Evol Biol (2013) 40:494–503
DOI 10.1007/s11692-013-9225-8

RESEARCH ARTICLE

Within-Population Developmental and Morphological Plasticity is Mirrored in Between-Population Differences: Linking Plasticity and Diversity

Frank Johansson · Alex Richter-Boix

Received: 22 January 2013 / Accepted: 5 March 2013 / Published online: 15 March 2013

Abstract It has been suggested that phenotypic plasticity can facilitate evolutionary diversification of organisms. If life-history and morphological diversification across a lineage is mirrored in diversification in the same traits due to phenotypic plasticity within a lineage it fulfils one of the expectations that are needed to support this diversification hypothesis. We carried out a laboratory study to examine development rate and morphology between and within populations of the parsley frog, *Pelodytes punctatus*. We found that frogs reared in the laboratory had a longer development time, relatively longer hind legs and relatively narrower heads under constant water level compared to those under decreasing water level simulating pool drying. This adaptive phenotypic plasticity response to pool drying was mirrored across populations because frogs from permanent waters had longer development times, relatively longer hind legs and relatively narrower heads compared to frogs from temporary waters. Hence the developmental and morphological plasticity observed within populations was also observed between populations as constitutive expressed traits. We suggest that the morphology pattern observed was driven by a common developmental process (time to metamorphosis), indicating that plasticity may contribute to evolutionary change, ultimately resulting in genetic accommodation of the morphological traits.

Keywords Development time · Morphology · Life history · Genetic accommodation · Pelodytes · Temporary pools

Introduction

The role of phenotypic plasticity in affecting biological diversification has received much attention recently (West-Eberhard 2005; Pfennig et al. 2010). Phenotypic plasticity is defined as the ability of a given genotype to produce different phenotypes in response to different environmental conditions (Agrawal 2001). When new environments impose phenotypic plasticity on a genotype, it can result in adaptive divergence of lineages (West-Eberhard 2005). This may occur because plasticity allows a population to survive long enough in the new environment for existing genetic variation, in combination with mutation and/or recombination, to respond to local selection conditions. Fore example, phenotypic plasticity may allow tadpoles to track the water duration period in the ponds they breed in, which should result in an optimal size and time at metamorphoses. Once a population is established, selection could favour genetic changes in size and time at metamorphoses optimal for the new condition. These changes might ultimately become constitutive. The rate at which this occurs or whether it may occur at all depends on several factors: the costs of plasticity, the genetic correlation between a trait and its plasticity, the degree of plasticity, and gene flow. Hence under some circumstances plasticity might dampen rather than facilitate diversification (Price et al. 2003; Pfennig et al. 2010).

The process of divergence through phenotypic plasticity is referred to as genetic assimilation and/or genetic accommodation (West-Eberhard 2005). Genetic assimilation

Electronic supplementary material The online version of this article (doi:10.1007/s11692-013-9225-8) contains supplementary material, which is available to authorized users.

F. Johansson (✉) · A. Richter-Boix
Department of Ecology and Genetics, Uppsala University, Uppsala, Sweden
e-mail: frank.johansson@ebc.uu.se

occurs when traits that were originally induced by the environment become unaffected by the environment (constitutive) and genetic accommodation is the same process but in this case the plasticity is not necessarily lost. Both of these processes allow a population to move from one adaptive peak to another. The theory of how phenotypic plasticity may facilitate biological diversity has been in existence for many decades (West-Eberhard 2005), and there is evidence that phenotypic plasticity, in the form of alternative phenotypes, can lead to diversification (West-Eberhard 2005; Gomez-Mestre and Buchholz 2006; Pfennig et al. 2010; Moczek et al. 2011). But, there have been few studies that systematically investigate this possibility taken into account changes in ancestral development mechanisms (Pfennig et al. 2010). In addition, there is plenty of evidence showing that phenotypic plasticity facilitates adaptation to heterogeneous environment that are unpredictable over time and that less heterogeneous environment selects for a constitutive trait (e.g. Boersma et al. 1998; Lind and Johansson 2007). However few studies have asked if a common developmental mechanism causing a corresponding response in a morphological trait is mirrored between species, within species and within populations in a system with a known ancestral stage. If such a pattern is found this would suggest evidence in support of a genetic accommodation mechanism.

One way of testing whether phenotypic plasticity facilitates evolutionary divergence of lineages is by examining whether phenotypic plasticity within a lineage is mirrored in diversity between lineages (Gomez-Mestre and Buchholz 2006; Pfennig et al. 2010; Moczek et al. 2011). For example, consider two genetic lineages that differ in their life history and morphology and assume that the difference in traits is an adaptation to the environments they inhabit. In addition, both lineages show some degree of adaptive phenotypic plasticity in life history and morphology because each lineage occasionally encounters the environment of the other. If the morphological difference between lineages is reflected in a similar morphological difference within a lineage (caused by phenotypic plasticity), this suggests that diversity among species has evolved to some extent as a correlative response to the changes seen within a lineage. Evidence for this process has been found in laboratory studies (e.g. Waddington 1953; Queitsch et al. 2002; Suzuki and Nijhout 2006), however, we have limited evidence from natural populations, but see (Losos et al. 1999; Gomez-Mestre and Buchholz 2006; Badyaev 2009; Moczek et al. 2011). Since the role of phenotypic plasticity in biological diversification is controversial (West-Eberhard 2005; Pfennig et al. 2010), more research, especially studies on natural populations, is needed before a firm conclusion about the contribution of phenotypic plasticity can be drawn (Moczek et al. 2011). Determining whether phenotypic plasticity has resulted in diversification is challenging using natural populations because it is difficult to know whether a trait that has become constitutive due to genetic change under selection or to environmentally induced phenotypic plasticity has led to one among several alternative trait states. Also, it must be established that plasticity is the ancestral stage which can be difficult.

