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COSTS AND LIMITS OF PHENOTYPIC PLASTICITY IN ISLAND POPULATIONS OF THE COMMON FROG *RANA TEMPORARIA* UNDER DIVERGENT SELECTION PRESSURES

Martin I. Lind[1,2] and Frank Johansson[1]

[1]*Department of Ecology and Environmental Science, Umeå University, 901 87 Umeå, Sweden*

[2]*E-mail: martin.lind@emg.umu.se*

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Costs and limits are assumed to be the major constraints on the evolution of phenotypic plasticity. However, despite their expected importance, they have been surprisingly hard to find in natural populations. It has therefore been argued that natural selection might have removed high-cost genotypes in all populations. However, if costs of plasticity are linked to the degree of plasticity expressed, then high costs of plasticity would only be present in populations where increased plasticity is under selection. We tested this hypothesis by investigating costs and limits of adaptive phenotypic plasticity in development time in a common garden study of island populations of the common frog *Rana temporaria*, which have varying levels of development time and phenotypic plasticity. Costs of plasticity were only found in populations with high-plastic genotypes, whereas the populations with the most canalized genotypes instead had a cost of canalization. Moreover, individuals displaying the most extreme phenotypes also were the most plastic ones, which mean we found no limits of plasticity. This suggests that costs of plasticity increase with increased level of plasticity in the populations, and therefore costs of plasticity might be more commonly found in high-plastic populations.

KEY WORDS: **Costs of plasticity, development time, limits of plasticity, pool permanence, size at metamorphosis.**

Adaptive phenotypic plasticity is the ability of a genotype to express different adaptive phenotypes in different environments (Pigliucci 2005). Theory shows that in the absence of constraints, adaptive phenotypic plasticity is generally favored over specialization in heterogeneous environments (e.g., Via and Lande 1985; Moran 1992; Van Tienderen 1997; Sultan and Spencer 2002). This has been supported in studies of species (Richter-Boix et al. 2006; Hollander 2008) and natural populations (Lind and Johansson 2007). However, phenotypic plasticity is not always present (Delasalle and Blum 1994; Pigliucci 1997), and often the degree of plasticity varies between populations (Donohue et al. 2000; Van Buskirk and Arioli 2005; Lind and Johansson 2007). Although the benefits of plasticity are clear, it is less clear what constrains plasticity (DeWitt 1998), although it is generally believed that the two most important factors that can constrain the evolution of phenotypic plasticity are fitness costs of the ability to be plastic and limits to the phenotypes that a plastic genotype can express (DeWitt et al. 1998).

Costs of plasticity imply that a plastic genotype has a fitness cost compared to a nonplastic genotype when expressing the same trait value (Pigliucci 2005). De Witt et al. (1998) have suggested a number of possible costs paid by plastic individuals but not by specialists, such as costs of maintaining the sensory and response pathways necessary for an accurate plastic response and extra costs of producing the trait not paid by the specialist. The fitness cost could either be constant, that is, independent of the level of plasticity of the genotype (e.g., Moran 1992; Sultan and Spencer 2002), or increase with the degree of plasticity (Van Tienderen

1997). Obviously, without some costs of plasticity, plasticity has only advantages and becomes the common outcome in theoretical models in which specialization is the alternative (Van Tienderen 1997). However, despite the assumed importance of plasticity costs, they have been surprisingly hard to find in natural populations (see a recent meta-analysis by Van Buskirk and Steiner 2009) and have only been detected in a fraction of measured traits in these experiments (DeWitt 1998; Scheiner and Berrigan 1998; Donohue et al. 2000; Dorn et al. 2000; Poulton and Winn 2002; Relyea 2002; Steinger et al. 2003; Callahan et al. 2005; Weijschedé et al. 2006; Weinig et al. 2006; Avramov et al. 2007; Steiner and Van Buskirk 2008), or only in a few measured environments and populations (Donohue et al. 2000; Merilä et al. 2004; Callahan et al. 2005). Many studies have also found significant positive relationships between plasticity and fitness (e.g., Dorn et al. 2000; Relyea 2002; Weijschedé et al. 2006; Weinig et al. 2006; Avramov et al. 2007; Steiner and Van Buskirk 2008), which have been interpreted either as costs of homeostasis (Dorn et al. 2000), or costs of canalization (Poulton and Winn 2002; Steiner and Van Buskirk 2008). Interestingly, the meta-analysis by Van Buskirk and Steiner (2009) found that costs of plasticity and canalization were about equally uncommon (costs of plasticity were only found in 14% of all published species, trait, and treatment combinations, costs of canalization in 11% of the published species, trait, and treatment combinations). Unfortunately, the importance of costs of canalization on the evolution of phenotypic plasticity has never been theoretically investigated.

Limits of plasticity mean that the plastic genotype can only produce phenotypes within a certain range. More extreme phenotypes cannot be achieved by plastic genotypes: they are only produced by specialized genotypes (Pigliucci 2005). Theory suggests that "the jack of all trades is the master of none" (Levins 1968), implying that although phenotypic plasticity allows individuals to deal with different environmental conditions, they are inferior to a nonplastic specialist in any particular environment (Newman 1992; DeWitt et al. 1998; van Kleunen and Fischer 2005). Thus, the theory assumes that if there is no stabilizing selection within each environment; the optimum phenotype will be within the range of the phenotypes that the plastic genotype can express, and the extreme phenotype is no longer the optimal phenotype. Limits to plasticity have received much less scrutiny than costs of plasticity: limits of plasticity have been investigated in a few studies only, and surprisingly neither DeWitt (1998) nor Relyea (2002) found evidence of limits. Instead, they found that the most plastic genotypes were also able to express the most extreme traits.

