

ADAPTIVE PHENOTYPIC PLASTICITY AND GENETICS OF LARVAL LIFE HISTORIES IN TWO *RANA TEMPORARIA* POPULATIONS

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Abstract.—Phenotypic plasticity provides means for adapting to environmental unpredictability. In terms of accelerated development in the face of pond-drying risk, phenotypic plasticity has been demonstrated in many amphibian species, but two issues of evolutionary interest remain unexplored. First, the heritable basis of plastic responses is poorly established. Second, it is not known whether interpopulational differences in capacity to respond to pond-drying risk exist, although such differences, when matched with differences in desiccation risk would provide strong evidence for local adaptation. We investigated sources of within- and among-population variation in plastic responses to simulated pond-drying risk (three desiccation treatments) in two *Rana temporaria* populations originating from contrasting environments: (1) high desiccation risk with weak seasonal time constraint (southern population); and (2) low desiccation risk with severe seasonal time constraint (northern population). The larvae originating from the environment with high desiccation risk responded adaptively to the fast decreasing water treatment by accelerating their development and metamorphosing earlier, but this was not the case in the larvae originating from the environment with low desiccation risk. In both populations, metamorphic size was smaller in the high-desiccation-risk treatment, but the effect was larger in the southern population. Significant additive genetic variation in development rate was found in the northern and was nearly significant in the southern population, but there was no evidence for genetic variation in plasticity for development rates in either of the populations. No genetic variation for plasticity was found either in size at metamorphosis or growth rate. All metamorphic traits were heritable, and additive genetic variances were generally somewhat higher in the southern population, although significantly so in only one trait. Dominance variances were also significant in three of four traits, but the populations did not differ. Maternal effects in metamorphic traits were generally weak in both populations. Within-environment phenotypic correlations between larval period and metamorphic size were positive and genetic correlations negative in both populations. These results suggest that adaptive phenotypic plasticity is not a species-specific fixed trait, but evolution of interpopulational differences in plastic responses are possible, although heritability of plasticity appears to be low. The lack of adaptive response to desiccation risk in northern larvae is consistent with the interpretation that selection imposed by shorter growing season has favored rapid development in north (~8% faster development in north as compared to south) or a minimum metamorphic size at the expense of phenotypic plasticity.

Key words.—Additive genetic variance, Amphibia, heritability, metamorphosis, phenotypic plasticity, pond desiccation, time constraints.

Received March 21, 2001. Accepted November 22, 2001.

Phenotypic plasticity, the capability of a genotype to produce different phenotypes when exposed to different environmental conditions, is ubiquitous phenomenon in nature (Stearns 1989; Schlichting and Pigliucci 1998). For organisms exploiting temporally and spatially heterogeneous environments, it provides one mechanism for adaptation (Bradshaw 1965; Levins 1968; Schlichting 1986; de Jong 1990; Schlichting and Pigliucci 1998), and it is now clear that in many cases phenotypic plasticity is adaptive, allowing organisms to exploit a wider range of environments than would otherwise be possible (Schlichting 1986; Newman 1992; Scheiner 1993; Gotthard and Nylin 1995).

In many organisms, development is limited by time constraints imposed by habitat duration, seasonality, or other climatic factors (Roff 1980; Rowe and Ludwig 1991; Newman 1992; Gotthard and Nylin 1995). Consequently, large-scale climatic heterogeneity may result in genetic geographic differentiation in critical life-history traits (Levins 1969; Berven et al. 1979; Roff 1980; Berven and Gill 1983; Conover and Schultz 1995; Arendt 1997). However, life-history theory predicts that when there is temporal variation in the time

constraints, age and size at the relevant life-history switch point (e.g., maturation or metamorphosis) should depend on these constraints (Ludwig and Rowe 1990; Rowe and Ludwig 1991; Abrams et al. 1996). Therefore, when there are geographic differences in temporal variability in the time constraints, there should be scope for evolution of geographic heterogeneity in phenotypic plasticity. For a plastic trait to respond to selection and evolve, there has to be genetic variation in the reaction norm (Via and Lande 1985; Scheiner and Lyman 1989; Gomulkiewicz and Kirkpatrick 1992). When selection is strong enough over evolutionary time, genetic variation in the reaction norm may become depleted and hinder further adaptation; but if the selection pressure varies in space and time, variation may be maintained (Stearns 1992; Roff 1997).

Genetic differences among populations that are correlated with identifiable agents of selection provide good evidence that the differences are adaptive (Endler 1986). Although a number of studies have demonstrated existence of genetic variation in plastic responses (Schlichting 1986; Scheiner 1993), only few have compared these responses among pop-

ulations (Scheiner 1993). This is especially true for animals, and vertebrates in particular. Furthermore, in many within-population vertebrate studies conducted so far, the genetic estimates of plasticity have been potentially distorted by the use of full-sibling analyses (but see Newman 1988; Semlitsch 1993), which tend to overestimate the amount of additive genetic variation (Falconer and Mackay 1996; Merilä and Sheldon 2001).