Gomez-Mestre and Buchholz (2006) provided some evidence about the way in which phenotypic plasticity has resulted in diversification in a spadefoot toad and parsley frog system. Using a phylogenetic framework they found that development time and body morphology within a species was correlated with environmentally induced differences in the larval period, and that this pattern was mirrored when a cross-species comparison was made. Long development times induced by temperature caused relatively longer hindlimbs and longer snouts within a species, and the same pattern was seen across species, since species with longer development times had relatively longer limbs and snouts (Gomez-Mestre and Buchholz 2006). The evidence found by Gomez-Mestre and Buchholz (2006) would be strengthened further if the same pattern they found within and between species could be observed by comparing within and between populations of a single species adapted to contrasted local conditions. Here we provide such evidence by presenting results of a study in which we examine development time and morphology of the parsley frog *Pelodytes punctatus*. We do this by determining whether the degree of phenotypic plasticity for development time and morphology associated with decreasing water levels parallels the variation of populations originating from populations adapted to local conditions across a hydroperiod gradient. Across the hydroperiod gradient we use the endpoint in water drying variation.

Development of larval amphibians is strongly influenced by environmental variables such as temperature and pool desiccation rates, and developmental differences have been found at the individual, population and species level (Newman 1992; Blouin and Brown 2000; Richter-Boix et al. 2011). It is well known that developmental plasticity shapes postmetamorphic morphology in frogs (Blouin and Loeb 1991; Gomez-Mestre et al. 2010; Tejedo et al. 2010). And this plasticity can be induced by temperature and water level manipulations (Tejedo et al. 2010). For example, individual tadpoles subjected to decreasing water levels in the laboratory typically speed up their development, resulting in a shorter time to, and different morphology at, metamorphosis compared to those of individuals subjected to constant water levels (e.g. Newman 1992; Richter-Boix et al. 2006a; Johansson et al. 2010). The environmental cue to the water level response seems to be the reduced water volume per se (Denver et al. 1998). Similarly, species, or populations within species, from temporary or vernal pools have a shorter development

time to, and a different morphology at, metamorphosis compared to those from permanent pools when they are raised in the laboratory under identical conditions (e.g. Morey and Reznick 2004; Gomez-Mestre and Buchholz 2006; Richter-Boix et al. 2006a; Lind and Johansson 2007).

These developmental patterns: faster development in response to natural or artificial pool drying and difference in development between pools that differ in water permanence are adaptive. For example not making it to metamorphosis before a pool dries up will inevitably result in death (Newman 1992). Similarly a longer development results in a larger size and therefore amphibians are assumed to optimize the trade-off between development and size (Newman 1992). A large size metamorphosis implies fitness benefits in amphibians, and is selected for under natural conditions (Altwegg and Reyer 2003).

In their comparative approach Gomez-Mestre and Buchholz (2006) found that morphometric differences between species were mirrored in within-species morphological variation caused by developmental plasticity. In this study we take the same approach as Gomez-Mestre and Buchholz (2006) but instead of comparing between species we compare between populations, using one of the species included in their study. We predict that: (1) individuals raised with constant water level should have a longer development time and longer hindlimbs compared to individuals raised with decreasing water level, (2) populations from permanent pools should have longer development times and longer hindlimbs than populations from temporary pools when raised under the same water level conditions, and (3) finally and most importantly, the difference in morphology seen among populations should be mirrored within populations. If this last prediction is supported it would provide evidence in the direction of genetic accommodation, i.e. phenotypic plasticity has facilitated diversity. If we find support for our predictions, such support in combination with the results from Gomez-Mestre and Buchholz (2006), would strengthen the hypothesis that plasticity facilitates morphological diversity in these frogs.

Materials and Methods

The Parsley Frog Study System

Pelodytes punctatus is widely distributed in Western Europe, inhabiting the eastern Iberian Peninsula and having a scattered distribution in France, Italy, Belgium and Luxemburg (Sánchez-Herráiz et al. 2000). The species inhabits forests, as well as semi-arid Mediterranean mountains and agricultural landscapes, and it breeds in freshwater habitats ranging from ephemeral pools to permanent pools (Richter-Boix et al. 2006b). Larval period is highly variable in this species, from around 40 days to 120 or almost 160 depending on ecological conditions (Richter-Boix et al. 2006a). For the present study we used eight populations (classifying each wetland area as a separate population) from a coastal Mediterranean region around Barcelona in the north-east of the Iberian Peninsula. See Richter-Boix et al. (2007) for more details about the study region and the amphibian habitat characteristics. These eight were chosen based on a prior survey including more than 100 populations in the area (Richter-Boix et al. 2007), and from that survey we chose the most extreme ones along a continuum of water permanence. Each wetland contained only one type of pool with regard to permanence. In 2001–2006, we monitored the duration of theses eight wetlands by visiting them twice a month to establish the hydroperiod of each, and counting the months during which they were filled with water. This allowed us to establish two different wetland categories: ephemeral-temporary pools (water bodies containing water for less than 8 months; hereafter referred to as temporary pools), and temporary-permanent pools (containing water for up to 10 months; referred to as permanent pools). During the 2001–2006 period the inter-annual variation observed in wetlands categorized as temporary pools was of 6.35 ± 1.13 SD months, whereas in permanent pools was of 11.12 ± 0.89 SD months (see Table S1 of Electronic Supplementary Material for detailed information per pool). Temporary pools flood after autumn storms (September), dry out from winter (December) onwards, and are flooded again from late February until June, with two dry seasons: winter and summer. Permanent pools flood at the end of summer and dry out in July–August; they become completely dry only during the warmest summer months in certain years. Hence, we refer to the pool categories as temporary and permanent. The populations we studied consisted of four permanent populations and four temporary populations. The mean distance between the populations was 15 km, with a minimum distance of 2.5 km and a maximum of 29.6 km. At this spatial scale *P. punctatus* population usually show some genetic differentiation at neutral genetic markers (Jourdan-Pileau et al. 2012) suggesting population differentiation.

