions are a significant source of phenotypic variation. The results reinforce the perspective that although the expression of genetic and maternal effects may be relatively homogeneous across different populations of the same species, local variation in environmental conditions can lead to significant variation in phenotypic expression of quantitative traits through genotype–environment and maternal effect–environment interactions.

1. Introduction

Intraspecific studies of geographic variation have provided convincing evidence for the occurrence of microevolution as a response to local ecological conditions (e.g. Endler, 1977; Chapin & Chapin, 1981; Conover & Schultz, 1995; Huey *et al.*, 2000; Reznick & Ghalambor, 2001). Differentiation in mean trait values may also be associated with population divergence in genetic architecture (e.g. Roff, 2000), leading to among-population heterogeneity in the heritability of different traits, and thereby also to differential capacity of populations to respond to selection in the future. Likewise, genotype–environment interactions are widespread (e.g. Schlichting & Pigliucci, 1998), and the expression of different causal components of phenotypic variance and heritability may differ widely depending on the environmental conditions under which they have been estimated (e.g. Hoffmann & Merilä, 1999). Environmental maternal effects comprise an additional source of phenotypic variation, which may vary in their strength across different populations and environments (e.g. Parichy & Kaplan, 1992; Einum & Fleming, 1999). However, in general,

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Table 1. *Mean age and size at metamorphosis and larval growth rate in five* Rana temporaria *populations in different temperature and food treatments*

Population	Restricted food level						Ad libitum food level					
	14 °C		18 °C		22 °C		14 °C		18 °C		22 °C	
	n	*x*	*n*	*x*	*n*	*x*	*n*	*x*	*n*	*x*	*n*	*x*
Age at metamorphosis (days)												
Lund	238	65·2	226	42·9	220	35·2	218	64·1	146	39·3	114	29·2
Uppsala	79	62·6	58	40·1	89	32·9	54	64·9	51	34·7	60	27·0
Umeå	232	60·3	256	38·4	181	29·5	188	59·4	203	33·8	107	23·9
Kiruna	133	55·4	150	37·3	161	25·3	85	54·5	109	30·7	129	21·1
Kilpisjärvi	182	52·4	159	35·7	163	22·8	125	51·7	118	29·8	163	19·4
Size at metamorphosis (g)												
Lund	238	0·58	226	0·29	220	0·23	218	0·77	146	0·51	114	0·41
Uppsala	79	0·66	58	0·33	89	0·27	54	0·99	51	0·65	60	0·49
Umeå	232	0·64	256	0·32	181	0·32	188	1·00	203	0·64	107	0·45
Kiruna	133	0·48	150	0·31	161	0·25	85	0·91	109	0·57	129	0·47
Kilpisjärvi	182	0·46	159	0·29	163	0·24	125	0·76	118	0·59	163	0·44
Growth rate (mg/day)												
Lund	238	8·9	226	6·8	220	6·5	218	12·0	146	13·0	114	14·0
Uppsala	79	10·5	58	8·2	89	8·2	54	15·3	51	18·7	60	18·1
Umeå	232	10·6	256	8·3	181	10·8	188	16·8	203	18·9	107	19·8
Kiruna	133	8·7	150	8·3	161	9·9	85	16·7	109	18·6	129	22·3
Kilpisjärvi	182	8·8	159	8·1	163	10·5	125	14·7	118	19·8	163	22·7

n, number of individuals measured.

differences in the relative roles of genetic, environmental and maternal influences on the phenotypic expression of quantitative traits across multiple populations have seldom been explored.

While line-cross studies have found evidence for among-population heterogeneity in the genetic architecture of trait expression (e.g. Armbruster *et al.*, 1997), only a few attempts have been made to assess the extent to which heritabilities and their underlying causal components vary between populations (but see: Morgan *et al.*, 2001; Etterson, 2004) or between environments (reviews in Hoffmann & Merilä, 1999). This is certainly the case for wild vertebrate populations; most studies of intraspecific variation in quantitative trait parameters in vertebrates have so far been restricted to two population comparisons (e.g. Laurila *et al.*, 2002; Uller *et al.*, 2002; but see: Berven & Gill, 1983; Haugen & Vøllestad, 2000) and designs not allowing the separation of additive effects from non-additive and maternal/common environment influences.

The aim of this study was to assess the relative importance of additive genetic, maternal and environmental effects on phenotypic variation in both age and size at metamorphosis as well as larval growth rate, under different environmental conditions in five common frog populations collected along a 1600 km long latitudinal gradient across Scandinavia. We did this by performing common garden experiments with a half-sib breeding design (termed a North

Carolina II design; Lynch & Walsh, 1998) at several temperature and food treatments, and subjecting the data to 'animal model' analyses. In particular, we were interested in investigating (1) the extent to which the five populations differed in the amount of genetic variation underlying larval life-history traits, (2) the effect of different environmental conditions on heritability and maternal effects in these traits and (3) how genotype–environment interactions influence phenotypic variation.