Temporary ponds are a classical example of a habitat showing high temporal variability (Newman 1992). Animals living in temporary ponds often face high mortality risk when the pond is about to dry out. In such situations, accelerated development assures that metamorphosis is reached before complete desiccation. Indeed, some anuran tadpoles (reviewed by Newman 1992; Gotthard and Nylin 1995) and insects (Chodorowski 1969; Juliano and Stoffregen 1994) are able to respond adaptively to pond drying by accelerating their development and metamorphosing earlier. Although the cues triggering accelerated development have been subject to some debate (Newman 1992; Tejedo and Reques 1994; Gotthard and Nylin 1995), recent studies suggest that tadpoles respond to the decreased water volume and not, for instance, to increased temperature in the drying ponds (Denver et al. 1998; Laurila and Kujasalo 1999; Merilä et al. 2000a). However, for the plasticity to be truly adaptive, staying in the pond longer when the conditions remain favorable also should be profitable. Large size at metamorphosis generally confers higher fitness through improved juvenile survival (Berven and Gill 1983; Goater 1994; Newman and Dunham 1994; Reques and Tejedo 1997) and higher adult reproductive success (Berven and Gill 1983; Scott 1994; Blanckenhorn 1998; Taylor et al. 1998). Indeed, when time is not constrained, individuals have been shown to extend their larval period and metamorphose at larger size (Denver et al. 1998).

The aim of this study was threefold. First, we investigated possible interpopulational differentiation in plastic responses to experimental pond drying in the common frog (*Rana temporaria*) originating from two populations differing both in the activity period and in the drying regime of the breeding pond. The activity period in the population originating from temperate latitudes is relatively long, but the breeding pond dries out at unpredictable intervals. In the other population, desiccation of the breeding pond is extremely rare, but the tadpoles face a severe time constraint due to the short activity period available in the northern boreal zone. Second, we investigated whether there was genetic variation in plastic responses to pond drying within these populations, and hence, scope for evolution of plastic responses in future. Although previous studies have shown that tadpoles of this species are able to accelerate their development in response to drying risk at the expense of metamorphic size (Laurila and Kujasalo 1999; Loman 1999; Merilä et al. 2000a), little is known about genetic variation in phenotypic plasticity in amphibians. Third, by using a North Carolina II breeding design, we investigated the relative importance of additive genetic, maternal effect, and dominance genetic sources of variation as determinants of within-population variation in larval life-history traits, and in particular, whether the relative importance of these factors differed between the two populations.

MATERIALS AND METHODS

Study Species and Populations

The common frog has a wide distribution that encompasses most of the Palearctic region, and in many areas of northern Europe it is the most common anuran species (Gasc et al. 1997). It breeds in a variety of freshwater habitats, including small ephemeral ponds that run a high desiccation risk (Gislén and Kauri 1956; Laurila 1998) and, in northern Europe, breeding occurs as soon as the ice in the ponds has melted. Consequently, the breeding activities in southern Sweden are initiated in late March to early April, whereas at high altitudes in northern Sweden breeding may not commence until late June (Elmberg and Lundberg 1991). Length of larval period is highly dependent on environmental conditions (e.g., food level, temperature), and in natural ponds in southern and central Scandinavia it typically lasts 35–70 days (A. T. Laugen, A. Laurila, and J. Merilä, unpubl. data).

Our first study population (hereafter called “southern population”) was located in Tvedöra (55°N, 13°E; for a map showing the location of study populations, see Merilä et al. 2000b) near the town of Lund in southern Sweden. The frogs were collected from a pond that dries up frequently but unpredictably (complete desiccation two times in 1990–1998 when still having tadpoles; J. Loman, pers. comm.), but breeding also occurs in nearby permanent ponds. The surrounding landscape is pastoral fields scattered with small woodlots. The second population (hereafter called “northern population”) was located in Esrange (68°N, 20°E) near the town of Kiruna in northern Sweden. This locality is situated about 1400 km north of Lund and 200 km north of the Arctic Circle. In this population, the frogs breed in a boggy extension of a small stream. The stream runs from a nearby small lake with many springs, suggesting that this breeding locality dries up extremely rarely (if ever), although we do not have long-term data on this. This locality and the nearby lake are the only breeding localities in the vicinity. The surrounding landscape is timberline coniferous forest. The populations differ in the experienced climatic conditions; growth season length in Lund (the southern population) is 217 days and the mean air temperature during the three months after egg laying is 11.3°C, whereas the corresponding figures for Kiruna (the northern population) are 113 days and 9.7°C (Alexandersson et al. 1991).

Laboratory Procedures

At the onset of the breeding season we collected breeding adults in both localities (March 26 and 27 in Lund and May 28 in Kiruna) and transported them to the laboratory in Uppsala. The artificial fertilizations were performed following the guidelines of Berger et al. (1994). In both populations, eggs of five females were fertilized with a set of five males on two consecutive days to create two 5 × 5 breeding matrices for both populations. However, in both populations, one female had not ovulated and, consequently, in one of the matrices only four females were used. Thus, the total number of crosses (full-sibling families) was 45 in both populations. The eggs from each cross were then divided into two groups and transferred to 0.9-L plastic vials filled with 0.75 L re-

constituted soft water (RSW; American Public Health Association 1985), where the embryos were allowed to develop at 14°C.

Hatching occurred relatively simultaneously within each of the breeding matrices. On the day the majority of the tadpoles had reached developmental stage 25 (Gosner 1960; day 0 of the experiment), we randomly took 18 tadpoles from each cross and transferred them to individual 0.9-L plastic vials filled with 0.75 L of RSW, where the tadpoles were allowed to develop until metamorphosis. The containers were arranged on 12 rows of shelves in a laboratory room. The tadpoles were fed every fourth day with a finely ground 3:1 mixture of rabbit pellets and aquarium fish flakes. At the start of the experiment, each tadpole was given 30 mg of food and thereafter 45 mg at each feeding time. The food level was determined to be ad libitum (i.e., uneaten food left after each feeding) from the experience from previous experiments (A. T. Laugen, A. Laurila, K. Räsänen, and J. Merilä, unpubl. ms.). The temperature in the laboratory room was 21°C. To simulate the higher mean temperature the tadpoles would experience in a drying pond (Newman 1989; Tejedo and Reques 1994), we maintained the temperature relatively high. Use of RSW allowed us to standardize the water quality during the experiment. The light period in the laboratory was 18:6 L:D, corresponding to the situation in central Sweden in May.