DeWitt (1998), Sultan and Spencer (2002), Callahan et al. (2005), and Weinig et al. (2006) have suggested that the failure of most studies to find costs of plasticity may be due to the action of natural selection, which might already have removed plastic genotypes with high costs. However, we argue that our inability to find strong evidence for costs and limits of plasticity might instead be due to the fact that these factors can differ among populations and environments. The current studies of costs and limits of plasticity have seldom included more than one population (but see Donohue et al. 2000; Merilä et al. 2004). Because selection pressures can differ over the landscape (Thompson 2005), populations can differ in both trait means (Reznick et al. 1997; Johansson et al. 2005) and plasticity (Donohue et al. 2000; Merilä et al. 2004; Lind and Johansson 2007). Thus, within a species, different populations can be subjected to opposing selection pressures, potentially influencing how the costs and limits of plasticity are expressed. If costs of plasticity are genetically linked to the degree of plasticity expressed, as assumed in the models of van Tienderen (1997), then high costs of plasticity would only be present in populations where increased plasticity is under selection. However, if all plastic genotypes pay the same costs, costs of plasticity would not differ between populations, as plastic genotypes pay the same costs of being plastic whether or not plasticity is expressed (De Witt et al. 1998). As for costs, limits of plasticity might also be population specific. It has been argued that limits of plasticity will not be detected if selection periodically alternates for and against specialized genotypes (Relyea 2002). Therefore, limits of plasticity should be most easily detected in populations specialized to an extreme environment, where the selection pressures may be expected to constantly favor extreme phenotypes.

Therefore, studies in which these factors are compared among populations that are expected to differ with regard to costs and limits of plasticity are needed. Temporary pools are excellent systems for the study of phenotypic plasticity (Newman 1992; De Block and Stoks 2005), and among organisms inhabiting these environments, amphibian metamorphosis is well studied (e.g., Wilbur 1987; Newman 1992; Denver et al. 1998; Brady and Griffiths 2000; Loman and Claesson 2003; Merilä et al. 2004). Time to, and size at, metamorphosis are important fitness components in amphibians; a large metamorphic size has substantial fitness benefits after metamorphosis (Smith 1987; Berven 1990; Morey and Reznick 2001; Altwegg and Reyer 2003), but is traded off against short development time in response to pool desiccation (Laurila and Kujasalo 1999). Therefore, we use metamorphic weight as our estimate of fitness. In this study, we investigate costs and limits of adaptive phenotypic plasticity in development time in isolated island populations of the common frog (*Rana temporaria* Linnaeus 1758) in an archipelago in the Gulf of Bothnia, northern Sweden. Because of the land uplift, the islands in the archipelago are relatively young, and the estimated ages of the frog populations in the area are between 23 and 267 generations (Johansson et al. 2005). The frogs breed in pools on the islands and these pools differs in drying regime. The rate of pool drying does not appear to be correlated with any other environmental variable associated

with islands (Lind and Johansson 2007). The populations show local adaptation in fixed development time as a response to the risk of pool desiccation so that tadpoles from islands with only temporary pools have a genetically shorter development time than those from islands with only permanent pools (Johansson et al. 2005; Lind and Johansson 2007; Lind et al. 2008). Moreover, the populations differ in their plasticity in development time, and the degree of plasticity is correlated with the within-island heterogeneity in pool-drying regimes (Lind and Johansson 2007). Thus, plasticity is adaptive and we can compare the costs and limits of plasticity in relatively young replicated natural populations with high or low levels of phenotypic plasticity in development time, and in populations locally adapted to the extreme environments (permanent or temporary pools). These factors are likely to influence the costs and limits of plasticity (Relyea 2002; Weinig et al. 2006).

We performed a common garden experiment with constant water level and artificial drying as treatments to estimate the costs and limits of phenotypic plasticity in development time. If natural selection quickly removes costly genotypes, and costs of plasticity are paid by all individuals expressing plasticity, then we predict no difference in costs between environments. However, if costs of plasticity are positively correlated to the degree of plasticity expressed, we expect to find more costs of plasticity in high-plasticity populations. We also predict stronger limits of plasticity on islands with more homogeneous environments (i.e., islands with either temporary or permanent drying regimes) because natural selection has favored specialists in these environments.