Laboratory Experiment

We collected four clutches (hereafter described as families) of *P. punctatus* from different areas within each pool to avoid collection of clutches laid by the same female. We assume that each clutch was fertilized by one male and this is probably very likely since this species is a non-explosive breeder with only few males and females present at a mating rendezvous at each night (Nöllert and Nöllert 1992;

Guyétant et al. 1999). Clutches were collected during the first week of March 2006 the day after they were deposited in the field. The age of deposited eggs can easily be determined by the relative size of the jellylike covering of the embryo. There was no temporal difference in egg collecting day between temporary and permanent pool clutches. Thereafter eggs were transported to the laboratory in Barcelona University, where they were kept in 10 L plastic tanks until individuals reached Gosner stage 25 (Gosner 1960). The environmental conditions in the laboratory were 14L:10D photoperiod and a water temperature of 22 °C. At Gosner stage 25, 10 individuals from each family were randomly selected in order to compare life-history traits and metamorphic morphological traits between and within populations. Tadpoles were raised individually in 1 l containers of dechlorinated tap water and fed with a mixture (4:1) of rabbit chow and fish food ad libitum every 4 days. Plasticity response to pool drying was analyzed using two treatments: a constant water level treatment and a drying treatment. The former simulated a permanent pool with no changes in water level during tadpole development, whereas the latter simulated a temporary pool by reducing water volume during larval development, following the reduction curve defined by Wilbur (1987). In the drying treatment, water level was adjusted every 4 days following the published drying curves over a period of up to 110 days. This approach of reducing water volume has been used successfully in a previous study on the same species (see Richter-Boix et al. 2006a for more details). Five tadpoles from each family were allocated to the constant treatment (C) and five to the drying treatment (D), with a total of 320 experimental units positioned randomly within the room.

The experiment ended when individuals completed metamorphosis and reached Gosner stage 46, i.e. when the tail was fully resorbed. At this stage we recorded the number of days from the start of the experiment to metamorphosis (larval development time), and photographed each toadlet from the ventral side from a standardized distance. Metamorphic morphological traits were determined from the images using the image processing software ImageJ (Abramoff et al. 2004). The morphological traits measured were: snout-vent length (SVL), head width (HW), femur length (FL), tibiofibula length (TL) and foot length (FL) (see Richter-Boix et al. 2006a for details). SVL was used as the metamorphic body size; other metamorphic traits were size-corrected for subsequent analyses by dividing each log-transformed morphological trait by SVL. As an alternative analysis we used SVL as a covariant, but since results were very similar to the ones obtained with the adjusted morphological traits (Table S2 of Electronic Supplementary Material), we chose to present the results from the adjusted morphological traits because they are easier to visualise and interpret. For logistical and ethical reasons our experimental design does not allow us to control for maternal effects on development time and morphology. Maternal effects are quite variable life history traits in amphibians. For example, Laugen et al. (2005) found that maternal genetic variance explained an average of 3.9 % of the total genetic variance over several population and traits in *Rana temporaria,* Lind and Johansson (2007) found that it accounted for 5 % in *Rana temporaria.*

Statistical Analyses

To determine whether wetland permanency (temporary or permanent) or drying treatment (constant or drying) predicted larval development time, metamorphic morphology and phenotypic plasticity we fitted a General Linear Mixed Model using a procedure in the "nlme" library (Pinheiro et al. 2008) in R version 2.10.1. We applied a top-down strategy to choose the fixed effects and the random effects (West et al. 2006; Zuur et al. 2009). We started with a "beyond optimal" model with the large number of explanatory variables that could contribute to the optimal model. This model included "treatment", "wetland permanency" and their interaction as fixed effects. In addition, we tested models with different structures of the random components, and nested random effect structures were compared using the AIC and REML likelihood test. We develop three random effect models: (1) a model without random structure, (2) a marginal model where we defined a general correlation matrix assuming that the residuals of the same family and population are not independent of each other, assuming that all observations from the same family and population are correlated (using the argument corCompSymm for the correlation option in the gls function in R); and (3) a hierarchical model with random effects where the induced correlation includes family nested inside population. The random effect structure best supported was the hierarchical which account for non-independence among individuals from the same family nested within the random effect of population. Once the random effect structure was determined, we compared models differing in their fixed effect structure with the likelihood ratio test using ML estimation. When comparison between models demonstrated that the model including the interaction between "treatment" and "wetland permanency" was not significantly better that the one without the interaction term, the interaction was removed (Crawley 2002). The final model for each trait was reanalyzed using REML. Since we were not interested in family effects and differences between pools within each pool category, the statistical results for these two effects are not presented. We did one analysis for each variable separately rather than using a PCA, because

we were interested in the morphology of each trait per see. This also allowed us to compare the result with those obtained by Gomez-Mestre and Buchholz (2006).

We examined the relationship between larval development time and hydroperiod by means of linear regression, using mean months with water per year (over the period 2001 to 2006) as a measure of hydroperiod. Separate regressions were calculated for larval development time under constant and drying treatments. In addition, we tested whether larval development time differences among populations could originate from genetic drift using a Mantel test procedure: correlation between two matrixes (Mantel 1967). First, we examined the relationship between hydroperiod differences between localities (measured as the Euclidean distances for months with water between different wetlands) and the logarithm of Euclidean geographic distance between localities, looking for a spatial distribution related to environment. Euclidean distance is defined as the ordinary distance between two points. Second, we tested for correlation between the geographic distances and the pairwise larval development time differences (Euclidean distances of larval development time between populations) for both treatments. Finally, we studied the relationship between hydroperiod differences and larval development time differences between localities. As spatial separation may be partly confounded by environmental factors, partial Mantel tests, were used to estimate the effects of geographic distances while incorporating a matrix describing phenotypic variation and hydroperiod differences. The Mantel test generated 100,000 randomizations and was conducted using the package "vegan" in R (Oksanen et al. 2009). We performed this test because in situations where the selection gradient can be viewed as one-dimensional (such as altitudinal/latitudinal clines), the balance between genetic drift and migration can generate patterns across environmental gradients (such as permanent and temporary pools) that closely mimic adaptive clines (Vasemägi 2006). This makes it difficult to distinguish the influence of drift and geographic isolation (Isolation By Distance [IBD]) from that of selection (Isolation By Adaptation [IBA]), because both are acting in the same direction (Nosil et al. 2007).