Water was completely changed every fourth day in conjunction with the feeding. At the same time, each tadpole was subjected to water-level manipulation according to one of the three water-level treatments. In the control treatment, water level was kept constant (750 ml) throughout the experiment. In the second treatment, water level was reduced by 15% at each water change. This treatment (henceforth "slow decrease") resulted in a water volume of 240 ml by day 28 of the experiment. In the third treatment, water level was reduced by 30% at each water change (henceforth "fast decrease"), resulting in a water volume of 62 ml by day 28.

Starting from 14 days from initiation of the experiment, the containers were checked twice a day (0800 and 1800 h) for metamorphosis. At metamorphosis (defined as the emergence of the first forelimb: stage 42 in Gosner 1960) the individuals were removed from the container, their total length from nose to tip of the tail was measured with a digital caliper to the nearest 0.1 mm, and they were weighed to the nearest 0.1 mg with an electronic balance. Length of larval period was defined as days elapsed from the start of the experiment (day 0) until metamorphosis. Individual growth rates were defined as metamorphic weight divided by length of larval period.

Statistical and Genetic Analyses

The experimental design was a factorial randomized block design, where the factors were population, water-level treatment, block, breeding matrix, male parent, and female parent. The blocking (six blocks corresponding to two rows of shelves each) was employed because of the vertical temperature stratification in the laboratory room, and each treatment combination (population \times treatment \times male \times female) was represented once in each of the six blocks. The terms population, water-level treatment, block, and crossing matrix

(nested within the population) were considered as fixed effects (Zar 1995; Lynch and Walsh 1998), and the effects of male and female parent (both nested within population and crossing matrix) were considered as random effects (Lynch and Walsh 1998). Interactions between fixed and random effects were considered to be random effects. A significant population \times treatment interaction would be indicative of genetic differentiation in plastic responses between the populations, whereas significant male parent \times treatment interaction would evidence for genetic variation in plastic responses within populations. Significant female parent \times treatment interaction could be evidence for genetic variation in plastic responses, but it could also arise due to interaction between environmental maternal effects and treatments. The data were analyzed with mixed model ANOVAs using Type III sums of squares as implemented in PROC GLM of SAS (ver. 6.12, SAS Institute 1996).

In addition to performing these overall tests for the combined data, we performed separate analyses for both populations to estimate heritabilities and magnitudes of causal components of variance. In these analyses, water-level treatment, block, and fertilization matrix were included into the models as fixed effects. Variance components were obtained by the restricted maximum-likelihood (REML) option in PROC VARCOMP in SAS. Because the design employed was North Carolina II, the among-sire component of variance (V_{SIRE}) estimates one-fourth of the additive genetic variance (V_A). Thus, V_A was estimated as $4V_{\text{SIRE}}$. The variance among dams (V_{DAM}) equals one-fourth of the V_A plus any maternal effects variance (V_M), whether of genetic or nongenetic origin. The size of the V_M was estimated as $V_M = V_{\text{SIRE}} - V_{\text{DAM}}$. The variance due to sire \times dam ($V_{\text{SIRE} \times \text{DAM}}$) interaction is assumed to estimate one-fourth of the dominance genetic variance (V_D), thus, $V_D = 4V_{\text{SIRE} \times \text{DAM}}$. However, the variance due to sire \times dam interaction could also be due to interactions between additive genetic and maternal effects (Kearsey and Pooni 1996). The residual variance is assumed to include $1/2V_A$, $3/4V_D$ plus the environmental variance (V_E ; Kearsey and Pooni 1996); and thus, V_E was estimated as $V_E = V_{\text{residual}} - 1/2V_A - 3/4V_D$. Because the variance components obtained with least square and REML methods were in good agreement, the significance testing of V_A and V_D was performed with PROC GLM. The significance of V_M was calculated by dividing the dam mean squares by the mean squares of sire (Lynch and Walsh 1998). Standard errors for V_A and V_D were calculated as in Lynch and Walsh (1998). Heritabilities (h^2), maternal effect coefficients (m) and dominance ratios (d) were estimated by dividing the respective causal components with total phenotypic variance of the given trait. Their standard errors ratios were calculated with a jackknifing procedure across sires (Sokal and Rohlf 1981).

Within-individual phenotypic correlations (r_p) between larval period and metamorphic mass were calculated as Pearson product-moment correlations separately within each desiccation treatment and population. Genetic (r_g) and environmental (r_e) correlations between larval period and mass at metamorphosis were calculated within each treatment from REML-estimated variance components. However, genetic correlations from REML estimates in the two decreasing water-level treatments in the southern population were not es-

timable, because the additive variance component in metamorphic mass equaled zero. Consequently, in these two cases genetic correlations were estimated by calculating product-moment correlations among sire means (r_m ; Roff 1997). Standard errors for environmental and genetic correlations were calculated with a jackknifing procedure across sires and their significance were tested with one-sample *t*-tests. The significance of product-moment correlations was determined directly from correlation coefficients and sample sizes (Lynch and Walsh 1998).

A critical assumption in studies aiming to estimate levels of genetic variability is that the estimates are not severely biased by differential survival of offspring before the variance components are estimated. This assumption seems to hold for this study, because the survival until metamorphosis was high in both populations (98.9% and 93.1% in southern and northern populations, respectively) and there was no heterogeneity in survival rates among sires within populations ($\chi^2 = 24.01$, $df = 18$, $P = 0.155$).

