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A comparative analysis of the adaptive developmental plasticity hypothesis in six Mediterranean anuran species along a pond permanency gradient

Alex Richter-Boix,* Gustavo A. Llorente and Albert Montori

Department of Animal Biology, Faculty of Biology, University of Barcelona, Av. Diagonal 645, E-08028 Barcelona, Spain

ABSTRACT

Question: Is developmental phenotypic plasticity an adaptive trait and therefore more flexible in variable and unpredictable environments?

Organism: The anuran larvae community encompassing *Alytes obstetricans*, *Pelodytes punctatus*, *Bufo bufo*, *B. calamita*, *Hyla meridionalis*, and *Rana perezi*.

Methods: In the field, we examined the ecological breadth (spatial and temporal variability) of the six species along a pond permanency gradient in 240 ponds. In the laboratory, we measured developmental plasticity (time to and size at metamorphosis) of each species using two treatments: (1) constant water level and (2) drying treatment. A comparative analysis was undertaken of developmental plasticity and the function of species ecological breadth and their phylogenetic relationship.

Results: Species that use a wide variety of habitats or unpredictable environments showed a greater plasticity of responses than those occurring in predictable habitats. At the two extremes of the hydroperiod (ephemeral and permanent ponds), specialist developmental phenotypes with limited plasticity occur, whereas species from variable habitats (temporary ponds) can be considered plastic strategists with asymmetric bet-hedging. Our results support the hypothesis that interspecific differences in developmental phenotypic plasticity are adaptive and are related to ecological breadth and unpredictability.

Keywords: habitat desiccation, metamorphosis, phenotypic plasticity, tadpoles.

INTRODUCTION

The role of phenotypic plasticity in adapting to natural variable environments has been the focus of much research (Schlichting and Pigliucci, 1998; DeWitt and Scheiner, 2004). To understand the evolution and adaptive nature of plasticity, it is necessary to study how plasticity is optimized and integrated with other strategies developed for dealing with variable and unpredictable environments. A comparative phylogenetic study among related or distant taxa can provide evidence of whether plasticity is correlated with differences in the

* Author to whom all correspondence should be addressed. e-mail: arichterboix@ub.edu

environment in which species occur (Doughty, 1995). Strong evidence of the adaptive significance of a trait is obtained from comparisons among populations and species (Endler, 1986). Although several studies have compared the plasticity of species (e.g. Schlichting and Levin, 1986; Bell and Sultan, 1999; Leips *et al.*, 2000), most have limited their focus to two closely related taxa, but with some exceptions (e.g. Pigliucci *et al.*, 1999; Richardson, 2002; Van Buskirk, 2002). Furthermore, few studies have contrasted the plasticity of species included in a guild or community in the same region (e.g. Lardner, 2000), and the contribution of plasticity and other strategies to community structure. To interpret phenotypic plasticity as an adaptive trait and to establish its contribution to species distribution, the environmental heterogeneity of species must be examined (Doughty and Reznick, 2004). In the present study, we examined the life-history response of tadpoles to desiccation in an anuran larvae community in the Mediterranean region as a function of habitat breadth and temporal variability.

Plasticity is often thought to be adaptive, enabling tadpoles to develop a suitable life-history phenotype to respond to habitat desiccation (Newman, 1992). Amphibian larvae exhibit plasticity in the timing of metamorphosis and capitalize on favourable conditions for growth while these conditions last. This plasticity may allow these larvae to match their phenotype to prevailing environmental conditions (Wilbur and Collins, 1973). Species that show phenotypic plasticity may have a higher probability of survival in unpredictable habitats than those with canalized development (Newman, 1992), and may occur in a wide range of habitats along the pond permanency gradient. Species do not show a random distribution and predictable assemblages are usually found along this gradient (Jeffries, 1994; Skelly, 1996; Babbitt *et al.*, 2003). Ponds with different permanency periods exert selective pressures on organisms, which, in response, develop a range of adaptive strategies (Brock *et al.*, 2003; Lake, 2003; Johansson and Suhling, 2004).

Here we addressed the following questions: (1) Does the magnitude of response of development time to pond drying differ among species in relation to habitat variability? (2) Is the evolution of phenotypic plasticity in response to habitat desiccation constrained by historical events (phylogenetic perspective)?

MATERIALS AND METHODS

Study area and habitat characteristics

To characterize the ecological breadth of the species, we surveyed a range of conditions and their frequency distribution of environmental states in nature. The field study was confined to a littoral Mediterranean region covering 22,645 ha around Barcelona in the north-east of the Iberian Peninsula that contains isolated ponds that vary in hydroperiod. The annual average rainfall there is around 600 mm and the annual average temperature is above 18°C. During the spring and summer of 2003, we surveyed a total of 246 isolated ponds as potential larval habitats of anurans. The localities surveyed spanned the range of aquatic breeding habitats of the species studied, including ephemeral pools and temporary and permanent ponds. The temporary ponds flood after strong autumn storms (September). The shallowest temporary ponds often dry out from winter onwards (December), whereas the deepest temporary ponds remain flooded until summer when they start to dry out. Many ephemeral and temporary ponds were flooded by rainfall in late February or early March and then dried up from mid-May to mid-July. The amphibian community of the area consists of six anuran species and one native urodela (*Salamandra salamandra*). The

anuran species are: *Alytes obstetricans* (Discoglossidae), *Pelodytes punctatus* (Pelodytidae), *Bufo bufo* (Bufonidae), *Bufo calamita* (Bufonidae), *Hyla meridionalis* (Hylidae), and *Rana perezi* (Ranidae).