Methodology

In a common garden experiment, we compared the costs and limits of phenotypic plasticity in populations with high or low natural levels of phenotypic plasticity, and populations originating from islands with temporary or permanent pools. To sample populations from a range of pool-drying regimes (from island with temporary to permanent pools), eggs were collected from nine islands in an archipelago outside Umeå, northern Sweden (Åhällan 63°40′N, 20°23′E; Ålgrundet 63°41′N, 20°25′E; Bredskär 63°39′N, 20°18′E; Lillklyvan 63°42′N, 20°26′E; Öster Hällskär 63°48′N, 20°37′E; Sävar-Tärnögern 63°45′N, 20°36′E; Stora Fjäderägg 63°48′N, 21°0′E; Storhaddingen 63°40′N, 20°25′E; Vitskär 63°40′N, 20°22′E) between 9 and 13 May 2006 (for a map, see Lind and Johansson 2007). To assess the pool-drying regime present on an island, the decrease in water level of the pools was estimated as follows. Maximum pool depth was measured at egg collection and at 16 and 17 August 2006. The percentage decrease in pool depth between the two sampling dates was then used as a proxy for the hydroperiod, following Lind and Johansson (2007). For two deep permanent pools (Stora Fjäderägg, Öster Hällskär), pool depth was not measured.

To estimate development time and plasticity, eggs were taken from the breeding poles on the island and transported to the laboratory. From 12 different egg clutches from each island, 40–50 eggs were sampled. If multiple pools were present on an island, eggs were taken from all pools in which breeding had occurred and the mean pool-drying regime of the breeding pools was calculated, to get a value for the average pool-drying regime of the island. Each egg clutch corresponds to the offspring of one female because female *R. temporaria* lay only one egg clutch per year in this region (Elmberg 1991). The eggs were kept at 4°C in the laboratory to slow down development, until all eggs were collected. This water temperature is at the mean temperature for spawning onset of *R. temporaria* in this region (Elmberg 1990). When all eggs were collected, the temperature of the laboratory was set to 22°C for the remainder of the experiment.

To separate the genetic and the environmental effects on development time and plasticity, both were estimated in a common garden, where replicate siblings from each egg clutch were subjected to one of two treatments. Development time was estimated in two laboratory treatments: constant water level (C) and artificial pool drying (D), and plasticity in development time was defined as the reduction in development time in the artificial drying treatment, see Lind and Johansson (2007). Six tadpoles from each female, three allocated to each water level treatment, were randomly chosen and placed individually into plastic containers (9.5 × 9.5 cm, height 10 cm). The experimental containers were filled with 750 mL of tap water, previously aged and aerated together with dried deciduous leaves, which were removed when the water was transferred to the experimental containers. This ensured sufficient oxygen levels for the tadpoles. In the simulated pool-drying treatment, the initial water volume of 750 mL was reduced by 33% every fourth day, starting at day 5 and continuing until day 25, after which the water volume was kept constant at 66 mL. The water temperature did not differ between the two treatments. The tadpoles were fed ad libitum every fourth day on a mixture (1:1) of finely ground fish food and rabbit chow. The tadpoles were given 15 mg food per tadpole at the beginning of the experiment, and it was increased to 30 mg at day 9, 45 mg at day 13, 60 mg at day 17, and 75 mg from day 21 to the end of the experiment, following Lind and Johansson (2007). Water was replaced every fourth day, prior to feeding. A light:dark cycle of 18:6 h was employed, which corresponds to the natural cycle in the area of egg collection. Experiments were started when the tadpoles entered Gosner stage 23 (active swimming) (Gosner 1960).

Maternal effects can always influence the phenotypic expression and needs to be controlled for. As maternal effects in *R. temporaria* most commonly are transferred through the size of

the egg (Laugen et al. 2002), we estimated the egg size of every clutch. Prior to hatching, at Gosner stage 10 (Gosner 1960), 10 eggs from each clutch were placed in a petri dish, completely covered with water, and photographed together with a scale using a vertically placed digital camera (Canon EOS 350D [Tokyo, Japan] with Tamron SP AF 90 mm F/2.8 Di Macro 1:1 lens [Tamron, Saitama, Japan]). The mean egg size of each female was then calculated using the image analysis program ImageJ version 1.36b (http://rsb.info.nih.gov/ij/), which later allowed us to control for egg-size-mediated maternal effects (Laugen et al. 2002) on development time and plasticity.

The experiment was terminated when the tadpoles entered Gosner stage 42 (Gosner 1960). At this stage, the forelimbs are visible and we define this as our estimate of metamorphosis. We used metamorphic weight as our fitness estimate because large size at metamorphosis is generally positively correlated with various aspects of fitness in amphibians (Smith 1987; Berven 1990; Morey and Reznick 2001; Altwegg and Reyer 2003). Therefore, the tadpoles were weighed (wet weight) and the time from the start of the experiment (at Gosner stage 23) until metamorphosis was recorded as development time. Plasticity in development time was defined as the mean development time for each family under constant water level conditions minus the mean development time under the artificial drying treatment, that is, how much the tadpoles were able to speed up the development time as a response too the artificial pool-drying treatment. Population level plasticity was defined as the mean plasticity in development time of the families in the populations and was used to distinguish high- and low-plasticity populations for analyzing costs and limits of plasticity.