Results

Our simulated pool drying treatment resulted in significantly shorter larval development time, and this was evident for individuals from permanent as well as temporary pool populations since both wetland categories showed the same degree of plasticity in development time (Fig. 1; Table 1). Constant water conditions resulted in significantly longer bodies, relatively longer femurs, tibias, and feet, and relatively narrower heads (Fig. 1; Table 1). The morphological pattern observed in the simulated drying conditions was mirrored when individuals from permanent and temporary pool populations were compared. Individuals from permanent populations had significantly longer development times and longer bodies (Fig. 1; Table 1). They also had significantly greater relative femur, tibia, and foot lengths, and narrower heads (Fig. 1; Table 1). Two traits showed significant interaction: relative femur and tibia length (Table 1). These traits did not differ between individuals from temporary and permanent populations under simulated pool drying conditions, but both traits showed significantly higher values in individuals from temporary and permanent populations under constant water conditions (Fig. 1). These relative differences in body morphology are not due simply to a greater body size under constant treatment or in individuals from populations from permanent waters, since there is considerable overlap in body size between individuals from constant and decreasing water treatment, and similarly between individuals from permanent and from temporary pools. We provide examples of plots showing the relationships between body length and two of our morphological variables in Figure S1 of Electronic Supplementary Material.

The regression of larval development time on hydroperiod was significant for both treatments: constant ($r^2 = 0.652$; $P = 0.0152$) and drying ($r^2 = 0.564$; $P = 0.031$) (Fig. 2a). A Mantel test revealed a significant negative relationship between geographic distance and phenotypic larval development time under the constant treatment ($r = -0.221$; $P = 0.035$), but not under the drying treatment ($r = -0.091$; $P = 0.313$; Fig. 2c). These negative correlations are consistent with the negative but not significant correlation between hydroperiod and geographic distance ($r = -0.182$; $P = 0.095$; Fig. 2b). In all cases the negative value of correlations indicates that differences in non-environmental phenotypic traits increase with geographic distance; this, together with the relationship between larval development time and wetland hydroperiod ($r = 0.467$; $P = 0.017$ and $r = 0.460$; $P = 0.037$ for constant and drying treatments, respectively; Fig. 2d) suggests that a major part of the phenotypic variation between localities has evolved in response to divergent selection across habitats. Because of the mosaic structure of the wetland network, the correlations that we observed between larval development time and water permanency cannot be explained by genetic drift alone. These relationships between development time and wetland hydroperiod differentiation remained even after controlling for geographic distance ($r = 0.445$; $P = 0.026$ and $r = 0.453$; $P = 0.033$ for constant and drying treatments, respectively). The distribution of the wetland hydroperiods exhibited no spatial autocorrelation, suggesting a lack of association between the environmental-selection gradient

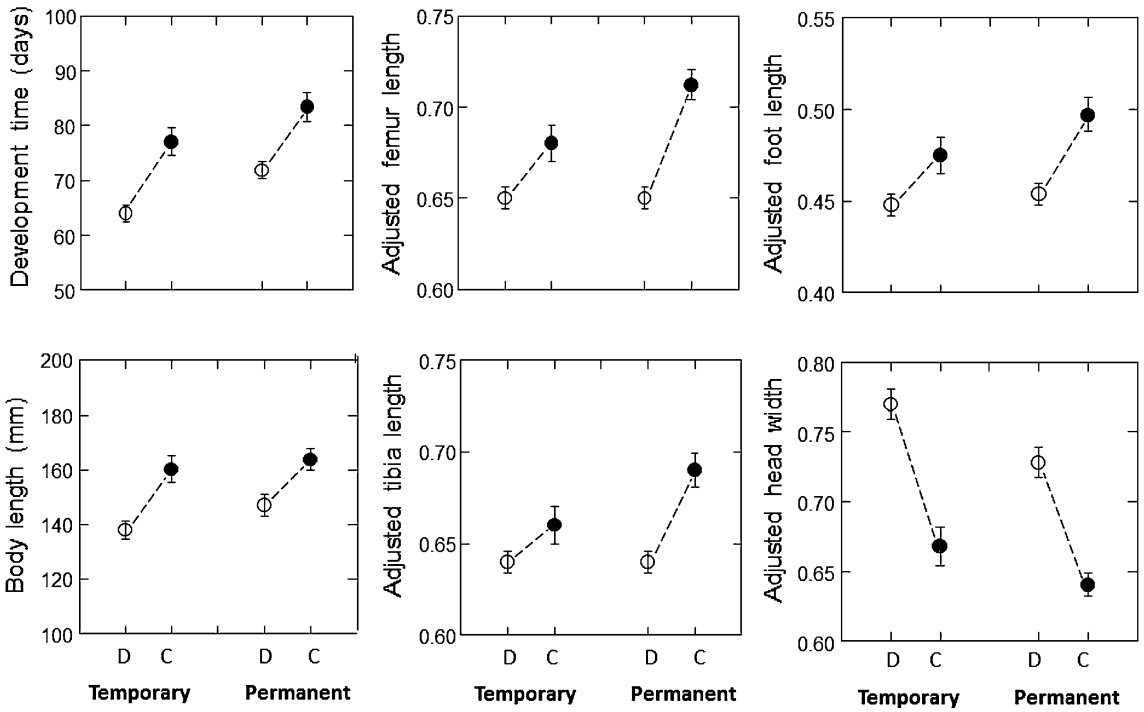

Fig. 1 Larval development time, and body length, relative femur, tibia, and toe length, and relative head width at metamorphosis for individuals from permanent and temporary *P. punctatus* populations raised under constant (*C*) water level and simulated pool drying (decreasing water) conditions (*D*) in the laboratory. *Error bars* are 95 % confidence intervals. *Hatched lines* between *dots* are drawn to facilitate visualization of phenotypic plasticity

(temporary and permanent pools) and any possible genetic drift resulting from geographic isolation (Fig. 2b; Table S3 of Electronic Supplementary Material).