We assessed the presence and successful reproduction of amphibians in the ponds by dip-netting and egg searches. For all ponds, sampling periods for amphibians were determined by preliminary surveys and accounted for differences in breeding activity between species and ensured that all species were detected. In the spring and early summer (a minimum of four visits, covering the breeding period of all species), we used a dip-net to sample tadpoles and predacious invertebrates. A minimum of 5–10 dip-net sweeps were made in potential tadpole microhabitats following standard techniques (Heyer *et al.*, 1994). All amphibian specimens were identified in the field and returned to water. Predacious invertebrates were identified to order only (except Odonate larvae, which were identified to family level). Because the water at the study site was generally clear, we determined fish presence through visual surveys in addition to dip-net captures. Egg searches were conducted throughout the same period as dip-netting and consisted of searching water and submerged vegetation within 3 m of the pond shore. We considered ponds successful breeding sites only when eggs and larvae were found. Eggs and larvae rather than adults were used to judge presence, so the data included actual breeding attempts.

Tadpoles inhabit ponds that vary along a gradient of permanency. This gradient has been studied extensively, and although it is continuous, two transitions have been identified (Wellborn *et al.*, 1996): (1) the 'permanence transition' between temporary and permanent ponds, and (2) the 'predator transition' between permanent ponds without fish and permanent ponds with fish. We did not make the latter distinction because all the ponds studied were isolated and did not hold native fish populations. We found only six ponds with fish, and these were excluded from our analyses. We limited our study to the 'permanence transition', and we adjusted the freshwater gradient to reflect three categories: (1) ephemeral ponds that dry within weeks (containing water for less than 60 days); (2) temporary ponds that dry every year during spring or summer (containing water for up to 180 days); and (3) permanent ponds, defined as containing water all year round (360 days). The ponds excluding fish ($n = 260$) were placed in one of these categories. We visited ponds approximately every 2 weeks throughout the year to establish the date of drying.

From 2001 to 2003, we periodically monitored 73 of these ponds, which represented all three categories. To establish the initiation of the hydroperiod, ponds were visited before heavy rains and were subsequently visited every week to establish the duration for each year. Thus, we established the variation of pond duration between years.

Laboratory experiments

Developmental phenotypic plasticity was measured in laboratory experiments during spring 2001 and spring 2002. In 2001, we conducted experiments with *Alytes obstetricans* and *Bufo bufo*. In 2002, we repeated the same experiments with *Pelodytes punctatus*, *Hyla meridionalis*, *Bufo calamita*, and *Rana perezi*. All experiments were conducted in the same environmental chamber at the University of Barcelona, at a constant water temperature of 21°C. Larvae from the six species were obtained from clutches collected in natural ponds from the study area (6 egg masses from *Alytes obstetricans*, 3 from *Pelodytes punctatus*, 6 from *Hyla meridionalis*, 3 from *Bufo bufo*, 3 from *Bufo calamita*, and 6 from *Rana perezi*). We collected clutches from temporary and permanent ponds to ensure a representative

sample of possible differences between populations, except for clutches of *Rana*, which were all from permanent ponds. Egg masses hatched in buckets and all experiments were started when tadpoles had reached Gosner stage 25.

We designed an experiment to analyse plastic response to drying using two treatments: a constant and a drying treatment. The former, which simulated a permanent pond without changes in water volume during tadpole development, had a larvae density of three individuals (each from distinct clutches to avoid population differences in phenotypic plasticity) per 2 litres. In contrast, the drying treatment simulated a temporary pond by reducing water volume during larvae development and had the same larval density. The fall in water level followed the curve $D_j = 1 - (j/t)^a P$ defined by Wilbur (1987), where D_j is the desired depth on day j, t is the target day for depth = 0 (110 days in our case, approximately the mean duration of temporary ponds in our study area), a is a shape parameter (0.4 in our treatment), and P is the water depth at the start of the experiment. Each treatment was replicated 20 times, with the exception of the *Pelodytes* treatment, which was replicated 38 times.