2. Materials and methods

(i) *The study species and populations*

The common frog (*Rana temporaria*) is the most widespread anuran in Europe (Fog *et al.*, 1997), and thereby experiences a wide range of environmental conditions throughout its distribution range (e.g. Miaud & Merilä, 2001). The five populations included in the laboratory study – situated along a latitudinal gradient from southern Sweden to northern Finland – were: Lund (55°42′N, 13°26′E), Uppsala (59°51′N, 17°14′E), Umeå (63°49′N, 20°14′E), Kiruna (67°51′N, 21°02′E) and Kilpisjärvi (69°03′N, 20°47′E). Laugen *et al.* 2003 provide a map of the study populations. All these populations bred in medium-sized ponds (maximum depth <1·6 m) with populations of 50–150 breeding females. The onset of the breeding season between the southern- and northernmost
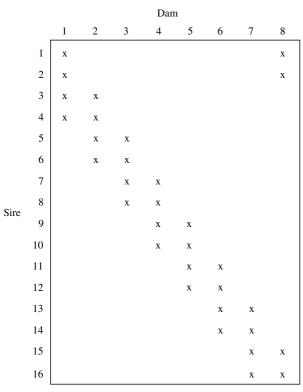
Dam

	1	2	3	4	5	6	7	8
1	x							x
2	x							x
3	x	x						
4	x	x						
5		x	x					
6		x	x					
7			x	x				
8			x	x				
9				x	x			
10				x	x			
11					x	x		
12					x	x		
13						x	x	
14						x	x	
15							x	x
16							x	x

Sire

Fig. 1. Schematic presentation of the partial North Carolina II breeding design utilized in this study.

localities differs by approximately 60 days, and there is a twofold difference in the length of growth season between these localities (see Laugen *et al.*, 2003, their table 1).

(ii) *Experimental design*

Tadpoles used in the experiment were obtained using laboratory crossings of adults collected from spawning sites at the onset of breeding season. We adopted a partial cross-classified breeding design (Fig. 1). Within each population, eight females were each mated to four of 16 males, and each male was mated to two females, yielding 32 maternal and paternal half-sib families. The Umeå tadpoles stem from 64 maternal half-sib families (16 females and 32 males used). Due to the large difference in the onset of spawning among the populations (see: Laugen *et al.*, 2003, their table 1), the starting dates for the experiment also differed. In the southernmost population (Lund), the fertilizations were performed on 9 April 1998, whereas in the northernmost population (Kilpisjärvi) the corresponding date was 4 June. However, progeny from the different populations were reared according to a common protocol (see below).

The crossings were carried out following the principles outlined in Laugen *et al.* (2002, 2003). One hour after fertilization, eggs were detached from the dish and a sample of 15–30 eggs from each family was stored in 4% formaldehyde for later determination of egg size. The remaining eggs were divided into three different temperature treatments (14, 18 and 22 °C [± 1 °C], two bowls per family in each temperature), where they were kept until hatching. These three temperature treatments were selected to represent the normal temperature range that tadpoles may experience in the wild throughout the geographical area included in this study. Water was changed every third day during embryonic development. When the majority of the embryos in a given temperature treatment had reached Gosner stage 25 (Gosner, 1960), eight randomly chosen tadpoles from each family and temperature were placed individually in 0·9 l opaque plastic containers in each of two food levels (restricted and *ad libitum*). Within each temperature treatment we assigned two tadpoles per family to each of four different blocks. The tadpoles were fed a finely ground 1:3 mixture of fish flakes (TetraMin, Ulrich Baensch, Germany) and rodent pellets (AB Joh. Hansson, Uppsala, Sweden) every seventh day. The amount of food given to each tadpole was 15 (restricted) and 45 (*ad libitum*) mg for the first week, 30 and 90 mg for the second week, and 60 and 180 mg per week thereafter until metamorphosis. The *ad libitum* level was selected to be such that in all temperatures the individuals could not consume all the food before the next feeding. In the restricted food treatment, the tadpoles in the two highest temperature treatments consumed all their food resources before the next feeding indicating food limitation, but in the low-temperature treatment tadpoles frequently had food left even after seven days from feeding. Tadpoles were raised in dechlorinated tap water, aerated and aged for at least 24 h before use, and water was completely changed every seventh day in conjunction with feeding. The light rhythm was 16L:8D. Close to metamorphosis, the tadpoles were checked once a day, and individuals that had reached Gosner stage 42 (Gosner, 1960) were noted.

Response variables measured from each tadpole were age at metamorphosis (defined as the number of days elapsed between Gosner stages 25 and 42), size at metamorphosis (blotted wet weight measured to the nearest 10^{-4} g) and mean larval growth rate (size divided by age).

(iii) *Statistical and quantitative genetic analyses*

The phenotypic variance (V_P) was partitioned as given in the following equation:

$$V_P = V_A + V_M + V_R$$

where V_A was the additive genetic variance, V_M was the effect due to variance between mothers, and V_R was the residual variance (Falconer & Mackay, 1996).

Table 2. *Log likelihood variance components for age, size and growth rate at/until metamorphosis (a) in five different Rana temporaria populations and (b) at different temperature and food level treatments*

	Age			Size			Growth		
(a)	V_A	V_M	V_R	V_A	V_M	V_R	V_A	V_M	V_R
Lund	5·44	0·37	15·05	9·01	1·02	32·19	4·77	0·00	45·89
Uppsala	2·91	0·00	6·58	11·88	0·00	17·75	8·10	3·53	25·68
Umeå	1·63	2·84	8·40	5·71	1·23	32·68	3·74	3·09	40·42
Kiruna	3·74	0·00	7·44	8·14	0·00	29·60	1·40	3·42	41·14
Kilpisjärvi	1·58	0·36	8·50	4·52	0·00	32·12	4·14	0·00	41·00
(b)	V_A	V_M	V_R	V_A	V_M	V_R	V_A	V_M	V_R
14 °C	4·77	1·36	8·00	8·58	1·39	32·66	10·86	3·30	44·28
18 °C	3·81	1·33	9·44	6·10	1·12	28·87	3·92	1·36	39·44
22 °C	5·23	0·61	8·64	10·26	0·65	40·63	4·27	1·21	46·31
Ad libitum	2·69	1·34	10·57	14·26	0·01	28·03	11·37	1·33	41·83
Restricted	3·30	0·90	14·11	3·82	0·72	42·93	1·64	1·63	58·06

Note that VCE4 does not report standard errors for variance components.