Discussion

As predicted, the morphological diversity caused by the plasticity response to pool drying within populations was mirrored between populations exposed to different hydroperiod environments, and this pattern was driven by development time. In the laboratory experiment we found that decreasing water conditions resulted in shorter development times compared to constant water levels. Such plasticity in responses in development time is common in frogs, reviewed in Richter-Boix et al. (2011). The shorter development affected the morphology of metamorphosed froglets, which had smaller size (shorter bodies), relatively shorter legs and relatively wider heads: a common pattern described in many frog species (Tejedo et al. 2010). This plasticity was mirrored across populations, in that frogs from populations with temporary water conditions had shorter development times and relatively shorter legs and wider heads than did individuals from habitats with permanent pools reared in the same conditions in the laboratory. Hence, the plastic response is mirrored in a constitutive response across populations, suggesting that diversity among populations has evolved to some extent as a correlated response in larval development rate. Several studies have shown that populations experiencing variable environments are more plastic in their trait expression compared to populations with low environmental variation (e.g. Conover and Heins 1987; Boersma et al. 1998; Ghalambor et al. 2007; Lind and Johansson 2007). Our result takes this plasticity effect one step further because we suggest that the induced plasticity in development time promoted the evolution of morphology divergence. That is, the morphology pattern observed was driven by a common developmental process: time to metamorphosis. However, mechanistic studies at the cellular and molecular level are needed to verify this but we note that Gomez-Mestre and Buchholz (2006) provide such evidence in their study.

Although our result does not prove that phenotypic plasticity facilitates evolutionary divergence of lineages, the trend is in the direction predicted by theory (West-Eberhard 2005; Pfennig et al. 2010). First, our result suggest that genetic accommodation has occurred, because it suggests that phenotypic plasticity allows the frogs to persist long enough in a new habitat for selection to favour genetic modification of new traits. Second, the morphological differences between populations that was mirrored within species in our study was also found at a higher

Table 1 Mixed model ANOVA for development time, body length, relative femur length, relative tibia length, relative foot length and relative head width

	df	F value	*P* value
Development time			
Pool	1, 6	37.6	<0.0001
Treatment	1, 264	133.6	<0.0001
Body length			
Pool	1, 6	6.8	0.04
Treatment	1, 264	89.3	<0.0001
Femur length			
Pool	1, 6	9.1	0.02
Treatment	1, 264	141.4	<0.0001
Pool × Treat.	1, 264	11.85	0.0007
Tibia length			
Pool	1, 6	8.3	0.03
Treatment	1, 264	98.0	<0.0001
Pool × Treat.	1, 264	12.49	0.0005
Foot length			
Pool	1, 6	4.5	0.07
Treatment	1, 264	37.2	<0.0001
Head width			
Pool	1, 6	8.5	0.03
Treatment	1, 264	354.8	<0.0001

Factors were pool wetland permanency: permanent and temporary populations and treatments were constant or decreasing water level in the laboratory

taxonomic level by Gomez-Mestre and Buchholz (2006), since they found that morphometric differences between species were mirrored in within-species morphological variation caused by developmental plasticity. We note that our study species is within the same clade (Pelobatoidea) as the species in that study and therefore the results seem to be general at several hierarchal levels (between species, within species and between populations. Phylogenetic studies suggest that *Pelodytes* represent the ancestral stage of developmental plasticity in spadefoot toads, and Gomez-Mestre and Buschholz (2006) suggested that the developmental acceleration has become genetically accommodated in more recent taxa. Hence plasticity seems to represent the ancestral stage in spadefoot toads. We do not know the ancestral population in our study area but we suggest the same mechanism could have worked at this level. That is, the developmental plasticity observed in some population may have been modified due to genetic accommodation. Since the pattern in development and morphology was similar at all these three levels it does not speak against a genetic accommodation mechanism but more research in certainly needed before firm conclusions can be drawn. Further support for the hypothesis that plasticity facilitates evolutionary divergence of lineages would have been found if our temporary pool population were more plasticity in development time since they probably experience more variation in water permanence in time. We did not find this, since there was no interaction term between pool category and treatment and we discuss this result below.

We hypothesize that the phenotypic plasticity observed within our populations allows individuals to survive in new environments that differ in water permanency and undergo variation in water level never previously experienced by the individuals. The phenotypic plasticity allows tadpoles to track the water duration period which should result in an optimal size and time at metamorphoses. Once a population is established, selection could favour genetic changes optimal for the new conditions and the changes might ultimately become constitutive (Price et al. 2003; West-Eberhard 2005). These constitutive changes might occur in in both directions: faster development in temporary and slower in permanent populations. Such genetic changes were reflected in our study since population categories differed in development time and morphology. Our evidence that phenotypic plasticity has facilitated adaptation to pool drying in our populations would be strengthened if we knew the phylogeny of our populations (Ledon-Retting et al. 2008). Unfortunately we do not have such information. However, we consider it unlikely that all four temporary populations are older lineages than the four permanent populations for statistical probability reasons and because they did differ in plasticity (and interaction between treatment and population for some traits). Instead, we suggest that in a population structure like ours, where there is large heterogeneity with respect to pool drying, does not cause unidirectional selection for adaptations to permanent or temporary pools. Instead, we suggest that phenotypic plasticity allows reciprocal adaptation from temporary pools to permanent pools and vice versa. Note that both population categories (temporary and permanent) show life-history phenotypic plasticity and that although there are no differences at the plasticity level there are differences in the mean value of the adaptive trait larval development time.