The experimental units consisted of plastic tubs (27 cm diameter) filled with 2 litres of dechlorinated tap water. To reduce the probability of infection and fouling, the water was changed approximately every 12 days. In the drying treatment, we adjusted the water level every 4 days following the planned drying curves. Tadpoles were fed periodically with a mixture (4 : 1) of rabbit chow and fish food *ad libitum* in relation to the number of tadpoles and their body size to avoid food accumulation and problems arising from water fouling. After the first metamorph was observed, the tubs were checked daily and all metamorphs were collected and kept in plastic boxes with 5 mm of water until tail resorption was complete. For all individuals, we measured: (1) time to metamorphosis, or time elapsed since the start of the experiment until forelimb protusion at Gosner stage 42 (potential plastic variable response to drying by accelerated development), and (2) mass at metamorphosis (tail resorption at Gosner stage 46) to 0.001 g precision [we used differences in mass at metamorphosis between treatments as a measure of cost of plasticity; mass at metamorphosis is crucial for post-metamorphic fitness in amphibians (Altwegg and Reyer, 2003)]. Survival to metamorphosis was expressed as the proportion of larvae per tub that completed development.

Ecological breadth of the species

We calculated the mean and variability in habitat use for each species after assigning numerical values to each pond category, as listed above: 1 = ephemeral ponds, 2 = temporary ponds, and 3 = permanent ponds. Variation in habitat use by species has two important components: spatial (among ponds) and temporal (within ponds but between years or seasons). Habitat variability can be determined by a generalist behaviour (those that breed along the entire freshwater gradient) or by the intrinsic temporal variability of the breeding habitat. Values of temporal variation within ponds were calculated from field data. We used data from the field surveys of 73 ponds in which we established the date of drying and duration over 3 years (2001–2003). Change in duration between years was used to examine variability in each pond category. For each species, we calculated an index of habitat variability, developed previously by Van Buskirk (2002). This index incorporates contributions from both sources. Spatial and temporal variation was calculated as $(p_e + 2p_t + p_p)$, where p_e is the occurrence score in ephemeral ponds, p_t is that in temporary ponds, and p_p is

that in permanent ponds. Temporary ponds (p_t) have the highest temporal variation (Fig. 2A), and the weightings in this equation ensured that temporary ponds contributed most strongly to habitat variability.

Magnitude of phenotypic plasticity and statistical analyses

The response of tadpoles to the two experimental treatments was studied by analysis of variance for each species. We used the mean individual response for each experimental unit to avoid a lack of independence of individual measures and thus pseudoreplication. Mass at and time to metamorphosis were $\log_e$ transformed because of heterogeneity of variances between treatments. As survival data were not a continuous trait (we only had four categories), we used the non-parametric Mann-Whitney *U*-test to make comparisons between treatments. We also examined correlations between larval period and mass at metamorphosis for each species.

We conducted the analyses with all data combined, and then repeated them using only the early 40% of tubs per treatment that had reached metamorphosis. This second analysis was performed to reduce bias promoted by the truncated distribution of those in the drying treatment, wherein time to metamorphosis was limited and survival could be reduced by this time limitation. This is a common problem in studies that use time horizons. Consequently, some authors readjust the data set (e.g. Tejedo and Reques, 1994), whereas others work with complete data sets. We performed both analyses to determine whether the use of complete data or truncated data alters the interpretation of results.

To compare the magnitude of phenotypic plasticity among species, we need a unitless proportional measure of plasticity. For this reason, we measured the plasticity of life-history traits by examining changes in traits that occurred between treatments divided by the mean value of the trait in the constant treatment ([drying – constant]/constant). In the drying treatment, negative values of plasticity reflect a decrease in the value of the trait (e.g. larval period), whereas positive values reflect an increase in this value.

If the traits measured affect species performance within a habitat type, then species from distinct habitats should differ in trait values. Therefore, we first tested for differences among the six species, regardless of habitat variability. To consider the effects of spatial and temporal variation in habitat, we ran analyses of variance using habitat as a fixed factor and species nested within habitat as a random factor. Species were nested within habitats following the index of habitat variability (see above). As we anticipated that the magnitude of plasticity would be a function of habitat variation and predictability, we considered two groups of species as a function of their index of habitat variability: constant habitats (predictable) and variable habitats (unpredictable). The six species were classified into one of these categories. Species with values ranging from 0.0 to 1.0 were considered to be from constant habitats, while those with values between 1.0 and 2.0 were considered to be from variable habitats. As in previous analyses, we calculated the magnitude of plasticity in order to perform a comparison between species using complete and truncated data sets (see above).

The trait values of species are influenced by shared common ancestry and thus species cannot be considered independent data points (Felsenstein, 1985). To determine whether the distribution of a particular species in phenotypic plasticity space is correlated with its phylogeny, we calculated Euclidean distances between species using standardized plastic traits values. The phylogenetic relationships between the six species were reconstructed.