Heritabilities (h^2) and maternal effects (m^2) were estimated using an animal model with a restricted maximum-likelihood (REML) estimation procedure (VCE4: Groeneveld & Kovac, 1990; Groeneveld, 1995; Lynch & Walsh, 1998). These REML-based mixed model analyses are more flexible and make fewer assumptions about the data than the conventional models used in estimating quantitative genetic parameters (Shaw, 1987; Lynch & Walsh, 1998). In particular, they have the advantages of allowing for highly unbalanced datasets and the inclusion of fixed effects. The phenotype of each animal was broken down into components of additive genetic value, maternal component and fixed effects:

$$Y = Xb + Za + Mc + e$$

where Y is a vector of phenotypic values; b is a vector of fixed effects; a and c are vectors of additive genetic and maternal effects, respectively; e is a vector of residual variance; and X, Z and M are corresponding design matrices relating records to the appropriate fixed and random effects.

We estimated genotype–environment and maternal effect–environment interactions by defining a trait measured in two environments as two different traits and using the genetic (maternal) correlation between the traits as an estimate of interaction (Falconer & Mackay, 1996). Values significantly less than 1 are indicative of significant genotype–environment interactions. This estimate includes only cross-environmental correlation and not the component attributable to differences in V_A(V_M). We will therefore refer to this as the cross-environmental correlation. *t*-tests were used to test whether pairs of heritabilities were significantly different. Since VCE4 does not report standard errors for variance components, these are tabled without standard errors. The data were log-transformed prior to analysis to meet the assumption of normality. Coefficients of additive genetic variance (CV_A; Houle, 1992) were used to compare absolute levels of genetic variance between different traits, populations and treatments.

Due to some mortality within families, the data were not sufficient to estimate separate variance components for each of the six treatment combinations in each of the populations. Consequently, we derived a set of four estimates of h^2, m^2, V_A, V_M and V_R for the overall and the main effects: (1) for the species in general by pooling all individuals across all populations, temperature and food treatments, (2) for each population by pooling over all temperature and food treatments, (3) for the different temperatures by pooling over food treatments and populations, and (4) for the different food treatments by pooling over all temperatures and populations. When pooling over different groups, differences in the mean between those groups were accounted for by fixed effects: for example, in analyses (1) above, population and treatment type were included in the model as fixed effects; for (2), treatment type was included, and so forth. Block was also included as a fixed effect in all analyses, to correct for any differences between the four blocks of the experimental design.

3. Results

The mean values for age, size and growth rate for the different populations in each treatment combination are listed in Table 1.

(i) *Heritabilities and cross-environmental genetic correlations*

The overall heritabilities ($h^2 \pm SE$) for age at metamorphosis, size at metamorphosis and growth rate estimated from the whole dataset, controlling for treatment and population effects, were $0·173 \pm 0·032$,

Table 3. *Heritabilities (a) and maternal effects (b) of age, size and growth rate at/until metamorphosis in five different* Rana temporaria *populations*

Population	Age			Size			Growth		
(a)	h^2 (SE)	CV_A	CV_R	h^2 (SE)	CV_A	CV_R	h^2 (SE)	CV_A	CV_R
Lund	0·26 (0·08)	3·3	9·1	0·21 (0·06)	23·9	85·4	0·09 (0·03)	2·3	22·5
Uppsala	0·31 (0·09)	1·8	4·1	0·40 (0·13)	38·0	56·8	0·22 (0·11)	4·2	13·3
Umeå	0·13 (0·04)	1·0	5·2	0·14 (0·06)	19·5	111·4	0·08 (0·04)	2·0	21·3
Kiruna	0·33 (0·08)	2·5	4·9	0·22 (0·06)	21·5	78·4	0·03 (0·03)	0·7	21·6
Kilpisjärvi	0·15 (0·06)	1·1	5·6	0·12 (0·05)	11·57	82·3	0·09 (0·05)	2·2	21·6
(b)	m^2 (SE)	CV_M		m^2 (SE)	CV_M		m^2 (SE)	CV_M	
Lund	0·02 (0·03)	0·2		0·02 (0·03)	2·7		0·00 (0·00)	–	
Uppsala	0·00 (0·00)	–		0·00 (0·00)	–		0·10 (0·08)	1·8	
Umeå	0·22 (0·06)	1·8		0·03 (0·03)	4·2		0·07 (0·03)	1·6	
Kiruna	0·00 (0·00)	–		0·00 (0·00)	–		0·07 (0·04)	1·8	
Kilpisjärvi	0·03 (0·03)	0·2		0·00 (0·00)	–		0·00 (0·00)	–	

Table 4. *Heritabilities (a) and maternal effects (b) for age, size and growth rate at/until metamorphosis in* Rana temporaria *raised in three different temperatures and two food levels*

