Analysis of the variations, at an intermediate regional scale, of life history traits of *Rhinella arenarum* using reciprocal transplant experiments

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RESUMEN: En los fenómenos de plasticidad, es necesario distinguir experimentalmente el componente genético de la variación inducida por el ambiente y de la interacción de ambos, que nos permita hacer interpretaciones acerca de la evolución y la naturaleza adaptativa de estos cambios. Entre las aproximaciones experimentales que pueden ayudarnos, suele utilizarse el experimento de trasplante recíproco, en el cual individuos de diferentes poblaciones son criados de manera conjunta en cada uno de los ambientes originales. En este trabajo, se presentan los resultados del estudio de las fuentes causales de la variación geográfica en caracteres de historias de vida durante la etapa larvaria en *Rhinella arenarum*. Con este propósito se diseñó un experimento de trasplante recíproco en los ambientes originales de Moldes (ambiente sur) y Salta (ambiente norte), ambos pertenecientes a la unidad geoestructural del Valle de Lerma, Salta, Argentina. Se observaron respuestas diferenciales de las poblaciones con relación al ambiente de crianza como así también influencia de este último en la edad, tamaño y tasa de crecimiento de los individuos metamórficos provenientes de las poblaciones estudiadas.

PALABRAS CLAVE: plasticidad fenotípica, *Rhinella arenarum*, transplante recíproco, tasa de crecimiento, metamorfosis.

ABSTRACT: In plasticity phenomena, it is necessary to experimentally distinguish between the genetic component, the variation induced by the environment, and the interaction between both, in order to make interpretations about evolution and the adaptive nature of these changes. Among the experimental approaches available, recyprocal transplants are often used, in which individuals from different populations are reared together in each of the native environments. This chapter presents the results obtained in the study of the causes of geographic variation in life history traits during the larval stage of *Rhinella arenarum*. For this purpose, a reciprocal transplant experiment was designed in the native environments Moldes (southern environment) and Salta (northern environment). The populations exhibited differential responses to the rearing environment, as well as an influence of this on age, size and growth rate of the individuals from the populations studied.

KEY WORDS: Phenotypic plasticity, *Rhinella arenarum*, reciprocal transplant experiment, growth rate, metamorphosis.

INTRODUCTION

One of the central aspects of the knowledge of phenotypical variability in nature is to what amount the phenotypical differences observed throughout the environment are caused by genetic variation and/or environmental influence (Conover and Schultz 1995, Wells 2007).

Thus, when dealing with plasticity phenomena, it becomes necessary to experimentally distinguish the genetic component from the environmentally induced variation, and the

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interaction between them, in order to interpret the evolution and adaptive nature of these changes (Berven and Gill 1983, Lardner 2000, Marangoni 2006). Generally, the environmental component quantifies the degree of phenotypical plasticity, whereas the population and population x environment components describe the genetic basis of the character (Reznick and Travis 1996). The interaction term (genotype x environment) indicates the existence of genetic variation in the plasticity (Stearns 1992, Schilichting and Pigliucci 1998) and denotes local adaptation (Lardner 2000).

Among the experimental approaches that can help measure the genetic and environmental components of phenotypical variation along an environmental gradient, the reciprocal transplant experiment is usually employed, in which individuals of different populations are reared together in each of the original environments (Via 1993, Harris 1999). Using this technique we can interpret the causes of phenotypical variation incorporating natural variation to all the environmental variables that can act collectively (Via 1993, Conover and Shultz 1995, Marangoni 2006).

Marangoni (2006) pointed that the design of reciprocal transplant experiments is an experimental approach that might allow the contrasting of three hypotheses within that conceptual context.

The first of these hypotheses is that of *genetic determination*, which proposes that the difference among populations throughout a gradient will be maintained independently from the environment. The second hypothesis is that of *phenotypical plasticity*, according to which populations will not differ from the native to the transplanted environment. Therefore, geographical variation in life history parameters might simply reflect differences induced by the local environment that determine, for instance, variations in growth rates but without implying genetic variations among populations.

The third hypothesis is that of *divergence in norms of reaction,* according to which the level of differentiation in phenotypical expressions of each genotype will be a function of the environment examined. In other words, there will be interaction between genotype and environment.