STATISTICAL ANALYSES

Costs of developmental plasticity imply that the plastic genotypes have fitness costs compared to nonplastic genotypes when expressing the same trait value (DeWitt et al. 1998), and were estimated using the following model (Van Tienderen 1991; Scheiner and Berrigan 1998):

$$W = X + X^2 + \mathrm{pl}X + X \times \mathrm{pl}X + X^2 \times \mathrm{pl}X, \qquad (1)$$

where W is the estimate of fitness (metamorphic weight in our case) in one environment, X is the age at metamorphosis (development time) in that environment and pl$X$ is the plasticity in age at metamorphosis, as measured between the two environments. X and X^2 estimate the linear and nonlinear selection components (Scheiner and Berrigan 1998). A negative regression component of the pl$X$ term indicates that the more plastic genotypes have a fitness cost when expressing the same trait value, that is, a lower metamorphic weight than a less plastic genotype at a given development time, whereas a positive regression coefficient implies that there is a fitness cost of possessing the ability to express a canalized (i.e., environmental insensitive) phenotype (Scheiner and Berrigan 1998; Poulton and Winn 2002). Egg size was included in the model as a covariate, to control for egg-size-mediated maternal effects (Laugen et al. 2002). Population was included in the model as a random effect. The model was fitted separately for the two treatments (constant water level and artificial pool drying), and for the three populations with highest or lowest mean plasticity in development time.

Limits of plasticity imply that the most plastic families cannot express the extreme traits. Therefore, limits of plasticity were investigated by correlating the development time of the families with their plasticity in development time in each environment (DeWitt 1998; DeWitt et al. 1998; Relyea 2002). Egg size was incorporated in the model as a covariate to control for egg-size-mediated maternal effects. To estimate whether plasticity was limiting the expression of the extreme trait value, we had to define the direction of the extreme trait value in each environment. In the time-constrained artificial pool-drying treatment, the adaptive developmental extreme is a short development time, whereas in the constant water level treatment, the adaptive extreme is an extended development time (Newman 1988). Thus, in the pool-drying treatment, limits of plasticity are present if we find a positive correlation between development time and plasticity in development time. In contrast, if there are limits of plasticity in the constant water level treatment, they will be identified by a negative correlation between development time and plasticity in development time. This does not mean that plastic genotypes must show negative, nonadaptive plasticity for limits to be present, rather than the limited plastic genotypes shall have a relatively shorter development time than the less plastic genotypes under constant water level conditions, and a relatively longer development time than the less plastic genotypes under artificial drying conditions.

Because our main aim was to estimate costs and limits of plasticity in populations that differ in regard to drying regime and in their degree of plasticity in development time, we chose populations from the extreme ends of the plasticity and pool permanence continuum for our analyses (see Table 1). Therefore, not all populations were used in the analyses; only the extreme populations in plasticity or pool-drying regime. Even if pool drying (lowering of the water surface) was observed for most pools, none of the extreme permanent pools has dried out completely in earlier years, whereas all pools on the temporary extreme are known to dry out regularly. Hence, for the costs of plasticity analysis, the three populations with the lowest and three populations with the highest mean family plasticity in development time were compared. For limits of plasticity, in addition to comparing populations with high and low plasticity's, the populations inhabiting the three islands with most temporary pools were compared to the populations originating from the three islands with most permanent pools (Table 1).

Table 1. Estimated pool drying, expressed as decrease in maximum pool depth between May and August 2006 in pools in which breeding had occurred. If multiple breeding pools were present within an island, the mean pool-drying regime was calculated. Population level plasticity in development time was measured in the laboratory as the decrease in development time between the constant water level and the artificial drying treatment. Number of families on each island in which individuals from both treatments survived to metamorphosis is indicated. For more detailed information, see Supporting Information Table S1.

Island name	Pool drying (%)	Plasticity in development time (days)±SE	Families
Åhällan	88.4	1.24±0.24	12
Ålgrundet	100	1.28±0.21	12
Bredskär	59.8	1.28±0.19	9
Lillklyvan	77.8	0.85±0.43	11
Öster Hällskär	0	2.99±0.82	9
Sävar-Tärnögern	100	1.40±0.32	12
Stora Fjäderägg	0	1.34±0.36	12
Storhaddingen	73.6	0.75±0.41	12
Vitskär	86.7	1.08±0.19	9

A Bayesian approach was used to calculate parameter estimates and confidence intervals of the models above, using Markov chain Monte Carlo (MCMC) methods. The models were fitted using the *lmer* function in the *lme4* library of the statistical package *R* (http://www.r-project.org). We used the *mcmcsamp* function to sample from the posterior density of the parameters of the fitted models. Two MCMC chains of 50,000 steps were run, and the first 1000 steps were discharged as burn-in samples. Confidence intervals were calculated as the region containing 95% of the posterior probability mass. Parameter estimates and 95% confidence intervals are presented in Figures 1 and 3, if the confidence interval overlaps zero, the parameter estimate does not differ from zero.