Treatment	Age			Size			Growth		
(a)	h^2 (SE)	CV_a	CV_R	h^2 (SE)	CV_A	CV_R	h^2 (SE)	CV_A	CV_R
14 °C	0·34 (0·03)	2·7	4·5	0·20 (0·04)	49·4	187·8	0·19 (0·05)	5·6	22·8
18 °C	0·26 (0·03)	2·4	6·1	0·17 (0·05)	15·2	72·0	0·09 (0·04)	2·0	20·1
22 °C	0·36 (0·05)	3·7	6·1	0·20 (0·05)	20·7	82·0	0·08 (0·04)	2·2	24·2
Ad libitum	0·19 (0·04)	1·7	6·8	0·34 (0·04)	67·4	132·4	0·21 (0·05)	6·4	23·6
Restricted	0·18 (0·03)	2·1	8·8	0·08 (0·02)	8·4	94·2	0·02 (0·01)	0·8	28·2
(b)	m^2 (SE)	CV_M		M^2 (SE)	CV_M		m^2 (SE)	CV_M	
14 °C	0·10 (0·02)	0·8		0·03 (0·02)	8·0		0·06 (0·03)	1·7	
18 °C	0·09 (0·02)	0·9		0·03 (0·02)	2·8		0·03 (0·02)	0·7	
22 °C	0·04 (0·02)	0·4		0·01 (0·02)	1·3		0·02 (0·02)	0·6	
Ad libitum	0·09 (0·02)	0·9		0·00 (0·00)	–		0·02 (0·03)	0·8	
Restricted	0·05 (0·02)	0·6		0·02 (0·01)	1·6		0·03 (0·01)	0·8	

0·145±0·024 and 0·062±0·013, respectively. All heritabilities were small to moderate in magnitude and very similar in all populations (Tables 2a, 3a). In all temperature treatments, heritabilities were largest for age at metamorphosis, intermediate for size at metamorphosis and smallest for growth rate (Tables 2b, 4a). There were no significant differences in heritability estimates between temperature treatments in any of the traits ($P > 0.05$ in all comparisons). A significantly larger heritability was found in the *ad libitum* food level than in the restricted food level (Table 2b) in size at metamorphosis ($t_{86} = 5.54$, $P < 0.01$) and growth rate ($t_{86} = 3.39$, $P < 0.01$). For age at metamorphosis, there was no difference in heritability among the food levels (Tables 2b, 4a).

Temperature treatments induced differential responses among genotypes in age and size at metamorphosis (Table 5a). Cross-environmental genetic correlations were significantly lower than unity in age at metamorphosis between all three temperatures (Table 5a), indicating the presence of genotype–environmental interaction. The effect was strongest between 18 °C and 22 °C and weakest between 14 °C and 18 °C. In size at metamorphosis evidence for genotype–environmental interaction between 14 °C and 18 °C and between 14 °C and 22 °C was also found, but not between the two highest temperatures (Table 5a). There was no evidence for differential response of genotypes to the two food levels in any of the traits (Table 5a).

There were no significant correlations between latitude and heritability in any of three traits ($r = -0.028$ to -0.48, $P > 0.40$ in all comparisons). The same was true in the case of CV_As ($r = -0.54$ to -0.62, $P > 0.26$) and CV_Ms ($r = -0.57$ to 0.25, $P > 0.66$).

(ii) *Maternal effects and cross-environmental maternal correlations*

The overall estimates for maternal effects ($m^2 \pm SE$) for age at metamorphosis, size at metamorphosis and growth rate were 0·060±0·021, 0·008±0·010

Table 5. *Cross-environment* (*a*) *genetic and* (*b*) *maternal effect correlations for different larval traits in* Rana temporaria *grown in three different temperatures and two different food levels*

	Trait		
Treatment combination	Age	Size	Growth rate
(a)	r_G (SE)	r_G (SE)	r_G (SE)
14 °C–18 °C	0·66 (0·1)*	0·78 (0·1)*	0·73 (0·2)
14 °C–22 °C	0·39 (0·1)*	0·74 (0·2)*	0·63 (0·3)
18 °C–22 °C	0·15 (0·1)*	1·00 (0·0)	0·93 (0·3)
Ad libitum–restricted	0·91 (0·1)	0·95 (0·1)	1·00 (0·0)
(b)	r_M (SE)	r_M (SE)	r_M (SE)
14 °C–18 °C	0·97 (0·1)	0·80 (0·3)	0·92 (0·3)
14 °C–22 °C	0·62 (0·3)	−0·12 (0·6)*	0·19 (0·4)*
18 °C–22 °C	0·80 (0·2)	0·50 (0·5)	0·10 (0·3)*
Ad libitum – restricted	0·88 (0·1)	1·00 (0·5)	0·82 (0·3)

* Correlation is significantly smaller than unity.

and $0·033 \pm 0·006$, respectively. For all three traits, estimates of maternal effects were generally much smaller than the additive genetic effects, generally explaining less than 10 % of the phenotypic variation in a given trait and being about 35 % lower in magnitude than heritability estimates. One exception to this pattern was the Umeå population, where maternal effects explained 22 % of the variation in age at metamorphosis, a value nearly twice the magnitude of the heritability (Tables 2a, 3b).

Maternal effects were present at all three temperatures in all three traits. Both m^2 and CV_M tended to decrease with increasing temperature (Tables 2b, 4b). Under restricted food conditions, maternal effects were slightly larger than under the *ad libitum* regime in size and growth rate, whereas the opposite was found in age at metamorphosis (Tables 2b, 4b).

As was the case with cross-environmental genetic correlations, food level did not induce any differential response in maternal effects in any of the traits, whereas temperature treatment had some effects (Table 5b). The clearest effects occurred in growth rate between 14 °C and 22 °C and between 18 °C and 22 °C. In size at metamorphosis we found evidence for interaction effects between 14 °C and 22 °C (Table 5b). No evidence for differential influence of maternal effects on growth rate was found among any of the temperature treatments.