Although the reciprocal transplant technique, because of its complex logistics requirements, can be even inviable in vertebrates (Conover and Shultz 1995), the greatest advantage of this essay is that, besides controlling the eventual environmental sources of phenotypical variation, it makes it possible (unlike regular environment experiments) to identify the sources of phenotypical variation attributable to the natural environment, the population of origin and the interaction between both terms.

In the present work, we present the results of our study of the sources causing geographic variation in life history traits during the larval stages of *Rhinella arenarum*. This species belongs to the worldwide spread family Bufonidae (Frost 2009). In Argentina the species is distributed from the northern limits to the south, near the Patagonia coasts, and it is present in several phytogeographic regions. Several populations of this species show morphological, physiological and behavioral traits that are locally different (Cei 1987, Hoffman and Blouin 2000). The creation of several subspecies has been proposed based on the remarkable polymorphism exhibited by these populations; nonetheless, according to Cei (1987), these are considered synonims to the *arenarum* Hensel form.

In this context, this widely spread species becomes an interesting candidate for analyzing the plasticity phenomenon. For this purpose an essay was designed consisting of a reciprocal transplant in the field of *R. arenarum* larvae, reared in natural bodies of water, into the native environments of Moldes (southern environment)

and Salta (northern environment) simultaneously, both environments belonging to the geo-structural unit of Valle de Lerma, Salta province, Argentina.

The main goals of this work were:

1. To analyze the geographic variation of life history traits during the larval stages of *R. arenarum*, such as growth rate and morphological parameters, developmental time (age) and size reached at metamorphosis.
2. To examine the norms of reaction of each population regarding the growth rate of the larvae, for the environments where reciprocal transplants were conducted.

MATERIALS AND METHODS

The environments selected for the reciprocal transplant experiments were, both in the northern and southern areas, two lagoons of similar size situated in Valle de Lerma, 70 km apart from each other. Valle de Lerma presents a marked environmental gradient, since precipitation records range from 359 mm in the southern area to 1615 mm in the northwestern area. Similarly, altitude, topography, exposure and the presence of transversal ravines determine modifications in the patterns of thermal distribution. Thus, in the northern and western areas of the valley summers are mild and winters cold, corresponding to a temperate climate, whereas in the southern and eastern areas the weather is warm and dry, with hot summers and cold winters (Baumgardner and Cozzi 1998).
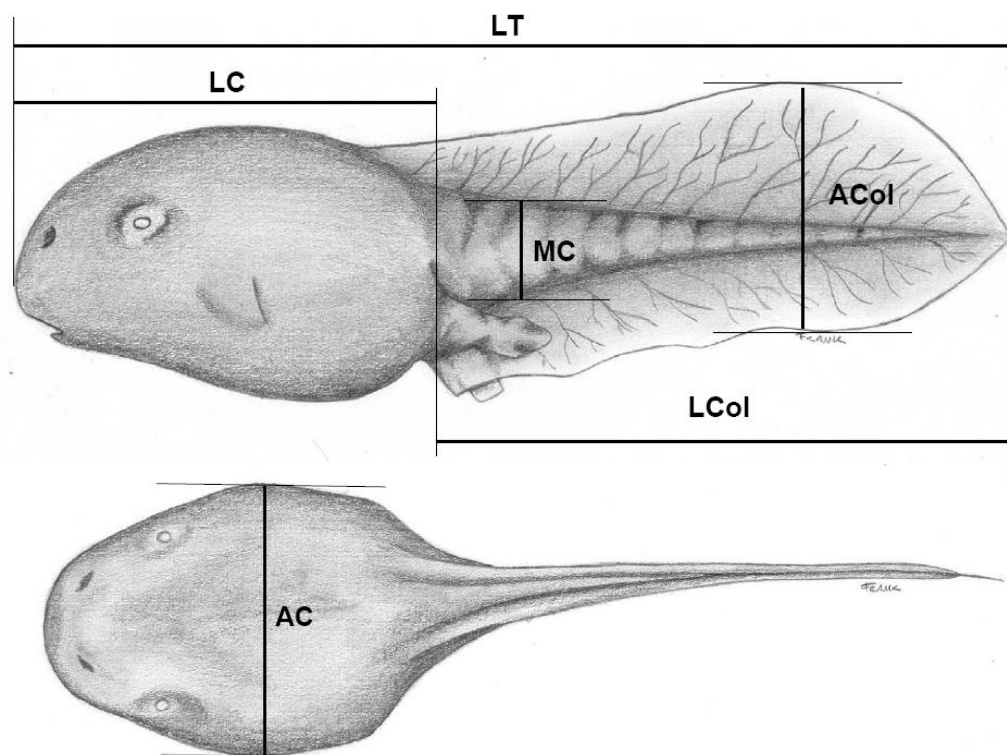

Figure 1. Sketch of a *Rhinella arenarum* larva showing the different measurements taken. LT: total length, LC: length of the body, Lcol: length of the tail, AC: width of the body, Acol: width of the tail, MC: width of the tail musculature.