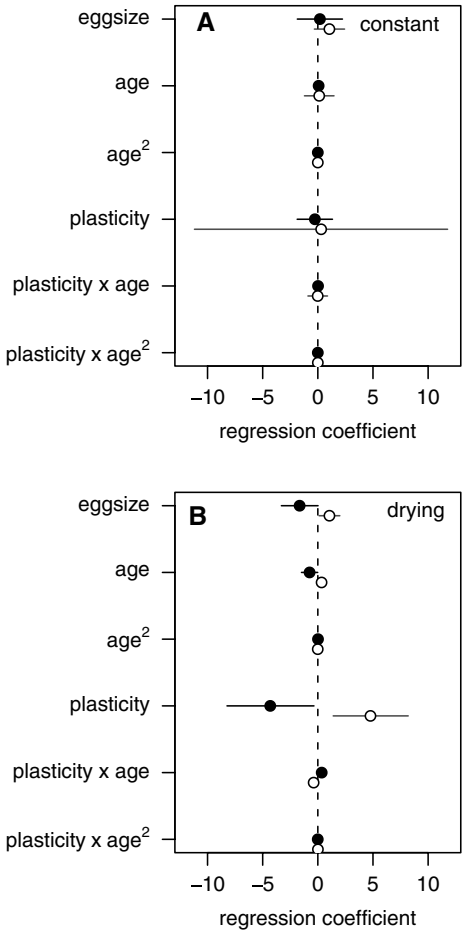

Figure 1. Mean and 95% confidence interval for the regression coefficients of parameters in equation (1) affecting weight at metamorphosis, our fitness estimate. Populations with high mean plasticity (filled circles) or low mean plasticity (open circles) under constant water level (A) or artificial drying treatment (B). If the confidence interval overlaps zero, the parameter does not differ from zero. A cost of plasticity is indicated as a negative effect of plasticity in development time on metamorphic weight, our fitness estimate.

Results

HATCHING SURVIVAL

Of the 800 tadpoles in the experiment, 719 survived until metamorphosis. Most cases of mortality were identified early in the experiment by developmental abnormalities and unusually slow development, before the treatments had any effect.

COSTS OF PLASTICITY

Costs of plasticity imply that the plastic genotypes have fitness costs (lower metamorphic weight, our fitness estimate) compared to nonplastic genotypes when expressing the same trait value (development time). The costs of plasticity were environment- as well as treatment-specific because the regression coefficient of the plasticity term (pl$X$ in equation (1)) differed between environments and treatments (Fig. 1). In the constant water level treatment, the degree of phenotypic plasticity in development time did not affect the final weight at metamorphosis, which was used as our fitness estimate (Fig. 1A). Hence no costs of plasticity or canalization were observed under the constant water level treatment. However, in the artificial drying treatment, a significant cost of plasticity was found in the three populations with highest mean plasticity in development time, as shown by the negative regression coefficient of the plasticity term (Fig. 1B). In contrast, in the three populations with the lowest degree of phenotypic plasticity in development time, the opposite pattern was found because a positive regression coefficient for the plasticity term was present (Fig. 1B) indicating a cost of canalization