4. Discussion

This study revealed that that all three larval traits were heritable in most populations and under all environmental conditions tested. At the same time, the impact of growth conditions on heritability estimates often exceeded that of population of origin. The heritabilities of the traits differed in their sensitivity to food treatments; size at metamorphosis and growth rate showed lower heritability in the low food regime whereas age at metamorphosis remained unaffected. Furthermore, although maternal effects were in general relatively small in most populations and treatments, their size exceeded the size of heritability estimates in one population. Cross-environmental genetic correlations significantly lower than unity were apparent among different temperature treatments, suggesting that different genes, or differential expression of the same genes, are important for the expression of phenotypic traits in different environmental conditions.

The heritability estimates for metamorphic traits revealed in this study fall within the range of earlier estimates obtained in amphibian studies (Table 6), and as in most of these studies, heritabilities of metamorphic traits were of small to moderate in magnitude. Studies of wild animal populations have revealed that the heritability of a trait is generally inversely related to its importance for fitness (e.g. Mousseau & Roff, 1987; Houle, 1992; Kruuk et al., 2000). While assessment of the relative importance of different traits for fitness remains a formidable task in almost any study (e.g. Kruuk et al., 2000), metamorphic traits are known to be closely related with fitness. Size at metamorphosis is positively related to probability of juvenile survival, size at maturity and fecundity (e.g. Semlitsch et al., 1988; Altwegg & Reyer, 2003). Growth rate is an important life-history trait defining the relationship between age and size at any given life-stage. Particularly important for temperate-zone ectotherms is the ability for rapid growth to attain a critical minimum body size for hibernation (reviewed by Arendt, 1997). Furthermore, rapid growth may evolve as a response to growth-suppressing environmental

Table 6. *Synopsis of heritability estimates (h^2) for amphibian larval life history traits. Estimates are for metamorphic traits unless otherwise stated*

Species	Age	Size	Growth	n_F	Design	Reference
Hyla cinerea	0·40	0·54$_{svl}$	0·50	68	HS	Blouin (1992)
Hyla crucifer	0·09	0·69$_w$	0·15	28	HS	Travis *et al.* (1987)
	0·08	0·10$_w$	0·14	24	HS	Woodward *et al.* (1988)
Hyla regilla	0·14	0·30$_{svl}$	–	90	HS	Watkins (2001)
	–	0·34$_{tl}$	–	90	HS	
Rana esculenta	0	0·23$_w$	0·04	36	HS	Semlitsch (1993)
Rana lessonae	0·26	0·18$_w$	0	36	HS	Semlitsch (1993)
Rana temporaria						
southern	0·11	0·17$_w$	0·25	45	HS	Laurila *et al.* (2002)
	–	0$_{tl}$	–	45	HS	
northern	0·26	0·40$_w$	0·26	45	HS	
	–	0·16$_{tl}$	–	45	HS	
southern	–	–	0·63	?*	FS	Uller *et al.* (2002)
northern	–	–	0·69	?*	FS	
lowland	1	0·06$_w$	–	16	HS	Sommer & Pearman (2003)
mountain	0·89	0·03$_w$	–	16	HS	
Lund	0·26	0·21$_w$	0·09	32	HS	This study
Uppsala	0·31	0·40$_w$	0·22	32	HS	
Umeå	0·13	0·14$_w$	0·08	64	HS	
Kiruna	0·33	0·22$_w$	0·03	32	HS	
Kilpisjärvi	0·15	0·12$_w$	0·09	32	HS	
Rana sylvatica	0·07	0·27$_?$	–	5	FS	Berven & Gill (1983)
lowland	0·27	0·08$_?$	–	?	HS	Berven (1981; in Berven & Gill, 1983)
mountain	0·34	0·58$_?$	–	?	HS	
lowland	0·36	0·07$_{vol}$	–	32	HS	Berven (1987)
mountain	0·35	0·66$_{vol}$	–	20	HS	
larval	–	0·51$_{svl}$	–	83	HS	Phillips (1998)
Bufo americanus	0·25	0·85$_{svl}$	–	48	HS	Howard *et al.* (1994)
Scaphiopus couchii	0·87	0$_{svl}$	–	20	HS	Newman (1988)
	0·40	0·58$_w$	–	ca. 57	FS	Newman (1994)
	–	0·32$_{svl}$	–			

Age, age at metamorphosis; Size, body size (w, weight; svl, snout–vent length; tl, total length; vol, volume); Growth, growth rate; n_F, number of families; Design, type of breeding design (HS, half-sib; FS, full-sib).
* $N = 79$ for the two populations jointly.

factors (countergradient variation; Conover & Schultz, 1995). Finally, even though the importance of the studied traits to fitness is not completely understood, we note that they exhibit heritabilities normally shown by fitness traits.

In many species, populations that inhabit recently deglaciated areas have been found to be less genetically variable than those inhabiting areas that remained unglaciated during the last ice ages (e.g. Sage & Wolf, 1986; Merilä *et al.*, 1996). In this study, we did not find evidence for consistent latitudinal trends in the amount of genetic variability in any of the investigated traits. This contrasts with the weak negative correlation between latitude and genetic variability in seven microsatellite loci across these very same populations (Palo *et al.*, 2003). However, since the correlation between genetic variability in marker loci and quantitative traits can be expected to be low for a number of reasons (e.g. Lynch, 1996; Reed & Frankham, 2001), this lack of latitudinal differentiation in levels of genetic variability in

quantitative traits may not be surprising. Furthermore, in several species, recolonization of Fennoscandia after the last ice age has proceeded both from the north through Finland and from the south via Denmark (e.g. Hewitt, 2000) creating hybrid zones in mid-Scandinavia where the last of the Scandinavian ice-cap melted around 9000 years ago. An investigation of the post-glacial recolonization of *R. temporaria* to the areas used in this study is needed to enable any predictions about the relationship between genetic diversity and latitude.