In each environment selected an oviposition was collected and then taken to the lab until the eclosion of larvae. When these reached the 25th stage (Gosner 1960). The cohort of siblings from the southern area of the valley was divided into two groups, one transplanted into the northern area and the other kept in the southern area. The same procedure was applied to the cohort from the northern area, that is, one group was transplanted into the southern area while the other was kept in the northern area.

Each cohort was taken to the field and placed in 1m long, 0,60m wide and 0.40m high structures, with their base and sides covered with a fine meshed fabric that allowed the entrance of water but prevented the larvae from escaping or other organisms, especially aquatic predators, from entering. The roof of each structure was covered with a metallic net, preventing the entrance of predators but allowing the light to pass through. The number of individuals per group was 200, taking into account the volume available within each exclusion structure as well as the number of samples per extraction (20 larvae).

Larvae were collected 12 and 24 days, captured and kept in 10% formaldehyde to be transported to the lab; this procedure was conducted simultaneously in both experimental environments. In the lab, the following morphometric variables were recorded: total body length and tail length, body width, tail height, height of tail musculature and developmental stage. Developmental time, size at metamorphosis and growth rate, were also recorded for those individuals that had already undergone metamorphosis (Fig. 1).

The growth rate for metamorphosed individuals was defined as:

$$\ln_{(weight\ at\ metamorphosis)} / \ln_{(time\ to\ metamorphosis)}\ (Kehr\ 1994)$$

Where:

Time to metamorphosis is the number of days from the beginning of the essay to the emergence of the fore limbs (stage 42 Gosner 1960), and weight at metamorphosis is the weight of the individual measured at the moment of the complete resorption of the tail.

For the larvae, the developmental rate was calculated as:

$$\ln_{(stage)} / \ln_{(time)}\ (modified\ from\ Harris\ 1999)$$

Where:

$\ln_{(stage)}$ is the natural logarithm of the stage at which the larva was collected, and $\ln_{(time)}$ the natural logarithm of the days from eclosion to the recording of data.

The growth rate for larvae was calculated as:

$$\ln_{(weight)} / \ln_{(time)}\ (Kehr\ 1994)$$

Length was recorded using a 0/100º, 0.01 mm digital caliper; weight was measured using a Metler Toledo AB 204 analytical scale.

The data were subjected to the modified Wilk-Shapiro normality test and, since some data did not adjust to a normal distribution, non-parametric statistics was applied (InfoStat/Professional version 2004i.1).

RESULTS
Comparison between populations in each experimental environment.

Southern experimental environment. In this environment, after 12 days after the beginning of the essay, no significant differences were observed either in the morphological variables of larvae between both population, or in the growth rate (Mann-Whitney, $W=405.00$ $p=0.8924$), however, the developmental stages, and consequently the developmental rate, exhibited significant differences (Mann-Whitney, $W=269.50$, $p=0.0001$), the larvae from

the northern population being at more advanced developmental stages (Table 1).

At 24 days, significant differences were recorded in the variables studied ($p<0.05$) (Table 1). The growth rate (Mann-Whitney, $W=337.00$, $p=0.0483$) and developmental rate (Mann-Whitney, $W=219.00$, $p<0.0001$) showed significant differences, with the larvae from the northern area (northern population) reaching the highest values, although a high dispersion of data could be observed.

Table 1. Variables analyzed by Mann-Whitney test at 12 and 24 days after beginning the essay in the southern experimental environment with populations from the southern and northern sites.