RESULTS

Differentiation between Populations

Developmental rate

The larvae from the northern population had about two days shorter larval period than those from the southern population (Table 1, Fig. 1), indicating genetic capacity for faster development in the north than in the south. The water-level treatments had a large effect on length of the larval period: Larval period was shorter in the fast-decrease treatment than in control and slow-decrease treatments, indicating adaptive plasticity in this trait (Table 1, Fig. 1). However, as indicated by the significant population \times treatment interaction (Table 1), the populations differed in their response to water-level treatment: Only the southern population exhibited adaptive plasticity in length of larval period, whereas water-level treatments had no effect in the northern population (Table 1, Fig. 1).

Size at metamorphosis

Because fast development in amphibians is usually associated with reduced size at metamorphosis (e.g., Newman 1992), we expected to find differences in size at metamorphosis between the populations and water-level treatments. The populations indeed differed both in metamorphic mass and total length, the slow-developing tadpoles from the southern population being somewhat heavier and larger at metamorphosis than the northern tadpoles (Table 1; Figs. 1, 2). In both populations, the metamorphic size was smaller in the fast-decrease treatment, whereas there was no difference between the control and slow-decrease treatments (Table 1; Figs. 1, 2). However, a significant population \times treatment interaction in all traits (Table 1) revealed that the reduction in metamorphic size in the fast-decrease treatment was considerably steeper in the southern, as compared to the northern population (Figs. 1, 2).

TABLE 1. ANOVA tables for length of larval period, mass, and total length at metamorphosis and growth rate. df_b , degrees of freedom in the denominator; df_n , degrees of freedom in the numerator.

	df_b	Larval period			Metamorphic mass			Total length			Growth rate		
		df_n	MS	F	df_n	MS	F	df_n	MS	F	df_n	MS	F
Population	1	21.30	837.83	18.86***	25.12	72322.31	5.29*	17.69	2230.59	46.39***	23.36	74.70	2.15
Treatment	2	9.31	29.16	20.76***	19.25	128635.93	93.12***	11.64	411.31	141.02***	15.87	151.19	53.74***
Block	5	1386.00	392.98	183.71***	1384.00	84210.23	85.26***	1385.00	131.56	36.13***	1384.00	5.40	2.23*
Population \times treatment	2	9.31	19.26	13.72**	19.25	17929.66	12.98***	11.64	100.77	34.55***	15.87	2.57	3.28†
Matrix(population)	2	21.29	44.36	1.66	25.11	1641.13	0.12	17.67	38.87	0.81	23.35	22.31	0.64
Sire(matrix \times population)	16	36.96	13.12	3.29***	25.42	7348.03	6.15***	33.75	12.96	2.16*	36.73	18.49	4.16***
Dam(M \times P)	14	31.96	17.95	5.07***	27.06	7597.50	4.74***	27.11	41.09	8.51***	33.66	20.59	4.89***
Sire \times dam(M \times P)	56	1386.00	4.14	1.94***	1384.00	1203.07	1.22	1385.00	5.78	1.59**	1384.00	4.25	1.76***
Treatment \times sire (M \times P)	32	1386.00	2.00	0.93	1384.00	980.57	0.99	1385.00	3.87	1.06	1384.00	2.61	1.08
Treatment \times dam (M \times P)	28	1386.00	1.54	0.72	1384.00	1389.62	1.41†	1385.00	2.69	0.74	1384.00	2.38	0.98

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

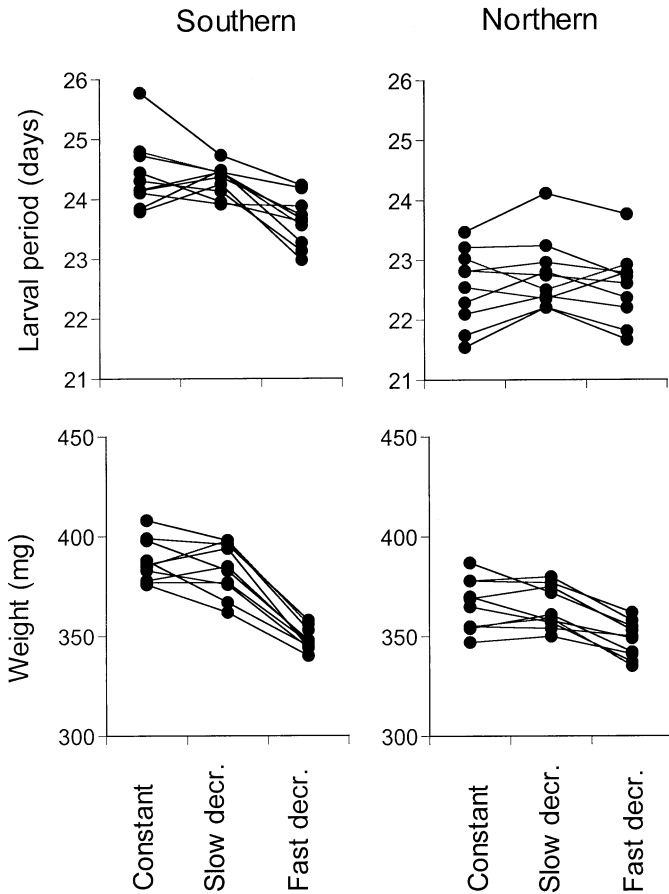


FIG. 1. Larval period and mass at metamorphosis in the three water-level treatments shown separately for the two populations. Mean values of each sire (calculated over dams) are shown.