Phylogenetic distance analyses were performed using the combined data set, which included three genes: 12S, 16S, and cyt *b*. Sequences were obtained from specimens in a personal collection (individuals collected and sequenced by Carranza) and from the GenBank database. All sequences were compiled, aligned, and refined manually using Sequence Navigator. Observed distances in pairwise comparisons were obtained using the software PAUP. We calculated the correlation between the two matrices: distance for plasticity values between species (Mahalanobis distances from a discriminant analysis) and phylogenetic distances between species. To assess the correlation between matrices, we applied a Mantel test. The correlation between the resulting evolutionary contrasts was repeated 5000 times and 95% confidence intervals were determined. Alternatively, we tested phylogenetic independence to larval development with the computer program 'Phylogenetic Independence 2.0' (Reeve and Abouheif, 2003). Tests For Serial Independence (TFSI) on continuous data were performed using the phylogenetic topology and node distances obtained from molecular reconstruction (Fig. 1). Topology was randomly rotated 2000 times to build the null hypothesis.

RESULTS

Ecological breadth of species

All pond categories were present in a similar proportion in the study area (60 ephemeral ponds, 100 temporary ponds, and 80 permanent ponds). Most of the species used the three categories of ponds. We tested if species differed in the frequency of use of the three pond categories with a Kruskal-Wallis analysis of variance by ranks. The species did not use the three habitats with the same frequency (*Alytes*: $H_{2,\ 31} = 30.0$, $P < 0.001$; *Pelodytes*: $H_{2,66} = 65.0$, $P < 0.001$; *Bufo bufo:* $H_{2,63} = 62.0$, $P < 0.001$; *B. calamita*: $H_{2,81} = 80.0$, $P < 0.001$; *Hyla*: $H_{2,56} = 55$, $P < 0.001$; *Rana*: $H_{2,31} = 30$, $P < 0.001$). Two species occupied habitats from the two extremes of the hydroperiod range (*B. calamita* the ephemeral end and *Rana* the permanent end), while the remaining species showed a preference for temporary ponds or occupied two categories indifferently (ephemeral and temporary, or temporary and permanent) (Fig. 2B). For each species, we calculated the index of habitat variability, which incorporates contributions from temporal (Fig. 2A) and spatial variation (Fig. 2B). *Rana*, *B. calamita*, and *B. bufo* were considered species from constant habitats (index values < 1.0), whereas *Hyla*, *Pelodytes*, and *Alytes* were considered species from variable habitats (index values > 1.0) (Fig. 2C).

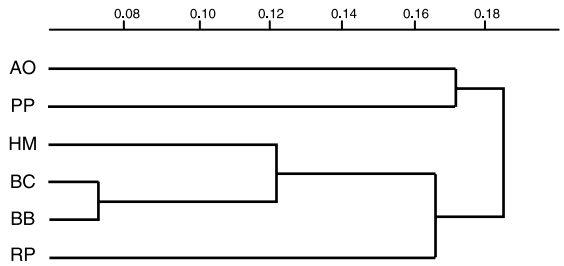

Fig. 1. Phylogenetic hypothesis depicting relationships between the six species on the basis of genetic distances for three genes: 12S, 16S, and cyt *b.* AO = *Alytes obstetricans*, PP = *Pelodytes punctatus*, BB = *Bufo bufo*, BC = *Bufo calamita*, HM = *Hyla meridionalis*, RP = *Rana perezi*.

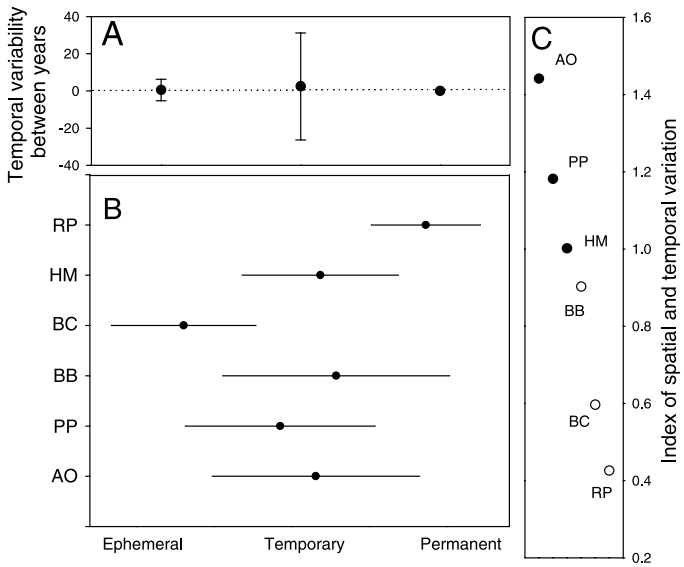

Fig. 2. (A) Temporal variability from each pond category (x-axis: ephemeral, temporary, and permanent) between years (2001–2003) using data from 73 ponds (mean and standard error). Open circles represent habitats with high temporal variability and filled circles habitats with low temporal variability. (B) Spatial use and variability of pond categories for each species (mean and standard error). (C) Value of index of spatial and temporal variability for each species after applying the model explained in the 'Materials and methods' section. Open circles represent species exposed to high spatial and temporal variability (values > 1.0), whereas filled circles represent those from more predictable environments (values < 1.0). AO = *Alytes obstetricans*, PP = *Pelodytes punctatus*, BB = *Bufo bufo*, BC = *Bufo calamita*, HM = *Hyla meridionalis*, RP = *Rana perezi*.