Variable	population	12 days				24 days			
		mean	DS	W	p	mean	DS	W	p
Total length	Southern	14.62	2.69	367	0.2447	26.26	4.43	256	<0.0001
	Northern	15.42	2.32			31.08	1.65		
Tail Length	Southern	8.29	1.77	363.5	0.2084	15.11	2.84	273.5	0.0002
	Northern	8.94	1.5			18.5	1.75		
Body length	Southern	6.33	1	388	0.5517	11.15	1.67	310.5	0.0071
	Northern	6.48	0.94			12.57	0.94		
Body width	Southern	3.91	0.61	430.5	0.5791	6.42	0.92	262	0.0001
	Northern	4.19	0.72			7.65	0.67		
Tail height	Southern	2.37	0.67	430.5	0.5791	4.49	0.83	271.5	0.0002
	Northern	2.16	0.34			5.33	0.33		
Height of tail musculature	Southern	1.06	0.26	398	0.7454	2.03	0.37	266	0.0001
	Northern	1.09	0.27			2.49	0.28		
Stage	Southern	27.7	1.89	269.5	0.0001	33.75	2.9	219	<0.0001
	Northern	30.35	1.57			38	1.08		
Weight	Southern	0.35	0.23	405	0.8924	0.38	0.25	337	0.0483
	Northern	0.32	0.14			0.47	0.25		

Northern experimental environment.
Contrary to what we observed in the southern environment, after 12 days after the beginning of the essay we detected significant differences in all the parameters studied in the northern environment, as well as in the growth rate (Mann-Whitney, $W=608.00$, $p<0.0001$) and the development rate (Mann-Whitney, $W=563.00$, $p<0.0001$); (Table 2).

The larvae from the southern population transplanted into the northern environment grew to a greater size and developed at a higher rate than the larvae native to the northern environment. These differences were statistically significant.

At 24 days, the trend varied remarkably, since no further significant differences in the parameters analyzed could be found, including the developmental rate (Mann-Whitney. $W=431.00$, $p=0.5284$), with the exception of weight which, reversing the observations of the previous record, was higher for the larvae native to the northern environment (Table 2).

Likewise, significant differences could be found in the growth rate, which showed an increase with respect to the previous record for the larvae native to this area, whereas the transplanted larvae exhibited a decrease in the aforementioned parameter when compared to the previous record.

Comparison between experimental environments for each population.

Southern population. The larvae belonging to the southern population responded differently to the experimental environment. Thus, 12 days after the beginning of the essay, significant differences were recorded for all the parameters studied (Table 3).

developmental rate (Mann-Whitney, $W=262.50$, $p=0.0001$).

This trend was maintained at 24 days after beginning the essay, since the variables recorded showed significant differences, including growth rate (Mann-Whitney, $W=276.50$, $p=0.0003$) and development rate (Mann-Whitney, $W=240.00$, $p<0.0001$), with the exception of body width, for which the larvae at the northern experimental environment presented higher values (Table 3).

Although, the larvae at the northern experimental environment grew more and at a faster pace, than their siblings at the native site, a high dispersion of data for these larvae was

A remarkable dispersion of data could be observed for the larvae transplanted into the northern experimental environment, which always exhibited higher values in the morphological variables. as well as in developmental stage, growth rate (Mann-Whitney, $W=264.50$, $p=0.0001$) and observed, as well as for the observations after 12 days. This happened with all the parameters except tail length, which exhibited a mirror-like behavior with respect to the first data record. Furthermore, we observed a deceleration in growth rate with respect to the previous record.

Northern population. The larvae belonging to the northern area of the Valle de Lerma showed a differential behavior after 12 days of beginning the essay. Significant differences were recorded in the variables studied, with the exception of tail height (Table 4), growth rate (Mann-Whitney. $W=490.00$, $p=0.0304$) and development rate (Mann-Whitney. $W=546.50$ $p=0.0002$).

Table 2. Variables analyzed by Mann-Whitney test at 12 and 24 days after beginning the essay in the northern experimental environment with populations from the southern and northern sites.

Variable	population	12 days				24 days			
		Mean	DS	W	p	mean	DS	W	p
Total length	Southern	19.35	4.24	605	<0.0001	31.49	1.91	429	0.6073
	Northern	12.47	1.88			31.09	2.2		
tail Length	Southern	11.24	2.47	603	<0.0001	18.78	2.78	427	0.6455
	Northern	4.74	3.39			18.36	1.3		
Body length	Southern	8.1	1.88	595	<0.0001	12.72	2.78	461.5	0.1635
	Northern	5.31	0.89			12.73	1.1		
Body width	Southern	5.06	1.03	602	<0.0001	6.89	0.51	419.5	0.7972
	Northern	3.43	0.55			6.87	0.6		
Tail height	Southern	2.94	0.85	570	<0.0001	5.09	0.22	415.5	0.8817
	Northern	2.08	0.31	.5		5.01	0.5		
Height of tail musculature	Southern	1.39	0.42	581	<0.0001	2.36	0.17	398.5	0.7556
	Northern	0.92	0.16	.5		2.38	0.2		
Stage	Southern	31.1	2.15	563	<0.0001	36.55	0.6	431	0.5284
	Northern	28.05	1.57			36.4	0.8		
Weight	Southern	0.64	0.21	608	<0.0001	0.74	0.28	309	0.0063
	Northern	0.22	0.07			1.06	0.5		