The strong association between larval development time and the geographical patterns of variation and pool habitat features suggests that larval development time differences between environments are of adaptive significance. It seems unlikely that a correlation between phenotype and environment would occur coincidentally when the environments are not spatially correlated (Nosil et al. 2005). Moreover, an association between larval development time and pool characteristics (e.g. hydroperiod, water temperature, forest canopy, predation) has been observed in other amphibian species as well (e.g. Relyea 2002; Orizaola and Laurila 2009; Richter-Boix et al. 2010; Lind et al. 2011), adding support to an interpretation

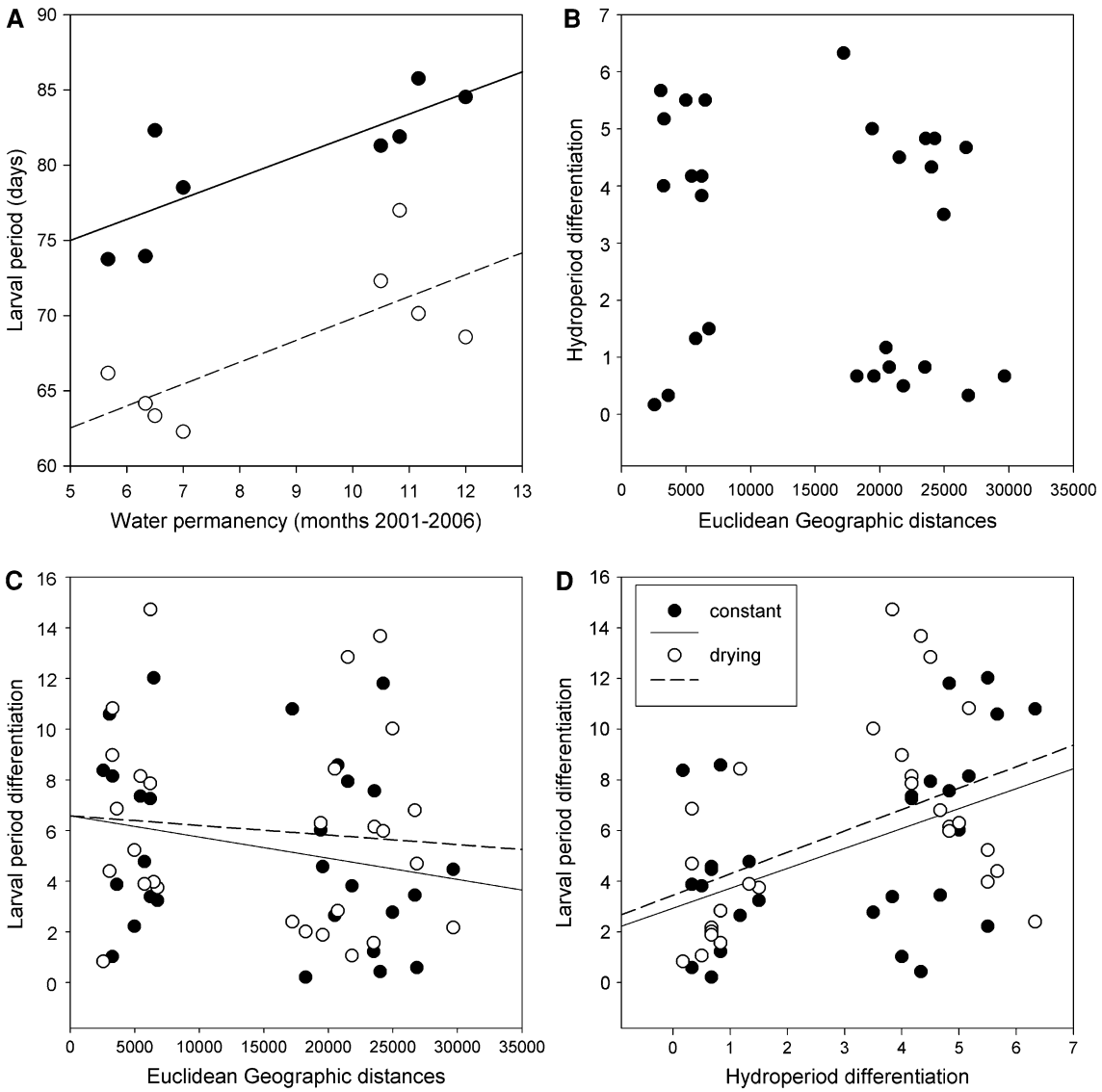

Fig. 2 **a** Relationship between larval period time (days) and water permanency of pools estimated as the mean value of months with water during the period 2001–2006. *Black circles* correspond to constant water level, and *white circles* to the drying treatment. **b** Non-significant relationship between the Euclidean geographic distances between pools and the hydroperiod differentiation between pools. **c** Relationship between larval development time differentiation (Euclidean distances between larval period between pairwise populations) and geographic distances. Note that correlation under drying treatment is not significant. **d** Relationship between larval development time differentiation and hydroperiod differentiation

invoking a natural selective effect. We do not have data on neutral genetic differentiation, but in another study on *P. punctatus* in a Mediterranean area with a similar pool structure and distance as in our study, the mean F_{st} between pools was 0.091 (± 0.044 S.E.), suggesting some degree of population differentiation at this geographical scale (Jourdan-Pileau et al. 2012). However, we cannot exclude that some of these other pool characteristics could affect the pattern we have observed and further work is needed to exclude the influence of alternative environmental factors. Stochastic events or historical processes such as genetic drift and colonization events, acting on gene frequencies within individual populations, can also generate and maintain complex patterns of geographic variation (Kondrashov 2003; Hallatschek and Nelson 2008). Drift alone, however, is unlikely to have produced these patterns, because we found the same pattern within the two pool drying categories in all four replicated pools. Instead, given the correlation between larval development time and the geographical pattern of hydroperiod distribution, adaptive evolution appears a more likely explanation.