There is evidence that stress-dependent changes in the expression of genetic variability are common (e.g. Hoffmann & Merilä, 1999). Although there is as yet no consensus as to whether heritable variation increases or decreases under stress, studies on birds and invertebrates have suggested certain trends. In birds heritability of size-related traits tends to decrease under unfavourable conditions, as a result of either decreased additive genetic contribution or an increase in the environmental variance (Hoffmann & Merilä,

1999). Studies of invertebrates have produced both increased and decreased heritabilities under nutritional stress (e.g. 1997; Hoffmann & Schiffer, 1998; Imasheva *et al.*, 1999; Bubliy *et al.*, 2001). We found evidence for lower heritability in both growth rate and size at metamorphosis in the restricted food level. This resulted from both a reduction in additive genetic variance and increased environmental variance. In contrast to the previous results on the same species (Uller *et al.*, 2002), we found no evidence for changes in the magnitude of heritabilities in respect to temperature treatment. This difference in results between these two studies could be due to the estimates in Uller *et al.* (2002) being broad-sense heritabilities, which did not account for the estimates of maternal and early common environment effects. The estimates of Uller *et al.* (2002) of the heritability for growth rate were also approximately 3 times higher than estimates from other amphibian studies (cf. Table 6), suggesting that full-sib estimates might be inflated by maternal/environmental effects. It is worth noting that the populations used in this study may differ in the extent to which they are stressed by the different environmental treatments. Since the data were not sufficient to obtain estimates of heritabilities and maternal effects for each population by treatment combination, further investigations are needed to reveal the nature of latitudinal differentiation in stress responses in this species.

Genetic variation in plasticity to different temperatures and resource levels has been found in several ectothermic species including dung flies (Blanckenhorn, 1998), fruit flies (Gebhardt & Stearns, 1993; David *et al.*, 1994) and anurans (e.g. Newman, 1988, 1994; Semlitsch, 1993; Sommer & Pearman, 2003). We found evidence for genotype–environment interaction between the different temperature treatments in age and size at metamorphosis, the interaction being particularly strong for age at metamorphosis. These results indicate that there is genetic variation in temperature-induced phenotypic plasticity and suggest that this plasticity can evolve in response to climatic variation. In accordance with this, previous studies have found adaptive variation in temperature plasticity in *R. temporaria* (Uller *et al.*, 2002). Genotype–environment interactions may also play a role in maintaining genetic variation within populations (e.g. Turelli & Barton, 2004) and contribute to the significant heritabilities found in fitness-related traits also in the present study.

Maternal effects have been found to influence offspring performance in many taxa (reviewed in Mousseau & Fox, 1998). In the case of amphibians, egg size is an important pathway for the expression of maternal effects in larvae (Kaplan, 1998), and substantial maternal influences on size during the larval stage or size at metamorphosis have been found in several studies (Berven, 1987; Phillips, 1998). We found that maternal effects were generally much weaker than the estimates of heritability, a result that concurs with previous studies of populations along the same latitudinal gradient (Laugen *et al.*, 2002, 2003). The only exception to this pattern was the Umeå population, a mid-latitude population in which maternal effects explained about twice as much of the total variation in age at metamorphosis as the effect of additive genetic variance. This could be explained by an egg size effect, but the eggs of Umeå females were not exceptionally large compared with the other populations (Laugen *et al.*, 2003). A strong maternal contribution to metamorphic size apparently independent of egg size was also found in a lowland population of *R. sylvatica* (Berven, 1987). Egg size does not necessarily reflect the energy content or other aspects of egg quality (Bernardo, 1996; McIntyre & Gooding, 2000). Thus, the disproportionately large maternal effects in this population could result from some particular environmental factor that affects egg composition in the Umeå population but none of the other populations.

A growing body of evidence suggests that consequences of maternal effects can vary significantly depending on the environmental conditions under which the offspring have been raised (Berven & Chadra, 1988; Gliwicz & Guisande, 1992; Parichy & Kaplan, 1992; Moran & Emlet, 2001). Unlike previous studies (Berven & Chadra, 1988; Gliwicz & Guisande, 1992; Parichy & Kaplan, 1992), we found strong cross-environmental maternal effect correlations between food levels (indicative of no maternal effect–environmental interactions), suggesting that maternal effects do not play a central role in the variation under different food resource levels. However, size at metamorphosis and growth rate exhibited weaker cross-environmental maternal correlations among certain temperature treatments, indicating the presence of maternal effect–environmental interactions. This concurs with previous results from studies of the frog *Bombina orientalis* where Kaplan (1992) found that maternal effects interacted with environmental temperature to produce morphological differences among larvae.

In conclusion, we found that three important larval life-history traits in the common frog exhibited significant heritabilities within five different populations and over several food level and temperature treatments. Although no consistent latitudinal trends or population differentiation in heritabilities and maternal effects were detected, the presence of significant cross-environmental genetic and maternal correlations may play an important role in maintaining phenotypic variation between and within populations.

References

Altwegg, R. & Reyer, H. U. (2003). Patterns of natural selection on size at metamorphosis in water frogs. *Evolution* **57**, 872–882.

Arendt, J. D. (1997). Adaptive intrinsic growth rates: an integration across taxa. *Quarterly Review of Biology* **72**, 149–177.

Armbruster, P., Bradshaw, W. E. & Holzapfel, C. M. (1997). Evolution of the genetic architecture underlying fitness in the pitcher-plant mosquito, *Wyeomyia smithii*. *Evolution* **51**, 451–458.

Bernardo, J. (1996). The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. *American Zoologist* **36**, 216–236.

Berven, K. A. (1987). The heritable basis of variation in larval developmental patterns within populations of the wood frog (*Rana sylvatica*). *Evolution* **41**, 1088–1097.