Table 3. Variables analyzed by Mann-Whitney test, at 12 and 24 days after beginning the essay for the southern population in the northern and southern experimental sites.

Variable	experimental sites	12 days				24 days			
		mean	DS	W	p	mean	DS	W	p
Total length	Southern	14.62	2.69	263	0.0001	26.26	4.43	250	<0.0001
	Northern	19.35	4.24			31.49	1.91		
Tail Length	Southern	8.29	1.77	264	0.0001	15.11	2.84	268	0.0001
	Northern	11.24	2.47			18.78	2.78		
Body length	Southern	6.33	1	282.5	0.0006	11.15	1.67	251	<0.0001
	Northern	8.1	1.88			12.72	2.78		
Body width	Southern	3.91	0.61	259	<0.0001	6.42	0.92	356	0.144
	Northern	5.06	1.03			6.89	0.51		
Tail height	Southern	2.37	0.67	310	0.0068	4.49	0.83	304	0.0041
	Northern	2.94	0.85			5.09	0.22		
Height of tail musculature	Southern	1.06	0.26	294	0.0017	2.03	0.37	285.5	0.0008
	Northern	1.39	0.42			2.36	0.17		
Stage	Southern	27.7	1.89	262.5	0.0001	33.75	2.9	240	<0.0001
	Northern	31.1	2.15			36.55	0.6		
Weight	Southern	0.35	0.23	264.5	0.0001	0.35	0.25	276.5	0.0003
	Northern	0.64	0.21			0.74	0.28		

Table 4. Variables analyzed by Mann-Whitney test at 12 and 24 days after beginning the essay for the northern population in the northern and southern experimental sites.

Variable	experimental sites	12 days				24 days			
		mean	DS	W	p	mean	DS	W	p
Total length	Southern	15.42	2.32	547	0.0002	31.08	1.65	392	0.6263
	Northern	12.47	1.88			31.09	2.17		
Tail Length	Southern	8.94	1.5	547	0.0002	18.5	1.75	425.5	0.6750
	Northern	7.16	1.12			18.36	1.27		
Body length	Southern	6.48	0.94	536	0.0006	12.57	0.94	379	0.4017
	Northern	5.31	0.89			12.73	1.11		
Body width	Southern	4.19	0.72	531	0.0011	7.65	0.67	529	0.0013
	Northern	3.43	0.55			6.87	0.61		
Tail height	Southern	2.16	0.34	440	0.4166	5.33	0.33	497.5	0.0179
	Northern	2.08	0.31			5.01	0.45		
Height of tail musculature	Southern	1.09	0.27	492.5	0.0255	2.49	0.28	466	0.1297
	Northern	0.92	0.16			2.38	0.21		
Stage	Southern	30.35	1.57	546.5	0.0002	38	1.08	555	0.0001
	Northern	28.05	1.57			36.4	0.82		
Weight	Southern	0.32	0.14	490	0.0304	0.47	0.25	282	0.0005
	Northern	0.22	0.07			1.06	0.47		

The differences always favored the larvae transplanted into the southern experimental environment with respect to their siblings living in their native site.

However, this trend was reverted for the variables related to body lengths at 24 days after beginning the experience (Table 4). Despite this behavior, the variables stage, weight, growth rate (Mann-Whitney, W=282.00, p=0.0005) and development rate (Mann-Whitney, W=555.00, p=0.0001) exhibited significant differences.