Response to experimental treatments

Survival to metamorphosis did not differ between treatments, with the exception of *Rana*, which showed a higher mortality in the drying treatment (Table 1, Fig. 3C). In general, species tended to reduce their larval period and reached metamorphosis with a lower body mass in the drying treatment (Fig. 3A, B, Table 1). However, the differences between treatments were not statistically significant for all species (Table 1). The two bufonids did not show a shorter larval period in the drying treatment, whereas the rest of the species did. A smaller size at metamorphosis in the drying treatment was observed in all species except *B. calamita* and *Rana*. A positive correlation between larval period and mass at metamorphosis was observed in all species except *B. calamita* (*Alytes*: $R = 0.471$, $P = 0.007$; *Pelodytes*: $R = 0.326$, $P = 0.008$; *B. bufo*: $R = 0.540$, $P = 0.001$; *B. calamita*: $R = 0.198$, $P = 0.277$; *Hyla*: $R = 0.711$, $P = 0.001$; *Rana*: $R = 0.558$, $P = 0.002$).

The statistical significance did not change greatly in the analysis of variance between treatments using the truncated data set (last two columns of Tables 1A, B). However, truncation of the data for the upper distribution tail (tubs with longer larval periods, because we only used the early 40% of tubs that had reached metamorphosis) changed the relationship between larval period and mass at metamorphosis in two species, *Pelodytes* and *Rana*, which do not show the positive correlation that is observed when the complete data is

Table 1. Descriptive statistics and results of analyses of variance and Mann-Whitney *U*-tests between treatments for each species

Species	Treatment	*n*	Mean	Variance	Standard error	Min.	Max.	*F*	*P*	Truncate *F*	Truncate *P*
A. Larval period (days)											
A. obstetricans	Constant	18	135.16	270.73	3.87	106	163	26.37	**<0.001**	23.10	**<0.001**
	Drying	14	109.35	116.39	2.88	97	110				
P. punctatus	Constant	38	73.89	429.77	3.36	47	105	4.73	**<0.05**	8.67	**<0.01**
	Drying	27	62.01	152.61	2.37	46	87				
B. bufo	Constant	17	92.17	37.91	1.49	83	101	0.6	0.453	2.49	0.135
	Drying	16	90.50	47.20	1.71	79	103				
B. calamita	Constant	16	61.18	96.29	2.45	48	77	2.69	0.113	0.29	0.594
	Drying	16	56.12	42.65	1.63	47	72				
H. meridionalis	Constant	19	144.47	366.71	4.39	108	171	60.75	**<0.001**	45.82	**<0.001**
	Drying	15	102.80	113.88	2.75	85	110				
R. perezi	Constant	18	129.16	242.03	3.66	104	153	21.79	**<0.001**	7.39	**<0.05**
	Drying	11	107.09	15.49	1.18	99	110				
B. Mass at metamorphosis (g)											
A. obstetricans	Constant	18	1.26	0.023	0.036	1.007	1.588	34.58	**<0.001**	42.56	**<0.001**
	Drying	14	0.99	0.012	0.029	0.856	1.274				
P. punctatus	Constant	38	0.21	0.004	0.011	0.108	0.385	1.18	**<0.001**	2.02	0.166
	Drying	27	0.16	0.002	0.008	0.084	0.253				
B. bufo	Constant	17	0.12	0.001	0.005	0.089	0.157	4.82	**<0.05**	5.57	**<0.05**
	Drying	16	0.10	0.001	0.005	0.078	0.158				
B. calamita	Constant	16	0.11	0.001	0.006	0.085	0.168	0.11	0.736	0.10	0.756
	Drying	16	0.10	0.001	0.005	0.086	0.148				
H. meridionalis	Constant	19	0.80	0.004	0.015	0.687	0.976	22.23	**<0.001**	31.38	**<0.001**
	Drying	15	0.68	0.006	0.021	0.572	0.827				
R. perezi	Constant	18	0.73	0.006	0.018	0.649	0.894	1.08	0.309	0.16	0.694
	Drying	11	0.71	0.005	0.021	0.594	0.801				

C. Survival to metamorphosis (%)*

A. obstetricans	Constant	20	83	66	100	0	100	1.82	0.083
	Drying	20	64	0	66	0	100		
P. punctatus	Constant	38	66	66	100	33	100	0.69	0.488
	Drying	36	66	0	100	0	100		
B. bufo	Constant	20	80	73	100	0	100	0.14	0.882
	Drying	19	80	66	100	0	100		
B. calamita	Constant	20	90	66	100	0	100	0.02	0.978
	Drying	20	80	80	100	0	100		
H. meridionalis	Constant	20	66	66	100	0	100	1.31	0.191
	Drying	20	66	33	100	0	100		
R. perezi	Constant	20	83	66	100	0	100	3.55	**<0.001**
	Drying	19	33	0	66	0	66		

Note: Results of analyses of variance for time to metamorphosis and mass at metamorphosis with the two data sets: complete and truncated data sets.
* Mann-Whitney *U*-test.