Berven, K. A. & Chadra, B. G. (1988). The relationship among egg size, density and food level on larval development in the wood frog (*Rana sylvatica*). *Oecologia* **75**, 67–72.

Berven, K. A. & Gill, D. E. (1983). Interpreting geographic variation in life-history traits. *American Zoologist* **23**, 85–97.

Blanckenhorn, W. U. (1998). Adaptive phenotypic plasticity in growth, development, and body size in the yellow dung fly. *Evolution* **52**, 1394–1407.

Blouin, M. S. (1992). Genetic correlations among morphometric traits and rates of growth and differentiation in the green tree frog, *Hyla cinerea*. *Evolution* **46**, 735–744.

Bubliy, O. A., Loeschcke, V. & Imasheva, A. G. (2001). Genetic variation of morphological traits in *Drosophila melanogaster* under poor nutrition: isofemale lines and offspring–parent regression. *Heredity* **86**, 363–369.

Chapin, F. S. III & Chapin, M. C. (1981). Ecotypic differences of growth processes in *Carex aquatilis* along latitudinal and local gradients. *Ecology* **62**, 1000–1009.

Conover, D. O. & Schultz, E. T. (1995). Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends in Ecology and Evolution* **10**, 248–252.

David, J. R., Moreteau, B., Gauthier, J. P., Petavy, G., Stockel, A. & Imasheva, A. G. (1994). Reaction norms of size characters in relation to growth temperature in *Drosophila melanogaster*: an isofemale lines analysis. *Genetics Selection Evolution* **26**, 229–251.

Ebert, D., Yampolsky, L. & Stearns, S. C. (1993). Genetics of life history in *Daphnia magna*. *Heredity* **70**, 335–343.

Einum, S. & Fleming, I. A. (1999). Maternal effects of egg size in brown trout (*Salmo trutta*): norms of reaction to environmental quality. *Proceedings of the Royal Society of London, Series B* **266**, 2095–2100.

Endler, J. A. (1977). *Geographic Variation, Speciation, and Clines*. Princeton, NJ: Princeton University Press.

Etterson, J. R. (2004). Evolutionary potential of *Chamaecrista fasciculata* in relation to climate change. II. Genetic architecture of three populations reciprocally planted along an environmental gradient in the Great Plains. *Evolution* **58**, 1459–1471.

Falconer, D. S. & Mackay, T. F. C. (1996). *Introduction to Quantitative Genetics*. Harlow, Essex: Longman Group.

Fog, K., Schmedes, A. & de Lasson, D. R. (1997). *Nordens padder og krybdyr*. Copenhagen: G. E. C. Gad.

Gebhardt, M. D. & Stearns, S. C. (1993). Phenotypic plasticity for life history traits in *Drosophila melanogaster*. I. Effect of phenotypic and environmental correlations. *Journal of Evolutionary Biology* **6**, 1–16.

Gliwicz, Z. M. & Guisande, C. (1992). Family-planning in *Daphnia*: resistance to starvation in offspring born to mothers grown at different food levels. *Oecologia* **91**, 463–467.

Gosner, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**, 183–190.

Groeneveld, E. (1995). REML VCE, a multivariate multimodel restricted maximum likelihood (co)variance component estimation package. Available via www.tzv.fal.de/institut/genetik/pub/eg/vce/manual/manual.html

Groeneveld, E. & Kovac, M. (1990). A generalized computing procedure for setting up and solving mixed linear models. *Journal of Dairy Science* **73**, 513–531.

Haugen, T. O. & Vøllestad, L. A. (2000). Population differences in early life-history traits in grayling. *Journal of Evolutionary Biology* **13**, 897–905.

Hewitt, G. M. (2000). The genetic legacy of quaternary ice ages. *Nature* **405**, 907–913.

Hoffmann, A. A. & Merilä, J. (1999). Heritable variation and evolution under favourable and unfavourable conditions. *Trends in Ecology and Evolution* **14**, 96–101.

Hoffmann, A. A. & Schiffer, M. (1998). Changes in the heritability of five morphological traits under combined environmental stresses. *Evolution* **52**, 1207–1212.

Houle, D. (1992). Comparing evolvability and variability of quantitative traits. *Genetics* **130**, 195–204.

Howard, R. D., Whiteman, H. H. & Schueller, T. I. (1994). Sexual selection in American toads: a test of the good-genes hypothesis. *Evolution* **48**, 1286–1300.

Huey, R. B., Gilchrist, G. W., Carlson, M. L., Berrigan, D. & Serra, L. (2000). Rapid evolution of a geographic cline in size in an introduced fly. *Science* **287**, 308–309.

Imasheva, A. G., Bosenko, D. V. & Bubliy, O. A. (1999). Variation in morphological traits of *Drosophila melanogaster* (fruit fly) under nutritional stress. *Heredity* **82**, 187–192.

Kaplan, R. H. (1992). Greater maternal investment can decrease offspring survival in the frog *Bombina orientalis*. *Ecology* **73**, 280–288.

Kaplan, R. H. (1998). Maternal effects, developmental plasticity, and life history evolution. An amphibian model. In *Maternal Effects as Adaptations* (ed. T. E. Mousseau & C. W. Fox), pp. 244–260. New York: Oxford University Press.

Kruuk, L. E. B., Clutton-Brock, T. H., Slate, J., Pemberton, J. M., Brotherstone, S. & Guinness, F. E. (2000). Heritability of fitness in a wild mammal population. *Proceedings of the National Academy of Sciences of the USA* **97**, 698–703.