It is interesting to highlight the reverse behavior of the growth rate with respect to the previous record, given that the significant differences recorded denote acceleration in the aforementioned rate for the larvae living in the native environment with regards to their siblings. On the other hand, we noted that despite this behavior, the analysis of developmental stages proved that the larvae transplanted into the southern environment. An area of greater hydric stress compared to their native site, presented a significantly more advanced developmental state.
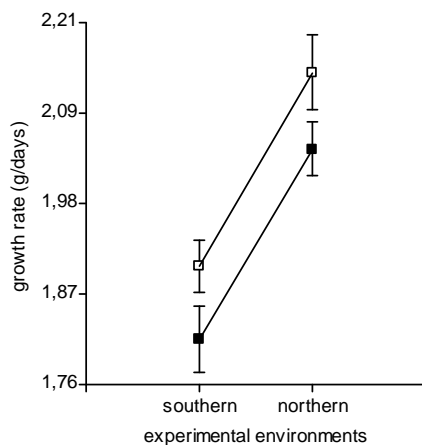

Figure 2. Norms of reaction of the population native to the southern (solid symbols) and northern (empty symbols) environments for the variable growth rate in both experimental environments.

Norms of reaction. In the larval stage, the factors environment and population exerted

significant variation in the growth rate (Kruskal-Wallis, H=28.58, p<0.0001). The environment significantly altered the growth rate, with the populations reared in the northern experimental environment presenting a higher growth rate than the populations reared in the southern environment (Fig. 2).
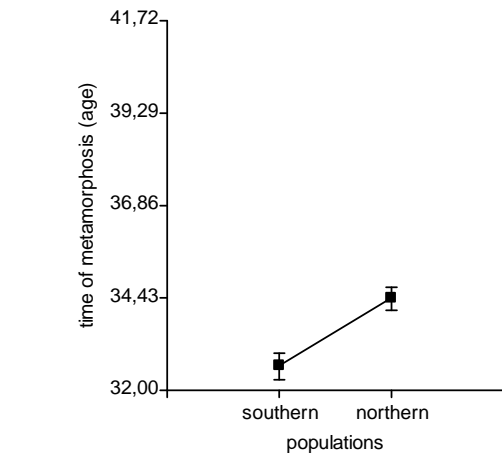
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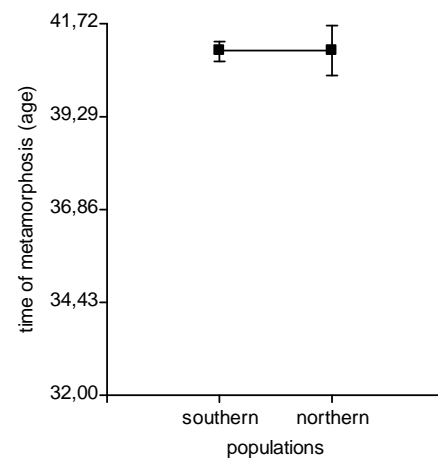
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Figure 3: Comparison the time of metamorphosis (age) between populations in each experimental environment for metamorphosed individuals. A. southern experimental environment. B. northern experimental environment.

Metamorphosed individuals

Metamorphosis time (age). The experimental environments imposed a different behavior on the metamorphosed individuals belonging to the

populations studied. Thus, the southern experimental environment significantly influenced the metamorphosis time for the populations studied, whereas the northern experimental environment did not influence the metamorphosis time (Table 5).

The developmental time of individuals transplanted into the southern environment was significantly greater than that of their co-specifics native to that environment. Nevertheless, in the northern environment, no differences were recorded between both populations, since a similar response was observed in both of them (Fig. 3).

When comparing the behavior of the metamorphosed individuals belonging to each population as a function of the rearing environment, we observed that those belonging to the southern population exhibited a significantly different response to the environment (Mann-Whitney, W=105, $p<0.0001$). Individuals in the transplanted environment reached metamorphosis after a longer time than their siblings in the native (southern) environment (Fig. 4). A similar differential behavior was observed among the individuals belonging to the northern population, which differed significantly according to the rearing environment (Mann-Whitney, W=338.5 $p<0.0001$). Thus, those individuals transplanted into the southern environment reached metamorphosis earlier than their siblings in the native (northern) site.

Size at metamorphosis. The experimental environment influenced the responses in size of individuals in each population studied. The differences were significant both in the northern and southern environments for the variables body length and weight (Table 6).

Also, significant differences indicated a greater size of the individuals belonging to the northern population, whether reared in the native

experimental environment or the transplanted one (southern environment).