Development time plasticity is a well-known adaptive phenomenon and we argue that the traits we have studied here are adaptive. It has been found that fast growth and short development time correlate with higher fitness when pools do not dry out (Altwegg and Reyer 2003), and shorter development time should be especially beneficial in temporary environments because it allows metamorphosis before the pool dries out (Newman 1992). Development time is probably a much more important fitness trait than the morphological traits because it affects survival directly (failing to metamorphose before a pool dries up is fatal), and therefore the variation observed in morphology might be to some extent a correlative response to development time. The adaptive value of the morphological patterns we found can be interpreted in the light of previous studies, which suggested that leg length affects jumping performance and hence ability to escape from predators (Blem et al. 1978; Nauwelaerts et al. 2007). Similarly, head shape has been shown to influence feeding capacity and the diet of froglets and toadlets (Emerson 1985). The allometric relationship between shape and size is not constant between populations, but rather is a function of development rate (Blouin and Loeb 1991). Thus, environment might induce morphometric variation by controlling the overall rates of growth and differentiation (Blouin and Brown 2000; Tejedo et al. 2010). Our data show that allometry could exist as a function of desiccation affecting hind limb length and head characters as described in other species (Blouin and Brown 2000; Tejedo et al. 2010). However, between-population differences in the interaction term for the size adjusted traits (treatment × population) suggest that populations differ in their degree of plasticity. One explanation for such a difference could be that populations differ in age, in which case genetic accommodation in morphological trait has not advanced to a similar degree. An alternative but not mutually exclusive explanation could be that pool drying variation differs among years between pools. That is, some pools are more and others are less variable which should result in more or less phenotypic plasticity.

In summary, our data suggest that plasticity promotes new phenotypes, since the phenotypic plasticity observed within populations in response to water permanence was mirrored in between-population differences with respect to pool drying. Together with the results of Gomez-Mestre and Buchholtz (2006), our study adds to the growing evidence that plasticity has the potential to promote divergence between populations, ultimately resulting in the formation of new species.

Acknowledgments We thank Anssi Laurila and German Orizaola for helpful comments on earlier drafts of the article. The research was funded by the Swedish Research Council to FJ. ARB was supported by a Spanish Ministry of Education and Culture postdoctoral grant (MEC2007-0944) and by a Beatriu de Pinós postdoctoral fellowship (2008 BP A 00032).

References

Abramoff, M. D., Magelhaes, P. J., & Ram, S. J. (2004). Image processing with ImageJ. *Biophotonics International, 11*, 36–42.

Agrawal, A. A. (2001). Phenotypic plasticity in the interactions and evolution of species. *Science, 294*, 321–326.

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

Badyaev, A. V. (2009). Evolutionary significance of phenotypic accommodation in novel environments: an empirical test of the Baldwin effect. *Philosophical Transactions of the Royal Society B: Biological Sciences, 364*, 1124–1141.

Blem, C. R., Steiner, J. W., & Miller, M. A. (1978). Comparison of jumping abilities of the cricket frogs Acris gryllus and Acris crepitans. *Herpetologica, 34*, 288–291.

Blouin, M. S., & Brown, T. S. (2000). Effects of temperature-induced variation in anuran larval growth rate on head width and leg length at metamorphosis. *Oecologia, 125*, 358–361.

Blouin, M. S., & Loeb, L. G. (1991). Effects of environmentally induced development-rate variation on head and limb morphology in the green tree frog, *Hyla cinerea*. *American Naturalist, 138*, 717–728.

Boersma, M., Spaak, P., & De Meester, L. (1998). Predator-mediated plasticity in morphology, life history, and behavior of Daphnia: the uncoupling of responses. *American Naturalist, 152*, 237–248.

Conover, D. O., & Heins, S. W. (1987). Adaptive variation in environmental and genetic sex determination in a fish. *Nature, 326*, 496–498.

Crawley, M. J. (2002). *Statistical computing: An introduction to data analysis using S-plus*. Chichester, UK: Wiley.

Denver, R. J., Mirhadi, N., & Phillips, M. (1998). Adaptive plasticity in amphibian metamorphosis: response of *Scaphiopus hammondii* tadpoles to habitat desiccation. *Ecology, 79*, 1859–1872.

Emerson, S. B. (1985). Skull shape in frogs – correlations with diet. *Herpetologia, 41*, 177–188.

Ghalambor, C. K., Mckay, J. K., Carroll, S. P., & Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology, 21*, 394–407.

Gomez-Mestre, I., & Buchholz, D. R. (2006). Developmetal plasticity mirrors differences among taxa in spadefoot toads linking plasticity and diversity. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 19021–19026.

Gomez-Mestre, I., Saccoccio, V. L., Iijima, T., Collins, E. M., Rosenthal, G. G., & Warkentin, K. M. (2010). The shape of things to come: linking developmental plasticity to postmetamorphic morphology in anurans. *Journal of Evolutionary Biology, 23*, 1364–1373.

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

Guyétant, R., Temmermans, W., & Avrillier, J. N. (1999). Phénologie de la reproduction chez Pelodytes punctatus Daudin, 1802 (Amphibia, Anura). *Amphibia-Reptilia, 20*, 149–160.

Hallatschek, O., & Nelson, D. R. (2008). Gene surfing in expanding populations. *Theoretical Population Biology, 73*, 158–170.

Johansson, F., Lind, M. I., & Lederer, B. (2010). Trait performance correlations across life stages under environmental stress conditions in the common frog, *Rana temporaria*. *PLoS One, 5*(7), e11680.

Jourdan-Pileau, H., David, P., & Crochet, P.-A. (2012). Phenotypic plasticity allows the Mediterranean parsley frog *Pelodytes punctatus* to exploit two temporal niches under continuous gene flow. *Molecular Ecology, 21*, 876–886.

Kondrashov, A. S. (2003). Accumulation of Dobzhansky-Muller incompatibilities within a spatially structured population. *Evolution, 52*, 151–153.

Laugen, A. T., Kruuk, L. E. B., Laurila, A., Räsänen, K., Stone, J., & Merilä, J. (2005). Quantitative genetics of larval life-history traits in *Rana temporaria* in different environmental conditions. *Genetical Research, 86*, 161–170.

Ledon-Retting, C. C., Pfennig, D. W., & Nascone-Yoder, N. (2008). Ancestral variation and the potential for genetic accommodation in larval amphibians: implications for the evolution of novel feeding strategies. *Evolution & Development, 10*, 316–325.