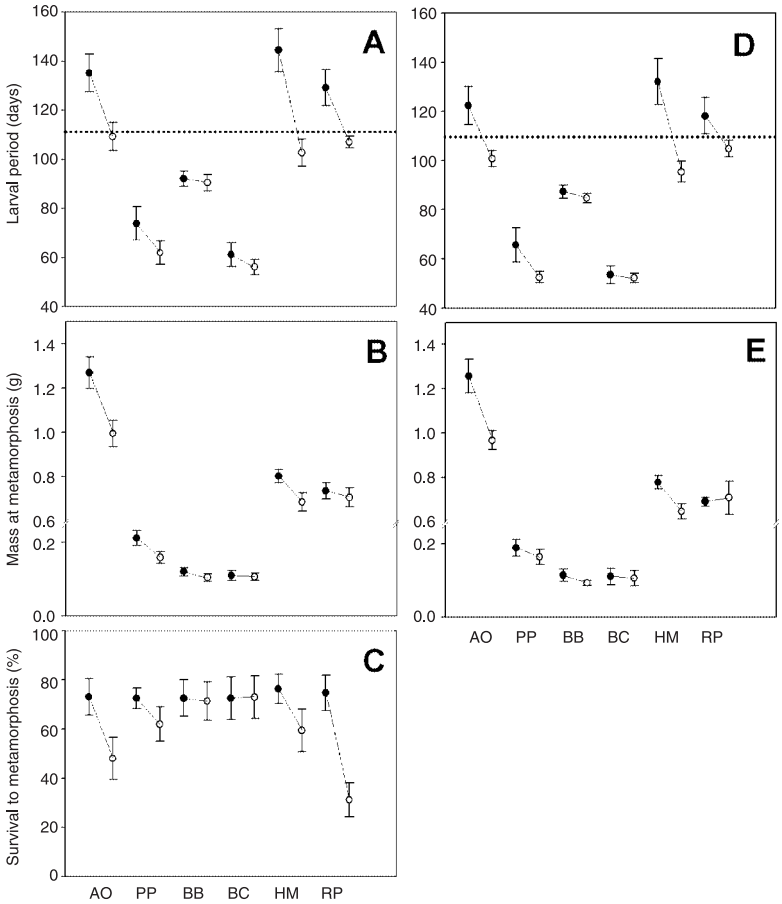

Fig. 3. Results for the traits measured for each species in the laboratory experiments (mean and standard error). Filled circles correspond to permanent treatment and open circles to drying treatment. Graphics in the first column were elaborated using all the data, whereas those from the second column were constructed using the truncated data set. (A) and (D) show changes in larval period, (B) and (E) mass at metamorphosis, and (C) survival to metamorphosis. The dashed line in (A) and (D) indicates the temporal horizon to drying treatment (110 days) when tubs were completely dry. Survival to metamorphosis was not estimated with the truncated data. AO = *Alytes obstetricans*, PP = *Pelodytes punctatus*, BB = *Bufo bufo*, BC = *Bufo calamita*, HM = *Hyla meridionalis*, RP = *Rana perezi*.

used (*Alytes*: $R = 0.633$, $P = 0.008$; *Pelodytes*: $R = 0.062$, $P = 0.729$; *B. bufo:* $R = 0.235$, $P = 0.362$; *B. calamita*: $R = 0.341$, $P = 0.196$; *Hyla*: $R = 0.655$, $P = 0.002$; *Rana*: $R = 0.173$, $P = 0.520$). Loss of individuals with a longer larval period by truncating the data also implied loss of larger froglets and toadlets.

Magnitude of phenotypic plasticity and habitat use

Species and the two groups considered as a function of habitat (constant habitats and variable habitats) differed in larval period phenotypic plasticity (Table 2). Species from

Table 2. Results of analysis of variance of the two standardized variables (unitless magnitude of phenotypic plasticity) with species nested within habitat

	Source of variation		
Variable	Species (SP) (d.f. = 5)	Habitat (HAB) (d.f. = 1)	Species (SP[HAB]) (d.f. = 4)
Larval period	10.8333**	28.1025**	6.2980**
Larval period 'truncate'	36.3371**	136.37**	8.3920**
Mass at metamorphosis	5.7779**	16.1107**	2.3163
Mass at metamorphosis 'truncate'	2.3547	5.1098**	2.0325

Notes: Six species, and constant vs. variable habitat species, are compared (Habitat). Table entries are *F*-ratios; d.f. for the error = 93 for larval period and mass at metamorphosis, d.f. for the error = 44 for larval period and mass at metamorphosis in the truncated data series.
** $P < 0.001$.

variable habitats showed a higher magnitude of phenotypic plasticity, especially for the larval period (Fig. 4A). Although the change in mass at metamorphosis differed among species, differences for species nested within the appropriate habitat affiliation were not significant. The truncated data set did not modify the results for larval period, while differences in mass at metamorphosis between species disappeared (Table 2, Fig. 4B).