The behavior of each population in relation to the rearing environment exhibited differential responses. The metamorphosed individuals from the southern populations exhibited a weight (Mann-Whitney, W=341.5, p=0.0007) and body length (Mann-Whitney, W=365.5 $p<0.0001$) that were significantly different depending on the rearing environment. The same behavior could be observed in the metamorphosed individuals belonging to the northern population, for which weight (Mann-Whitney, W=78, $p<0.0001$) as well as body length (Mann-Whitney, W=80.0, $p<0.0001$) were significantly different (Table 6).
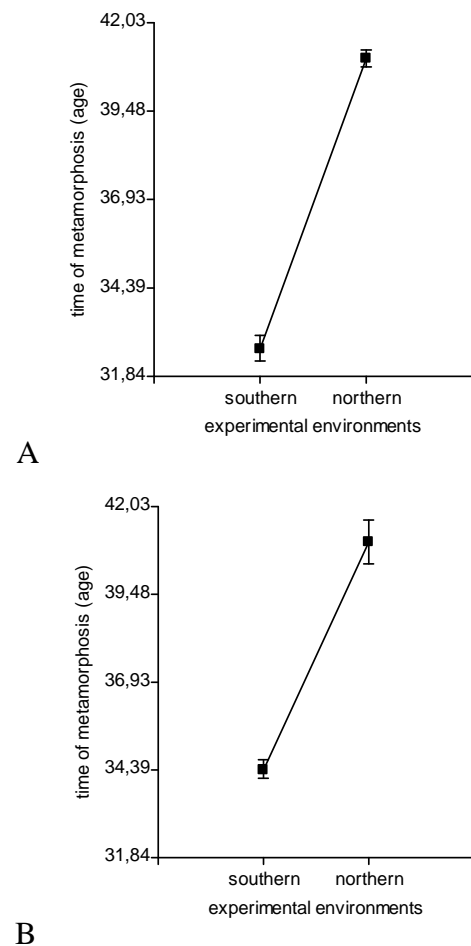

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Figure 4: Comparison the time of metamorphosis (age) between experimental environments for metamorphosed individuals belonging to each

population. A. southern population. B. northern population.

The behavior of both populations was similar with respect to the rearing environment, the southern environment evidencing a strong influence on the weight and body length reached. However, the northern population reached higher values in the transplanted environment than the southern population, which reached the highest values only in its native environment (Table 6).

Table 6. Results of Mann-Whitney test for metamorphosed individuals belonging to the two populations studied in both experimental environments, considering the variables 'weight' and 'body length'.

Variable	Experimental environments	Population	Mean	DS	*W*	*p*
Weight	Southern	Southern	0.18	0.04	105	<0.0001
		Northern	0.47	0.07		
	Northern	Southern	0.14	0.02	306	<0.0001
		Northern	0.20	0.03		
Body length	Southern	Southern	12.74	0.41	105	<0.0001
		Northern	16.18	0.86		
	Northern	Southern	11.33	0.75	313	<0.0001
		Northern	13.74	0.60		

The behavior of both populations was similar with respect to the rearing environment, the southern environment evidencing a strong influence on the weight and body length reached. However, the northern population reached higher values in the transplanted environment than the southern population, which reached the highest values only in its native environment (Table 6).

Growth rate. The type of environment influenced the growth rate of metamorphosed individuals belonging to the populations inhabiting each of them. Thus, significant differences were recorded both in the southern and the northern experimental environments (Table 7).

In each environment the response was significantly higher for metamorphosed individuals native to the northern population (Fig. 5), although the southern experimental environment imposed a faster pace in growth rate when compared to the northern environment.

Table 7. Results of Mann-Whitney test for metamorphosed individuals belonging to the two populations studied in both experimental environments, considering the variable 'growth rate'.

Variable	experimental environments	population	mean	DS	*W*	*p*
Growth rate	Southern	Southern	1.48	0.05	105.00	<0.0001
		Northern	1.74	0.04		
	Northern	Southern	1.33	0.04	309.00	<0.0001
		Northern	1.42	0.05		