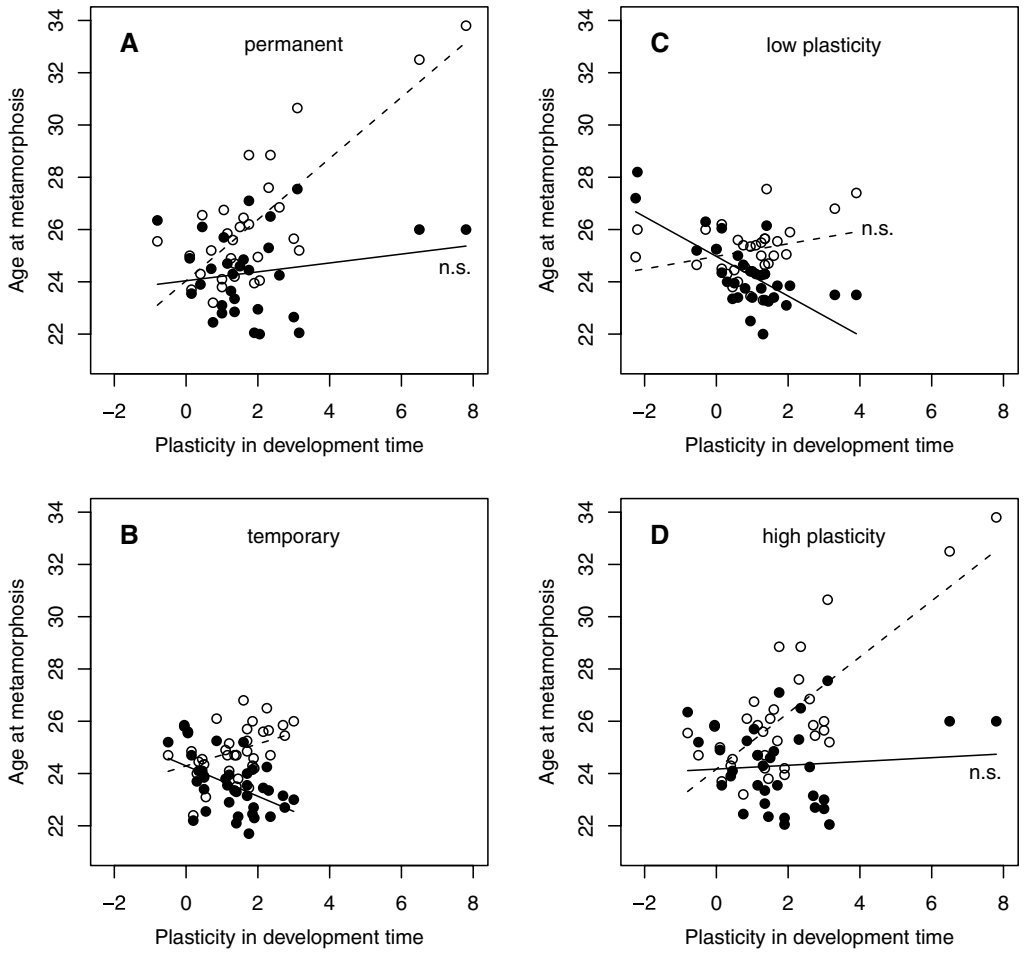

Figure 2. The relationship between phenotypic plasticity in development time and development time under artificial drying (filled circles and solid lines) or constant water level treatment (open circles and broken lines). Plasticity is limiting the expression of extreme phenotypes if we find a positive correlation between plasticity and the phenotype in the artificial drying treatment and a negative correlation in the constant water level treatment. (A) The three populations from the islands with most permanent pools, (B) the three populations from the islands with most temporary pools, (C) the three populations showing least plasticity in development time, (D) the three populations showing most plasticity in development time. For significance of parameters, see Figure 3.

because the most plastic genotypes also had the largest metamorphic weights when expressing the same development time. No other coefficients showed any significant effect on weight at metamorphosis (Fig. 1). Because removing the nonlinear selection coefficient X^2 and its interaction significantly reduced the model fit it was retained in the model, even though its confidence interval includes zero.

LIMITS OF PLASTICITY

Limits of plasticity mean that the plastic genotypes are not able to express the extreme trait. For plasticity to limit the expression of the extreme development times, a negative correlation between plasticity and the trait value was expected under the constant water level treatment (long development time is optimal), whereas a positive correlation was expected under the artificial pool-drying treatment (short development time is optimal). However, the most plastic families did not, independent of treatment or origin, display a limited developmental range compared to the less plastic individuals (Figs. 2 and 3). Under constant water level conditions, the tadpoles from the most plastic families originating from islands with permanent pools were also able to express the most extended development times; however, the development time of the most plastic families were neither more nor less extended than the development time of the less plastic families under artificial drying (Fig. 2A). In contrast, the most plastic families from islands with temporary pools were able to express the shortest development time under artificial drying as well as most extended development time under constant water level conditions in the laboratory (Fig. 2B).

The most plastic families originating from populations with the lowest mean plasticity values in development time were able to express the shortest development times, whereas the degree of

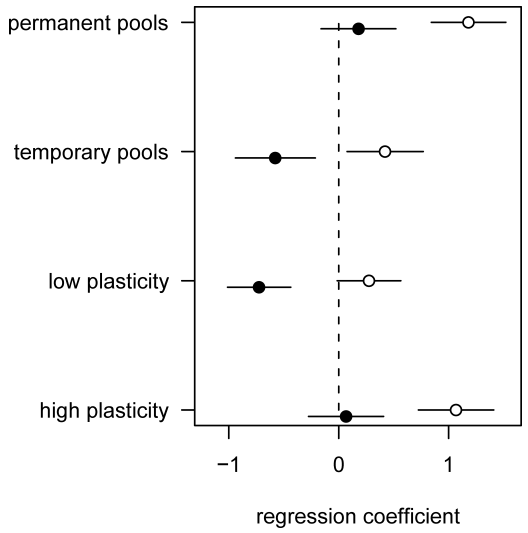

Figure 3. Mean and 95% confidence interval for the regression coefficient of plasticity in development time when investigating the relationship between phenotypic plasticity in development time and development time under artificial drying (filled circles) or constant water level treatment (open circles). If the confidence interval overlaps zero, the parameter does not differ from zero. Data shown for the three populations from the islands with most permanent pools, the three populations from the islands with most temporary pools, the three populations showing least plasticity in development time, and the three populations showing most plasticity in development time.

family plasticity was not related to the development time under constant conditions in these populations (Fig. 2C). The pattern in populations with high mean values of phenotypic plasticity was the opposite: the most plastic families were able to express the most extended development time under constant water level, whereas plasticity was not related to development time under artificial drying conditions (Figs. 2D and 3). Egg size was not influential in any analysis and was excluded in the model simplification process.

In summary, we found little evidence for limits of plasticity. Of the eight correlations performed, five were opposite in sign to those predicted, that is, the most plastic genotypes expressed the most extreme trait values. The other three correlations showed no significant relationship between the trait value and the plasticity in the trait.

Discussion

COSTS OF PLASTICITY

Costs of plasticity have been surprisingly hard to find in natural populations (e.g., Scheiner and Berrigan 1998; Donohue et al. 2000; Weijschedé et al. 2006; Steiner and Van Buskirk 2008, Van Buskirk and Steiner 2009). The general failure to find costs of plasticity in the majority of traits has led to the hypothesis that natural selection quickly removes costly genotypes (DeWitt 1998; DeWitt et al. 1998; Sultan and Spencer 2002; Callahan et al. 2005; Weinig et al. 2006). However, if the costs of plasticity are positively related to the degree of plasticity expressed (Van Tienderen 1997); then failure to find costs of plasticity might be because we are examining populations with too low degree of plasticity for the costs to be detected. This hypothesis has, however, never been critically examined. As the first study designed to test this, we compared the costs of plasticity in development time for three island populations with a high and three island populations with a low mean plasticity in development time. Costs were measured as a negative correlation between the degree of developmental plasticity and metamorphic weight (our fitness estimate), when expressing the same development time. Costs of plasticity were only found in populations with high mean plasticity, implying that natural selection had not removed the costly genotypes in these populations. Moreover, in the three populations expressing the lowest plasticity, the most plastic genotypes had a fitness benefit, higher metamorphic weight, compared to the less plastic genotypes when expressing the same development time (Fig. 1B). We cannot therefore support the hypothesis that selection against costly genotypes explains why costs of plasticity are hard to find in natural populations. Instead, our study suggests that plasticity is maintained in a population with high degree of phenotypic plasticity despite an increased fitness cost (lower metamorphic weight) with increased plasticity in development time in the population, that is, the costs seems to be genetically linked to the extent of plasticity.