Growth rate

There was no difference in mean growth rate between the populations, but in both populations the fast-decrease treatment reduced growth rates (Table 1, Fig. 2). Because there was only a nonsignificant population \times treatment interaction (Table 1), there was only weak evidence that the reduction in growth rate in the northern population tended to be smaller than in the southern population (Fig. 2).

Sources of Variation within Populations

Genetic variation in plasticity

The preceding analyses suggest that the two populations have diverged genetically in their capacity to respond to desiccation risk by accelerated development. Furthermore, metamorphic size was reduced in the fast-decreasing treatment in both populations. For this type of divergence in plastic responses to evolve, genetic variation in this trait is required. However, we found no evidence for genetic variation in plastic responses in any of the traits, as indicated by nonsignificant male \times treatment interactions for all traits (Table 1). The same is true for female \times treatment interactions, although there was nonsignificant among-female heterogeneity in plastic responses in total length (Table 1). Consequently, we

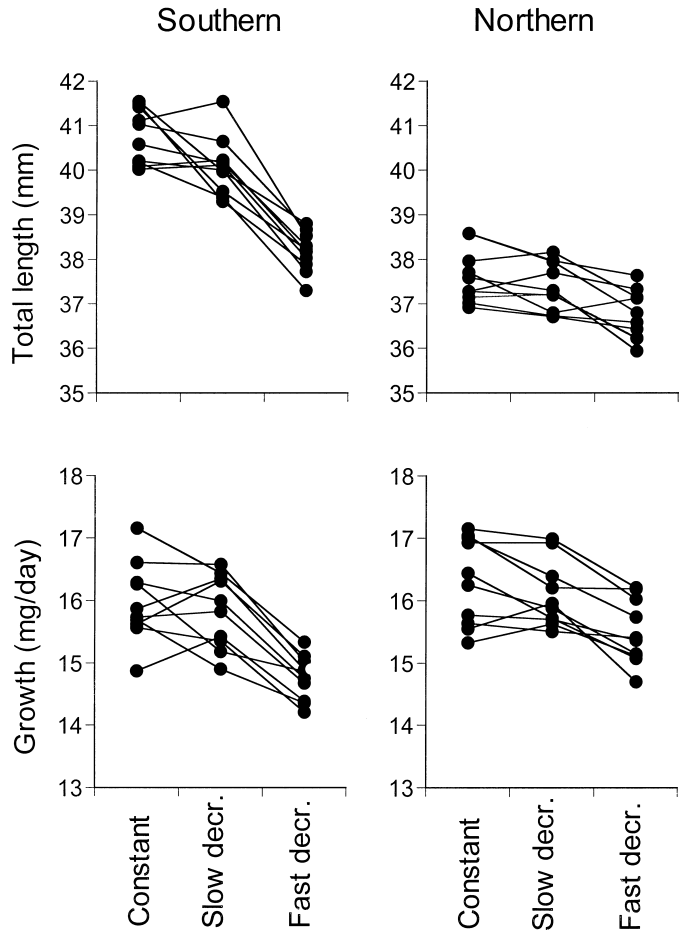


FIG. 2. Total length at metamorphosis and growth rate in the three water-level treatments shown separately for the two populations. Mean values of each sire (calculated over dams) are shown.

pooled variation due to both male and female \times treatment interactions in V_p in Table 2.

Additive genetic, maternal, and dominance variance

Both male and female parent contributions for all traits were significant in the combined analyses of both populations (Table 1), indicating that all traits were heritable. However, maternal effect coefficients were small, suggesting that maternal effects were not important determinants of larval performance in these experiments (Table 2). A significant male \times female parent interaction existed in all traits except in mass at metamorphosis (Tables 1, 2), indicating a significant role for dominance interactions in determining variation in larval traits.

Separate analyses for the two populations were largely in agreement with the results of the combined analyses, but some important differences emerged. In general, additive genetic variance components in larval traits tended to be moderately large and significant, the only exception being total length in the southern population, where no additive genetic variation was detected (Table 2). Additive genetic variances were generally somewhat higher in the northern population, although the difference was significant only in total length,

TABLE 2. Maximum-likelihood estimates of causal variance components for larval period length, different measures of size at metamorphosis (mass, body length, and total length), and growth rate in the two *Rana temporaria* populations. V_A , additive genetic; V_M , maternal; V_D , dominance; V_E , environmental; V_P , total phenotypic variance (see Materials and Methods for definitions). Variance due to parent \times treatment interactions has been pooled to V_P (see Results); t -values refer to t -tests for differences in variance components between the populations.

Trait	Population	V_A (SE)	V_M	V_D (SE)	V_E (SE)	V_P
Larval period	southern	0.2942 (0.120) \dagger	0.1040	0.4784 (0.248)**	1.6778 (0.116)	2.5675
	northern	0.6474 (0.191)**	0	0.4791 (0.252)**	1.3717 (0.113)	2.4805
		$t_{16} = -1.57$		$t_{56} = -0.00$	$t_{1386} = 1.89\dagger$	
Mass	southern	2.2213 (0.701)*	0	1.0916 (0.966)	9.6989 (0.613)	12.7873
	northern	4.0909 (1.018)***	0.3543	0	5.7894 (0.432)	10.3563
		$t_{16} = -1.43$			$t_{1384} = 5.15***$	
Total length	southern	0	0.2705*	0.6039 (0.374)**	3.0898 (0.204)	4.0380
	northern	0.7462 (0.226)**	0.3985 \dagger	0.3608 (0.326) \dagger	3.0570 (0.189)	4.5713
				$t_{56} = 0.48$	$t_{1385} = 0.12$	
Growth rate	southern	0.6755 (0.210)*	0	0.4899 (0.264)**	1.6894 (0.131)	2.7500
	northern	0.7846 (0.222)**	0.1819	0.4048 (0.251)*	1.6617 (0.126)	3.0331
		$t_{16} = -0.36$		$t_{56} = 0.23$	$t_{1384} = 0.15$	

$\dagger P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

in which variance equaled zero in the southern population (Table 2). The largest heritabilities were detected in the northern population for metamorphic mass, growth rate, and larval period, whereas in the southern population heritability was largest in growth rate (Table 3).