Laugen, A. T., Laurila, A. & Merilä, J. (2002). Maternal and genetic contributions to geographical variation in *Rana temporaria* larval life-history traits. *Biological Journal of the Linnean Society* **76**, 61–70.

Laugen, A. T., Laurila, A., Räsänen, K. & Merilä, J. (2003). Latitudinal countergradient variation in larval life history traits of common frog (*Rana temporaria*) developmental

rates: evidence for local adaptation. *Journal of Evolutionary Biology* **16**, 996–1005.

Laurila, A., Karttunen, S. & Merilä, J. (2002). Adaptive phenotypic plasticity and genetics of larval life histories in two *Rana temporaria* populations. *Evolution* **56**, 617–627.

Lynch, M. (1996). A quantitative-genetic perspective on conservation issues. In *Conservation Genetics* (ed. J. C. Avise & J. L. Hamrick), pp. 471–501. New York: Chapman & Hall.

Lynch, M. & Walsh, B. (1998). *Genetics and Analysis of Quantitative Traits*. Sunderland, MA: Sinauer.

McIntyre, G. S. & Gooding, R. H. (2000). Egg size, contents, and quality: maternal age and -size effects on house fly eggs. *Canadian Journal of Zoology* **78**, 1544–1551.

Merilä, J., Björklund, M. & Baker, A. J. (1996). Genetic population structure and gradual northward decline of genetic variability in the greenfinch (*Carduelis chloris*). *Evolution* **50**, 2548–2557.

Miaud, C. & Merilä, J. (2001). Local adaptation or environmental induction? Causes of population differentiation in alpine amphibians. *Biota* **2**, 31–50.

Moran, A. L. & Emlet, R. B. (2001). Offspring size and performance in variable environments: field studies on a marine snail. *Ecology* **82**, 1597–1612.

Morgan, K. K., Hicks, J., Spitze, K., Latta, L., Pfrender, M. E., Weaver, C. S., Ottone, M. & Lynch, M. (2001). Patterns of genetic architecture for life-history traits and molecular markers in a subdivided species. *Evolution* **55**, 1753–1761.

Mousseau, T. A. & Roff, D. A. (1987). Natural selection and the heritability of fitness components. *Heredity* **59**, 181–197.

Mousseau, T. A. & Fox, C. W. (1998). *Maternal Effects as Adaptations*. New York: Oxford University Press.

Newman, R. A. (1988). Genetic variation for larval anuran (*Scaphiopus couchii*) development time in an uncertain environment. *Evolution* **42**, 763–773.

Newman, R. A. (1994). Genetic variation for phenotypic plasticity in the larval life history of spadefoot toads (*Scaphiopus couchii*). *Evolution* **48**, 1773–1785.

Palo, J., O'Hara, R., Laugen, A., Laurila, A., Primmer, C. R. & Merilä, J. (2003). Latitudinal divergence of common frog (*Rana temporaria*) life history traits by natural selection: evidence from a comparison of molecular and quantitative data. *Molecular Ecology* **12**, 1963–1978.

Parichy, D. M. & Kaplan, R. H. (1992). Maternal effects on offspring growth and development depend on environmental quality in the frog *Bombina orientalis*. *Oecologia* **91**, 579–586.

Phillips, P. C. (1998). Genetic constraints at the metamorphic boundary: morphological development in the wood frog, *Rana sylvatica*. *Journal of Evolutionary Biology* **11**, 453–463.

Reed, D. H. & Frankham, R. (2001). How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. *Evolution* **55**, 1095–1103.

Reznick, D. N. & Ghalambor, C. K. (2001). The population ecology of contemporary adaptations: what empirical studies reveal about conditions that promote adaptive evolution. *Genetica* **112/113**, 183–198.

Roff, D. (2000). The evolution of the G matrix: selection or drift? *Heredity* **84**, 135–142.

Sage, R. D. & Wolf, J. O. (1986). Pleistocene glaciations, fluctuating ranges, and low genetic variability in a large mammal (*Ovis dali*). *Evolution* **40**, 1092–1095.

Schlichting, C. D. & Pigliucci, M. (1998). *Phenotypic Evolution: a Reaction Norm Perspective*. Sunderland, MA: Sinauer.

Semlitsch, R. D. (1993). Adaptive genetic variation in growth and development of tadpoles of the hybridogenetic *Rana esculenta* complex. *Evolution* **47**, 1805–1818.

Semlitsch, R. D., Scott, D. E. & Peckmann, J. H. K. (1988). Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. *Ecology* **69**, 184–192.

Shaw, R. G. (1987). Maximum-likelihood approaches applied to quantitative genetics of natural populations. *Evolution* **41**, 812–826.

Sommer, S. & Pearman, P. B. (2003). Quantitative genetic analysis of larval life-history traits in two alpine populations of *Rana temporaria*. *Genetica* **118**, 1–10.

Travis, J., Emerson, S. B. & Blouin, M. (1987). A quantitative-genetic analysis of larval life-history traits in *Hyla crucifer*. *Evolution* **41**, 145–156.

Turelli, M. & Barton, N. H. (2004). Polygenic variation maintained by balancing selection: pleiotropy, sex-dependent allelic effects and G × E interactions. *Genetics* **166**, 1053–1079.

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

Watkins, T. B. (2001). A quantitative genetic test of adaptive decoupling across metamorphosis for locomotor and life-history traits in the pacific tree frog, *Hyla regilla*. *Evolution* **55**, 1668–1677.

Woodward, B. D., Travis, J. & Mitchell, S. (1988). The effects of the mating system on progeny performance in *Hyla crucifer* (Anura, Hylidae). *Evolution* **42**, 784–794.