Therefore, we argue that a main reason that costs of plasticity have been difficult to find in natural populations is that the costs are mostly expressed in populations in which the individuals have a high degree of phenotypic plasticity in the trait we measure. If the populations we are using for estimating costs of plasticity are not selected for high plasticity, we will be less likely to find costs of plasticity. Although most models of the evolution of phenotypic plasticity assume the costs of plasticity to be independent of the level of plasticity expressed, Van Tienderen (1997) considers the cost of plasticity to be linearly related to the magnitude of plasticity. Support for this interpretation also comes from a study of *R. temporaria* populations from southern and northern Sweden (Merilä et al. 2004). The authors found that the populations in southern Sweden were more plastic in their development time, and also had a significant cost of plasticity, which was absent in the less plastic northern populations. Nevertheless, Donohue et al. (2000) found no evidence of plasticity costs in shade avoidance traits when comparing two populations of *Impatiens capensis* that differed in plasticity, contrary to what would been expected. If our result is general, it could be that the *I. capensis* populations studied by Donohue et al. (2000) showed too little difference in plasticity to be able to detect costs of plasticity.

Our study adds to the numerous examples showing that, even if plasticity costs are present in a trait, these costs are treatment dependent (Dorn et al. 2000; Relyea 2002). This is of special concern because theoretical modeling has shown that only global costs (costs expressed over multiple environments) will influence the evolution of plasticity (Sultan and Spencer 2002). The fitness cost of plasticity in development time was expressed only in the time-stressed artificial drying treatment, which is predicted (Van Tienderen 1991) and commonly found in studies on animals (Van Buskirk and Steiner 2009) because individuals in stressful treatments might be unable to allocate enough resources to both maintenance of developmental systems underlying plasticity and growth (Van Tienderen 1991; DeWitt 1998; Dorn et al. 2000; Steinger et al. 2003; van Kleunen and Fischer 2005; Weinig et al. 2006, but see Merilä et al. 2004; Steiner and Van Buskirk 2008).

It has been argued that, if the degree of plasticity is coupled to the trait mean (Via 1993), an increase in the trait mean would also increase the degree of plasticity and potentially lead to the accumulation of costs (Weijschedé et al. 2006). This does not occur in our system because the trait mean (development time) and the degree of plasticity in development time are not correlated under any treatment in our study system (Lind and Johansson 2007). It should also be noted that there might be costs of plasticity unrelated to our fitness estimate (metamorphic size) and that they therefore went undetected in our analysis. However, we emphasize that metamorphic weight is positively correlated to a number of fitness measures in amphibians (Smith 1987; Berven 1990; Morey and Reznick 2001; Altwegg and Reyer 2003). Another general problem with studies using the regression approach to detect potential plasticity costs is that costs might be hard to detect if the plastic genotypes are not able to express the extreme phenotypes because the fitness function will be heavily weighted by these extreme nonplastic phenotypes. However, that is not a problem in the current study, as the most plastic genotypes also expressed the extreme phenotypes and no limits of plasticity were present.

In addition to a fitness cost of plasticity for individuals from populations with high plasticity, a fitness benefit of plasticity was found in the populations with lowest mean plasticity in development time (Fig. 1B), indicating a cost of canalization (Dorn et al. 2000; Poulton and Winn 2002; Steiner and Van Buskirk 2008). Although costs of canalization have been found in a number of studies of both animals and plants (e.g., Dorn et al. 2000; Relyea 2002; Weijschedé et al. 2006; Weinig et al. 2006; Avramov et al. 2007; Steiner and Van Buskirk 2008), canalization costs have not been considered as a factor influencing the cost/benefit trade-off in the evolution of phenotypic plasticity (Van Tienderen 1991; DeWitt et al. 1998; Scheiner and Berrigan 1998). Yet the empirical evidence for costs of canalization suggests that they are common (Van Buskirk and Steiner 2009) and might influence the evolution of plasticity and specialization.

LIMITS OF PLASTICITY

The hypothesis that limits of plasticity exist is based on the assumption that plastic genotypes have higher fitness costs compared to nonplastic ones. In models in which the population has access to two environments, increased costs of plasticity generally lead to a shift from plastic generalists to specialists on the two environments, with more extreme phenotypes (e.g., Moran 1992; Wilson and Yoshimura 1994; Sultan and Spencer 2002). However, our study adds to the previous empirical evidence (DeWitt 1998; Relyea 2002) that limits of plasticity do not exist in natural populations and that the developmental ranges of plastic genotypes are not smaller than those of the fixed specialists (Figs. 2 and 3). If plasticity is not costlier than specialization, as experimental evidence suggests (e.g., Scheiner and Berrigan 1998; Relyea 2002; Steiner and Van Buskirk 2008), there is no reason for limits of plasticity to exist, and this is what we find.