Variation due to maternal effects was usually much less than additive genetic variation (Table 2) and accounted for a much smaller proportion of the total phenotypic variation than the additive effects (at best 9% in total length in the northern population; Table 3). In fact, maternal effect variation was significant only in total length in the northern population (Table 2), and there were no significant differences between the populations, as indicated by the generally non-significant maternal effect tests (Table 2).

The magnitude of dominance variance was in most cases intermediate between additive and maternal variances (Table 3). Among individual traits, the contribution of dominance variance to total variance was highest for larval period and growth rate (Table 3). There were no differences between the populations in dominance variance in any of traits (Table 2). The proportion of total variance explained by environmental influences varied between 55% and 75%. Environmental variance was significantly larger in metamorphic mass in the southern than in the northern population (Table 2). Larval period showed a nonsignificant trend in the same direction.

Phenotypic and genetic correlations

Within-individual phenotypic correlations between larval period length and metamorphic mass were positive, indicat-

ing that large metamorphs also tended to be older, and differed significantly from zero in both populations and in all treatments (Table 4). However, the genetic correlations were negative in both populations, and all except one (southern population, fast decrease) were fairly large and significant (Table 4). All environmental correlations were positive, although only four of eight correlations reached statistical significance (Table 4).

DISCUSSION

Adaptive Plasticity and Variation in Larval Traits

In the southern population, *R. temporaria* tadpoles exhibited adaptive phenotypic plasticity by metamorphosing earlier and at smaller size in the fast-decrease treatment. This result is in accordance with the previous studies on *R. temporaria* from the same population (Loman 1999; Merilä et al. 2000a), as well as from one southern Finnish population (Laurila and Kujasalo 1999). In the northern population, however, larval period was not influenced by the water-level treatments, although metamorphic size was smaller in the fast-decrease treatment. This suggests that the population difference in the plastic responses reflect differences in the environment the populations live in: Pond desiccation is common both in the southern population of this study and in the Finnish population (Laurila and Kujasalo 1999), whereas the northern breeding pond desiccates only rarely, if ever. Thus, if plasticity is costly (DeWitt et al. 1998), one may expect that the nonplastic genotypes have been favored in the north-

TABLE 3. Heritabilities (h^2) maternal effect coefficients (m), and dominance ratios (d) for four metamorphic traits in *Rana temporaria*. All estimates are based on variance components presented in Table 2.

Trait		h^2 (SE)	m (SE)	d (SE)
Larval period	southern	0.114 (0.107)	0.041 (0.047)	0.186 (0.104)
	northern	0.261 (0.188)	0	0.193 (0.198)
Mass	southern	0.174 (0.116)	0	0.085 (0.126)
	northern	0.395 (0.123)	0.034 (0.044)	0
Total length	southern	0	0.067 (0.034)	0.150 (0.073)
	northern	0.163 (0.172)	0.087 (0.049)	0.079 (0.069)
Growth rate	southern	0.246 (0.137)	0	0.178 (0.067)
	northern	0.259 (0.120)	0.06 (0.059)	0.133 (0.079)

TABLE 4. Phenotypic (r_p), genetic (r_g), and environmental (r_e) correlations between larval period length (age at metamorphosis) and mass at metamorphosis within different treatments in the two populations of *Rana temporaria*.

	r_p	r_g	r_e
Southern population			
Control	0.156 (0.052)*	-0.765 (0.279)*	0.317 (0.218)
Slow	0.254 (0.046)***	-0.458 (0.192)†*	0.325 (0.239)
Fast	0.481 (0.032)***	-0.091 (0.322)†	0.541 (0.071)***
Whole data	0.332 (0.024)***	-0.430 (0.121)**	0.459 (0.085)***
Northern population			
Control	0.271 (0.047)***	-0.355 (0.054)*	0.559 (0.130)**
Slow	0.220 (0.049)***	-0.385 (0.092)**	0.457 (0.260)
Fast	0.241 (0.048)***	-0.427 (0.170)*	0.494 (0.244)
Whole data	0.240 (0.028)***	-0.383 (0.087)**	0.543 (0.166)**

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

† Genetic correlation estimated from product-moment correlation on sire means.

ern population. Although our analyses are based on only two geographically widely separated populations and more definite conclusions will need autecological studies within the two populations as well as comparisons of larger number of populations, we suggest here two scenarios of how these differences may have evolved.