Lind, M. I., Ingvarsson, P. K., Johansson, H., Hall, D., & Johansson, F. (2011). Gene flow and selection on phenotypic plasticity in an island system of *Rana temporaria*. *Evolution, 65*, 684–697.

Lind, M., & Johansson, F. (2007). The degree of adaptive phenotypic plasticity is correlated with spatial environmental heterogeneity experienced by island populations of *Rana temporaria*. *Journal of Evolutionary Biology, 20*, 1288–1297.

Losos, J., et al. (1999). Evolutionary implications of phenotypic plasticity in the hindlimb of the lizard Anolis sagrei. *Evolution, 54*, 301–305.

Mantel, N. (1967). The detection of disease of clustering and a generalized regression approach. *Cancer Research, 27*, 209–220.

Moczek, A. P., Sultan, S., Foster, S., Ledon-Rettig, C., Dworkin, I., Nijhout, H. F., et al. (2011). The role of developmental plasticity in evolutionary innovation. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 278*, 2705–2713.

Morey, S. R., & Reznick, D. N. (2004). The relationship between habitat permanence and larval development in California spadefoot toads: field and laboratory comparisons of developmental plasticity. *Oikos, 104*, 172–190.

Nauwelaerts, S., Ramsay, J., & Aerts, P. (2007). Morphological correlates of aquatic and terrestrial locomotion in a semi-aquatic frog, *Rana esculenta*: no evidence for a design conflict. *Journal of Anatomy, 210*, 304–317.

Newman, R. A. (1992). Adaptive plasticity in amphibian metamorphosis. *BioScience, 42*, 671–678.

Nöllert, A., & Nöllert, C. (1992). *Die Amphibien Europas*. Stuttgart: Franckh-Kosmos.

Nosil, P., Egan, S. P., Funk, D. J., & Hoekstra, H. (2007). Heterogeneous genomic differentiation between walking-stick ecotypes: "Isolation by Adaptation" and multiple roles for divergent selection. *Evolution, 62*, 316–336.

Nosil, P., Vines, T., & Funk, D. J. (2005). Perspective: Reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution, 59*, 705–719.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G. L., Solymos, P., et al. (2009). *Community Ecology Package*, version 1.15-3. http://vegan.r-forge.r-project.org/.

Orizaola, G., & Laurila, A. (2009). Microgeographic variation in temperature-induced plasticity in an isolated amphibian metapopulation. *Evolutionary Ecology, 26*, 979–991.

Pfennig, D., Wund, M. A., Snell-Rood, E. C., Cruickshank, T., Schlichting, C. D., & Moczek, A. P. (2010). Phenotypic plasticity's impacts on diversification and speciation. *Trends in Ecology & Evolution, 25*, 459–467.

Pinheiro, J., Bates, D.,DebRoy, S., Sarkar, D. & R. C. team. (2008). *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-90.

Price, T. D., Qvarnström, A., & Erwin, D. E. (2003). The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 270*, 1433–1440.

Queitsch, C., Sangster, T. A., & Lindquist, S. (2002). Hsp90 as a capacitor of phenotypic variation. *Nature, 417*, 618–624.

Relyea, R. A. (2002). Local population differences in phenotypic plasticity: Predator-induced changes in wood frog tadpoles. *Ecological Monographs, 72*, 77–93.

Richter-Boix, A., Llorente, G. A., & Montori, A. (2006a). Effects of phenotypic plasticity on post-metamorphic traits during pre-metamorphic stages in the anuran *Pelodytes punctatus*. *Evolutionary Ecology Research, 8*, 309–320.

Richter-Boix, A., Llorente, G. A., & Montori, A. (2006b). A comparative analysis of the adaptive developmental plasticity hypothesis in six Mediterranean anuran species along a pond permanency gradient. *Evolutionary Ecology Research, 8*, 1139–1154.

Richter-Boix, A., Llorente, G. A., & Montori, A. (2007). Structure and dynamics of an amphibian metacommunity in two regions. *Journal of Animal Ecology, 76*, 607–618.

Richter-Boix, A., Tejedo, M., & Rezende, E. (2011). Evolution and plasticity of anuran larval development in response to desiccation: a comparative analysis. *Ecology and Evolution, 1*, 15–25.

Richter-Boix, A., Teplitsky, C., Rogell, B., & Laurila, A. (2010). Local selection modifies phenotypic divergence among *Rana temporaria* populations in the presence of gene flow. *Molecular Ecology, 19*, 716–731.

Sánchez-Herráiz, M. J., Barbadillo, L. J., Machordom, A., & Sanchiz, B. (2000). A new species of Pelodytid frog from the Iberian Peninsula. *Herpetologica, 56*, 105–118.

Suzuki, Y., & Nijhout, H. F. (2006). Evolution of polyphenism by genetic accommodation. *Science, 311*, 650–652.

Tejedo, M., Marangoni, F., Pertoldi, C., Richter-Boix, A., Laurila, A., Orizaola, G., et al. (2010). Contrasting effects of environmental factors during larval stage on morphological plasticity in post-metamorphic frogs. *Climate Research, 43*, 31–39.

Vasemägi, A. (2006). The adaptive hypothesis of clinal variation revisited: Single-locus clines as a result of spatially restricted gene flow. *Genetics, 173*, 2411–2414.

Waddington, C. H. (1953). Genetic assimilation of an acquired character. *Evolution, 7*, 118–126.

West, B., Welch, K. B., & Galecki, A. T. (2006). *Linear mixed models: A practical guide using statistical software*. Boca Raton: Chapman & Hall/CRC.

West-Eberhard, M. J. (2005). Developmental plasticity and the origin of species differences. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 6543–6549.

Wilbur, H. M. (1987). Regulation of structure in complex systems: Experimental temporal pond communities. *Ecology, 60*, 1432–1437.

Zuur, A. F., Leno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R*. New York, USA: Springer.