Position in phenotypic space (using all variables measured) was closely linked to habitat type, but not to lineage. The Mantel test did not show any matrix correlation between the phenotypic matrix and the phylogenetic matrix after 5000 random permutations ($P_{(\text{random } Z \leq \text{observed } Z)} = 0.5349$ and $P_{(\text{random } Z \geq \text{observed } Z)} = 0.4659$). Also, the magnitude of developmental plasticity was phylogenetically independent (C-statistics = 0.0471 with $P = 0.4052$) according to the test for serial independence.

DISCUSSION

Magnitude of plasticity and habitat unpredictability

The species showed significant differences in ecological distribution along the freshwater gradient described in other amphibian communities (Skelly, 1996; Babbitt *et al.*, 2003; Van Buskirk, 2003). The environmental heterogeneity of species (in space and time) is an early step by which to recognize and interpret phenotypic plasticity as an adaptation (Doughty and Reznick, 2004). Variability in desiccation risk is predicted to vary more within and between years in temporary ponds of intermediate duration than in permanent ponds and ephemeral pools (Leips *et al.*, 2000). The magnitude of response follows the pattern predicted by models (Moran, 1992; Van Tienderen, 1997). Tadpoles of species that use a wide variety of habitats, while typically breeding in temporary ponds (*Alytes*, *Pelodytes*, and *Hyla*), showed major plastic responses in life-history traits and a tendency towards a reduced larval period than those occurring in constant or predictable habitats. A positive relationship between plasticity and environmental heterogeneity is expected when divergent selection promotes the evolution of plasticity within a species and when species differ in the extent to which they experience this

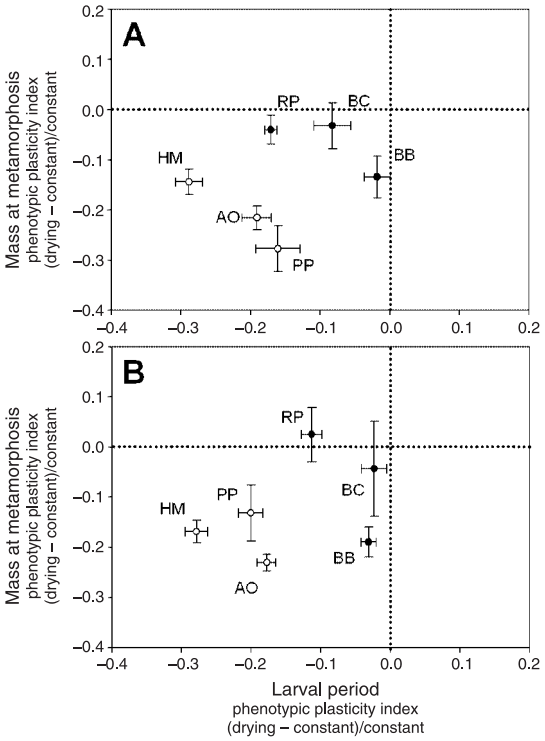

Fig. 4. Phenotypic plasticity index (proportional changes between treatments) of larval period and mass at metamorphosis for each species (mean and standard error). (A) Graphic constructed with the complete data set, and (B) with the truncated data set. Dashed lines indicate the case in which there was no plasticity. Open circles represent species exposed to high spatial and temporal variability, and filled circles species from more predicable environments. AO = *Alytes obstetricans*, PP = *Pelodytes punctatus*, BB = *Bufo bufo*, BC = *Bufo calamita*, HM = *Hyla meridionalis*, RP = *Rana perezi*.

selection. At the two extremes of the hydroperiod range, evolution may favour the development of specialist phenotypes with limited plasticity. Fast development rates were selected in predictable ephemeral ponds to escape the risk of drying (e.g. *B. calamita*). In contrast, in predictably permanent water bodies, slower development has evolved to optimize larval growth opportunities (e.g. *Rana*).

The acceleration of metamorphosis is clearly advantageous when the pond is at risk of drying. This response has an associated cost, as there is a trade-off between development rate and size at metamorphosis (Wilbur and Collins, 1973; Newman, 1992). Individuals that develop faster are typically smaller than those that develop more slowly. Small size at metamorphosis may reduce resistance to parasites (Goater, 1994), may lead to a major risk of water loss during post-metamorphic life (Newman and Dunham, 1994), may affect locomotor performance and the metabolic rates of metamorphs (Beck and Congdon, 2000; Richter-Boix *et al.* 2006), and, finally, may reduce reproductive fitness (Smith, 1987; Semlitsch *et al.*, 1988). Consequently, these studies indirectly support the contention that larval period plasticity implies an associated cost (but see Loman and Claesson, 2003, for a discussion of cost models). It will be necessary to measure other variables to search possible costs associated with developmental plasticity [e.g. morphological changes independently of size (Newman, 1992, Richter-Boix *et al.*, 2006)].