One proposed explanation for the lack of plasticity limits is that alternating selection pressures would select against the nonplastic extreme specialists (Relyea 2002). However, no limits of plasticity were present in the populations expressing the lowest levels of phenotypic plasticity in this experiment, despite the fact that individuals in these populations were more specialized. Moreover, plasticity was not limiting the expression of the extreme development times in the populations from temporary or permanent environments, despite the fact that individuals from these populations are locally adapted in their development time to the pool-drying regime present (Johansson et al. 2005; Lind and Johansson 2007; Lind et al. 2008). Therefore, we found no support for the hypothesis that limits of plasticity should be present in populations with low plasticity or local adaptation to an extreme environment.

Another explanation to the lack of studies finding limits of plasticity is that, for limits to be found, the most extreme phenotype must also be the most optimal phenotype in each environment, for example, directional and not stabilizing selection on the trait must take place within each environment. Stabilizing selection is the most likely scenario in our system because the specialist genotypes often expressed a development time in between the extremes of the plastic genotypes. Because the degree of phenotypic plasticity and the trait mean can evolve as independent characters (Scheiner and Lyman 1991; Pigliucci and Schmitt 1999); the adaptation to temporary pools seems to have been driven by a shift in the trait mean (Lind and Johansson 2007). Yet plasticity around that mean development time has been retained, as only the most plastic individuals have been able to express the extreme development time. Therefore it will be hard to find limits of plasticity in the frog system studied.

Both canalization and phenotypic plasticity were found in our study system because development times and plasticity in development times are not correlated (Lind and Johansson 2007). Within an island population, genotypes adapt either by development time becoming canalized to the mean drying regime on the island, or by increased plasticity in development time. As a consequence of this local adaptation to the local environment in every population, the individuals within a population with one environment will appear as specialists to that environment *when compared to* individuals from island populations with contrasting environmental conditions. Possibly, the shifts in the trait mean when the frogs have invaded the islands with temporary pools have been accommodated by phenotypic plasticity in development time, making them able to express the shorter development time needed to survive in those temporary pools through a Baldwin effect (Crispo 2007).

The analysis of limits of plasticity also provides insight into the direction of the plastic response in development time to pool drying. Although the most plastic individuals from populations with temporary pools can both speed up and slow down their development times depending upon treatment (Fig. 2B), the most plastic tadpoles from islands with permanent pools do not develop faster than the less plastic individuals under artificial drying, but have longer development times under constant water level conditions (Fig. 2A). Because the risk of complete pool drying is very low in these pools, there is probably no selection pressure to decrease the development time. The observation that the most plastic tadpoles were also able to express the longest development times in the permanent conditions in these pools indicates that plasticity in development time is an important trait in these permanent pools. Therefore, despite the stable environment in permanent pools, plasticity is maintained by selection because only the most plastic tadpoles can express the extreme trait (Fig. 2D). Because no cost of being plastic was found in the constant water level treatment, plasticity in development time is not selected against in populations inhabiting islands with permanent (constant) pools.

CONCLUSIONS

This study is the first to test the general hypothesis that the lack of ubiquitous presence of plasticity costs is the consequence of natural selection removing costly genotypes. Our results, however, showed that high levels of plasticity occurred in populations despite increasing costs of plasticity. Taken together, these results suggest that phenotypic plasticity can evolve despite increasing costs with high levels of plasticity and that costs of plasticity therefore are most likely to be found in populations in which the individuals express a high level of phenotypic plasticity. Moreover, costs of canalization were found, a cost that has not been included in models of phenotypic plasticity. The study also adds to the existing empirical evidence that limits of plasticity are uncommon in natural populations. Together, these results suggest that the current theories of costs and limits of plasticity need to be extended, to include varying costs between environments (Steinger et al. 2003) and to investigate the reason why plasticity costs seem to increase, rather than decrease, with increased plasticity in a population (Merilä et al. 2004). This may indicate that plasticity costs are not important constraints for the evolution of plasticity as previously believed (DeWitt et al. 1998). Moreover, our study also highlights the fact that costs of plasticity are population specific (see also Merilä et al. 2004) and can differ substantially between populations adapted to different environments. We agree with Steiner and Van Buskirk (2008) that the reason that we observe different levels of plasticity in different populations is that they are maintained by differences in environmental selection affecting plasticity (or drift), rather than costs of plasticity. In addition to costs of plasticity, another strong predictor if plasticity or specialization will evolve in theoretical models is the accuracy of the response to the environment (e.g., Moran 1992; Sultan and Spencer 2002). We hope that more experimental studies will investigate the accuracy of the plastic response, as this might be one of the most important constraints on the evolution of phenotypic plasticity in natural populations.

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Supporting Information

The following supporting information is available for this article:

Table S1: Mean and standard error of the metamorphic weight and development time for all islands.

Supporting Information may be found in the online version of this article.
(This link will take you to the article abstract).

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