In amphibians, adaptive plasticity in response to pond drying involves the cost of smaller body size at metamorphosis, as was shown in this study. Similarly, Laurila and Kujasalo (1999) found that acceleration of development by two days was coupled with a 30% reduction in metamorphic size. Size at metamorphosis is an important determinant of fitness in amphibians because it may strongly affect adult reproductive parameters (Scott 1994), and plastic changes in metamorphic size directly influence juvenile survival, with smaller individuals having higher mortality (Reques and Tejedo 1997; J. Hjelm and P. Eklöv, unpubl. ms.). Size at the onset of hibernation is an important variable determining overwintering success in many organisms living in seasonal environments (Arendt 1997), and, although no direct data are available, it is reasonable to expect that metamorphic size is an especially important parameter for juvenile frogs in high-latitude populations, where the time available for foraging and growth after metamorphosis is strongly limited as the winter comes early and the hibernation period lasts eight months. Hibernating amphibians lose weight over winter (Holenweg and Reyer 2000), and in *R. temporaria* overwintering success is positively correlated with body size (J. Hjelm and P. Eklöv, unpubl. ms.). Therefore, it seems likely that there is a minimum metamorphic size for a successful hibernation and selection for sufficient metamorphic size may have precluded the plastic responses in development time. This explanation is especially plausible in the view that size and age at metamorphosis were shown to be negatively genetically correlated: Selection for large size at metamorphosis is thus expected to lead to faster development as a correlated response, leaving little room for any further acceleration in development in the face of pond-drying risk. However, metamorphic size has not become a phenotypically fixed trait in the northern population, as desiccation treatments were shown to have an effect on it, although much weaker than in the southern population.

Another perspective to this issue opens if we consider the

observation that the larval period was about 8% shorter in the northern than in the southern population. This finding corroborates the results of the earlier studies (Merilä et al. 2000b; Laugen et al., unpubl. ms.) demonstrating a steadily increasing developmental rate toward higher latitudes across the Scandinavian Peninsula. This finding was framed in terms of the countergradient variation hypothesis (Levins 1969; Berven and Gill 1983; Conover and Schultz 1995), which postulates that genetic capacity for higher developmental and growth rates is expected to evolve in situations where decreasing temperatures and/or shortening growth season tend to suppress or constraint development. The theory on the impact of season length on life histories also has produced qualitatively very similar predictions (Roff 1980), and these have been verified in several insect species (reviewed by Nylin and Gotthard 1998). Consequently, the other explanation for the discrepancy in plastic responses between the two populations found in this study is that the more severe climatic conditions in the north have selected the development rate to the level where it is not possible to accelerate development any more in response to desiccation. Thus, given the negative genetic correlation between size and age metamorphosis, we suggest that selection on either trait might explain the lack of adaptive plasticity in developmental rates of northern frogs.

Plasticity of amphibian metamorphosis has been the subject of much research since the seminal paper by Wilbur and Collins (1973). One of the key predictions of the Wilbur-Collins model is that the initiation of metamorphosis is highly plastic and dependent on the recent growth history of the tadpole. However, there should be a developmental (or body size) threshold only after which metamorphosis is possible (Wilbur and Collins 1973). Most recent studies have used one of two methods, varying food or water level, to manipulate metamorphosis (food level: Alford and Harris 1988; Hensley 1993; Leips and Travis 1994; Newman 1994b, 1998; Denver et al. 1998; Morey and Reznick 2000; water level: Denver et al. 1998; Laurila and Kujasalo 1999; Leips et al. 2000; for reviews on earlier work, see Newman 1992; Gotthard and Nylin 1995). The general result from these experiments is that, whereas larval life histories are highly plastic, the responses depend on the nature of manipulation. For instance, constant, low food level usually increases the length

of larval period, whereas changes from high to low food levels tend to decrease it, indicating that tadpoles respond to changes in recent growth history (Hensley 1993; Leips and Travis 1994; Newman 1998). Water-level changes do not always produce an adaptive response (Brady and Griffiths 2000; Leips et al. 2000), and there is evidence that tadpoles exposed to experimental treatments too early in the development (before Gosner stage 34) may enter developmental stasis and fail to respond to the treatments (Denver 1998; Morey and Reznick 2000). The results of the present study are broadly in accordance with Wilbur and Collins' model; however, it is noteworthy that we started the water-level manipulations well before the critical development stage found in the other studies. Yet, the tadpoles in the southern population responded with accelerated development and the northern tadpoles did not enter developmental stasis. This may indicate that metamorphosis in *R. temporaria* is more plastic than in the other species studied so far. Clearly, additional studies focusing explicitly on developmental thresholds in *R. temporaria* would be useful in relating the present results to the earlier work.

Genetics of Plasticity

Although most of the measured traits exhibited significant additive genetic variation, we found no evidence for existence of genetic variation in plastic responses within populations. This contrasts with the fact that many studies looking for genotypes \times environment interactions in a variety of organisms have found them (reviews in Scheiner 1993; Schlichting and Pigliucci 1998). Furthermore, because the difference in plastic responses between the two populations most likely represents a genetically based difference, there must have been some genetic variation in plastic responses in past for these differences to evolve. One possible explanation for the observed lack of genetic variation in plasticity is that natural selection has eroded variation in plastic responses, perhaps because there is only one way of responding adaptively to desiccation risk in the southern population, whereas the northern larvae have been selected for maximum possible developmental rate. To this end, our results support the general conjecture that trait heritabilities are usually higher than the heritabilities of plasticity in the same traits (Scheiner 1993; Scheiner and Yampolsky 1998). Consequently, even if selection is acting directly on plasticity, evolution of plasticity is likely to be slower than evolution of trait means.

It is well known that quantitative genetic studies need large sample sizes (Falconer and Mackay 1996; Lynch and Walsh 1998). In the present study, the number of parents screened was relatively modest and we cannot rule out the possibility that the absence of genetic variation in plastic responses is due to small sample sizes. Nevertheless, we note that our sample size was much larger, in terms of independent families, than those used in previous genetic studies of phenotypic plasticity in larval amphibians (Newman 1988, 1994a; Semlitsch 1993; Reques and Tejedo 1997), in which evidence for genetic variation in plasticity have been found. However, experimental designs in these previous studies have not always allowed for full distinction between additive, nonadditive, and environmental effects. Many genetic studies on

phenotypic plasticity have used full-sibling designs (e.g., Newman 1994a; Reques and Tejedo 1997; but see Newman 1988; Semlitsch 1993), and effects due to maternal environment and gene interactions have therefore potentially confounded the estimates. In the present study, maternal effects were generally considerably weaker than additive genetic effects, suggesting that maternal effects may not be important determinants of plastic responses. However, dominance variation was significant in many traits, and care should be taken when interpreting the results of experiments based on full-sibling designs (Crnokrak and Roff 1995).