As in previous studies (Richardson, 2001; Van Buskirk, 2002), phylogeny was not correlated with phenotypic plasticity and did not contribute to the identification of a phylogenetic pattern of plasticity evolution. However, this result should be treated with caution, as it could be a reflection of the limited power of the statistical analyses. In some statistical tests, the phylogenetic signal is significant only if the number of species is large, which obviously was not the case here. However, differences in developmental plasticity among species from the same genera have been reported (Morey and Reznick, 2000, 2004). These authors suggested that variability of response within families implies that plasticity evolves over relatively short time-scales. The results of the present study provide support for the hypothesis that interspecific differences in phenotypic plasticity to pond desiccation are adaptive and are related to temporal unpredictability of habitat and niche breadth; however, recognizing the role of phylogeny in the evolution of plasticity requires a great number of species. In our study, we assumed no geographic variation within species among populations from temporary and permanent ponds, but recent studies with tadpoles have described geographic variations in plasticity within species (Gómez-Mestre and Tejedo, 2003; Van Buskirk and Arioli, 2005).

A potential weakness of this study is that the same slow decrease in water level, which is typical of a temporary pond, was applied for all species. Consequently, this treatment may not have been sufficient to stimulate a response in species with short larval periods like bufonids. However, Brady and Griffiths (2000) obtained similar results with the two bufonid species and reported that the timing of metamorphosis was unaffected, whereas Tejedo and Reques (1994) found a positive response of *B. calamita*. Several variables are informative cues of environmental drying: increments of conspecific density, reductions in swimming volume, reduced food, and changes in chemical and physical properties of water (reviewed in Denver *et al.*, 2002). The six species studied here may not have responded in the same manner to these factors, and in the case of bufonids, for example, the density of treatments may have been insufficient to generate a stress response in species that normally develop in high-density cohorts in nature. Bufonids may have responded to desiccation through detecting a marked increase in density. For example, *Bufo americanus* and *B. bufo* accelerate development rate at high density but not at low density (Wilbur, 1987; M. Tejedo and R. Reques, unpublished data).

An additional problem was the difficulty encountered in measuring plasticity in species in which the larval period was truncated in the drying treatment by their longer larval periods. In the case of *Alytes* and *Hyla*, we hypothesize an acceleration of metamorphosis and, as a result, survival to metamorphosis was unaffected between treatments. However, in the case of *Rana*, for which there was a high mortality during the drying treatment, the time horizon resulted in a truncation of data, which may have overestimated plasticity (observe position change of *Rana* and *Alytes* between Figs. 3A and 3B). The use of the truncated data set (the early 40% of replicas per treatment) in analyses helps to minimize this problem by working with the same proportion of early tubs that reached metamorphosis in the two treatments. Nevertheless, this data set underestimates the cost of plasticity. Removal from the analyses of individuals with longer larval periods also implies removal of larger individuals and, consequently, a modified positive correlation between time of and size at metamorphosis. The use of both data sets can help us to interpret the results correctly.

Habitat breadth and strategies for environmental heterogeneity

Following the categories of possible strategies for environmental heterogeneity, described by DeWitt and Langerhans (2004), species from variable habitats can be considered plastic

strategists with asymmetric bet-hedging. In this strategy, the terrestrial environment is supplied continuously with metamorphs provided the water body does not dry out and, consequently, there is large variance in larval period. If desiccation occurs in a very short time, thus not allowing tadpoles to react, as occurs in early summer in the Mediterranean region (where ponds can dry in less than one week), the faster developing individuals of the cohort will have reached the terrestrial phase (Lane and Mahoney, 2002; Thumm and Mahoney, 2002). Alternatively, in ponds that dry during spring at a slower and more constant velocity, all individuals react in the same manner by increasing development rate, as in our drying treatment, and metamorphosing more synchronously (low variance of larval period with respect to the constant water treatment).

Specialized species, such as *B. calamita*, did not show a change in mean larval period but their variance values did differ across environments. *Bufo calamita* showed low variance in its specialized environment (drying treatment) and some optimal level of variance in its non-specialized environment (permanent waters). This strategy allows some individuals to optimize growth opportunities. This integrated solution increases the fitness of specialists across environments (DeWitt and Langerhans, 2004). As breeding amphibian populations occur as networks of subdivided populations connected by migration of long-lived and mobile adults, which can breed in patches of distinct variability, maintenance of variance in these traits is expected to persist at a range of magnitudes in all species. These strategies may ensure that individuals with some magnitude of plasticity can successfully colonize a wide range of habitat types. Developmental plasticity is ecologically significant in that it permits a widening of the niche breadth of species with a metapopulation structure.

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