Genetic Organization of Larval Traits

Although the existence and magnitude of heritability of fitness-related traits (life-history traits) has been a matter of some controversy during the past two decades (e.g., Gustafsson 1986; Mousseau and Roff 1987; Roff and Mousseau 1987; Houle 1992), it now appears clear that life-history traits often harbor considerable amounts of genetic variation (Houle 1992; Merilä and Sheldon 1999). In amphibians, size at and timing of metamorphosis have been repeatedly identified as traits influencing fitness (see introduction), and growth rate is a critical trait influencing both size at and timing of metamorphosis (Wilbur and Collins 1973; Rowe and Ludwig 1991). Studies of amphibian larvae have usually found significant heritabilities in length of larval period and size at metamorphosis (Berven and Gill 1983; Berven 1987; Travis et al. 1987; Newman 1988; Blouin 1992; Semlitsch 1993). In the present study, additive genetic variance components were in most cases significant, and the magnitude of the heritability values of larval traits conformed to values typical for life-history traits (Mousseau and Roff 1987).

Berven and Gill (1983) found that heritability of development rate was considerably lower in a tundra population as compared to two more southern populations of *R. sylvatica*, supporting the idea that selection had been eroding the genetic variation in the north. Our results do not support this idea because the additive genetic components and heritabilities of larval period (and other traits) were, if anything, larger in the northern population. Although the reasons for this pattern remain unclear, our results parallel the findings of Armbruster et al. (1998), who found higher amounts of additive genetic variance in life-history traits of the northern as compared to the southern populations of the mosquito *Wyeomyia smithii*. This result was framed in terms of release of additive genetic variance from nonadditive by genetic drift and the relatively recent colonization history of the north (Armbruster et al. 1997, 1998).

Arguments have been put forth to predict that nonadditive genetic contributions to fitness-related traits should be high (Roff 1997; Merilä and Sheldon 1999). One reason for this is that selection is expected to deplete additive genetic variation in fitness traits fast, and most of the remaining genetic variation should be attributable to interaction sources of variance. In line with this prediction, we found significant contributions of dominance variance in most traits. However, the dominance ratios tended to be smaller than those typical for life-history traits (Crnokrak and Roff 1995) and, in general, smaller than or of similar magnitude of heritabilities. Despite

the fact that larval life-history traits are considered to be important fitness-related characters, only two (Travis et al. 1987; this study) of the five amphibian quantitative genetic studies (Newman 1988; Blouin 1992; Semlitsch 1993) using a breeding design capable to detect dominance variance have found significant male \times female interactions in larval growth and development rates. Although this could suggest that the amount of dominance variance in these traits may be typically low, we note that the power to detect these interaction effects may be low due to small number of parents used in all these studies.

Genetic Correlations

We found that the genetic correlation between size and age at metamorphosis was negative, suggesting that selection for faster development (i.e., metamorphosis at younger age) would result in increased size at metamorphosis as a correlated response and vice versa. Because fitness is likely to be maximized by metamorphosing early at large size, this finding contradicts the popular idea that genetic correlations between traits positively correlated with fitness (in this case metamorphic size and development rate) should be predominantly negative (for reviews, see Roff 1996, 1997). However, although genetic correlations among life-history traits are more likely to be negative than those among morphological traits, positive genetic correlations among fitness traits are common (Roff 1996). For instance, two of the five published estimates of genetic correlations between size and age at metamorphosis in amphibians have been negative (Berven and Gill 1983; this study) and the rest positive (Berven and Gill 1983; Travis et al. 1987; Blouin 1992). As pointed out by Houle (1991), such heterogeneity in the sign of genetic correlation among fitness traits may not actually be surprising, but depends especially on the fitness function and mutational variance of the traits in question. The moderately strong and consistent positive environmental correlations between age and size at metamorphosis indicate that environmental factors that tend to delay metamorphosis will also increase size at metamorphosis.

Conclusions

In summary, our results demonstrate clear interpopulation divergence in important larval life-history traits and phenotypic plasticity in a vertebrate species. These findings are consistent with the interpretation that time constraints arising from the differences in growth season length may have selected for faster developmental rate in the north as compared to the south, and that the selection for faster development in the north has been accompanied by reduced ability to respond adaptively to pond drying, perhaps also because of costs (reduced size at metamorphosis) involved with further acceleration of development. To this end, the results indicate that adaptive phenotypic plasticity is not a species-specific fixed trait, but evolution of population differences in the degree of phenotypic plasticity are possible even though the heritability of plasticity appears to be low.

ACKNOWLEDGMENTS

F. Söderman helped in catching the frogs and M. Pahkala, K. Räsänen, and A. T. Laugen gave invaluable help during the initial phases of the experiment. Helpful comments by J. Jokela, A. T. Laugen, S. Nylin, K. Räsänen, and an anonymous reviewer on earlier drafts of the manuscript are acknowledged. Our research was funded by the Swedish Natural Science Research Council (to JM), the Academy of Finland (AL) and the European Union (AL).

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Corresponding Editor: G. Wallis