The environment also influenced the responses of each population. The individuals from the southern population exhibited significant differences according to the environment in

which they were reared (Mann-Whitney, W = 385.00 p<0.0001), with a higher growth rate in their native site when compared to the transplant site (Fig. 5). Furthermore, the northern population behaved in the same manner, exhibiting significant differences (Mann-Whitney, W = 78.00 p = <0.0001), with higher values for the individuals in the transplant site (southern environment) (Fig. 6).
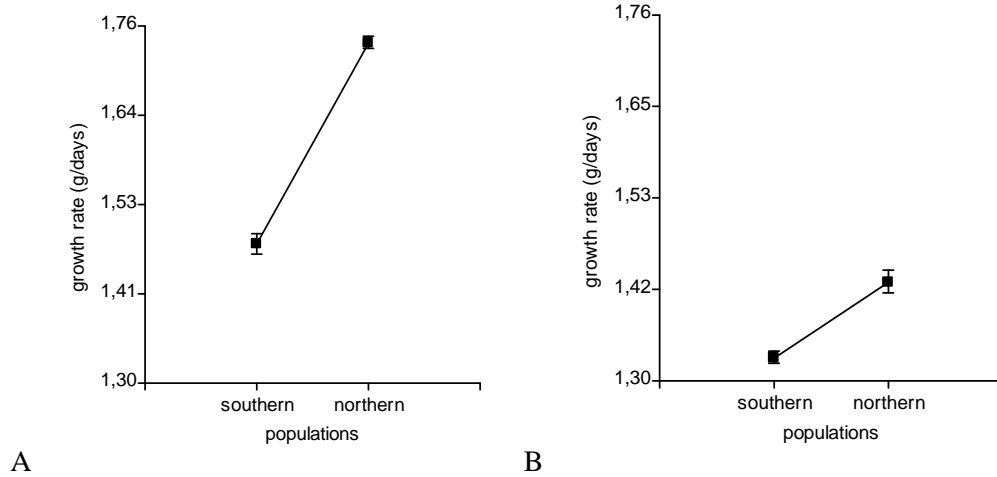

Figure 5. Graphic comparing the growth rate (g/days) between populations in each experimental environment for the metamorphosed individuals. A. southern experimental environment. B. northern experimental environment.
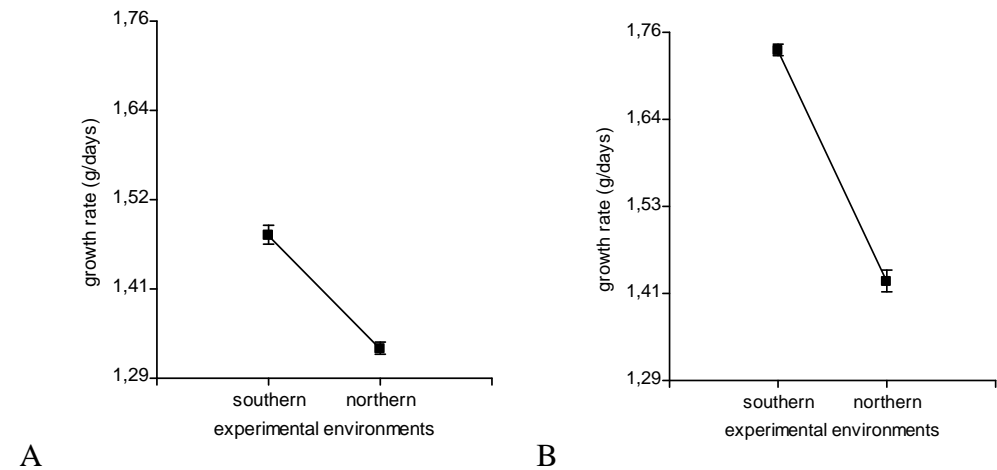

Figure 6: Graphic comparing the growth rate (g/days) for metamorphosed individuals belonging to each population and reared in different environments. A. southern population. B. northern population.

DISCUSSION

The variations that can be established in a phenotypic range can generally be explained either as a result of the influence of the environment, which prompts modifications during the development of the character through phenotypic plasticity, or of the local adaptation to the particular conditions of each environment (Berven *et al.* 1979, Marangoni 2006). In fact, according to Stearns (1992) the variations in life history traits that appear between different populations of the same species are usually interpreted as a group of multiple evolutionary adaptations to different environments.

In our reciprocal transplant experiments the factor 'environment' had significant effects on the variables studied, although the behavior was different depending on the experimental environment in question. In the southern experimental environment, the differences were significant only for the metamorphic stage, with the transplanted larvae showing a clearly more advanced stage although, as development progressed, differential responses could be observed for all the variables except weight; nonetheless. The trend favoring the transplanted larvae was maintained. This might be explained as a response to the type of environment, which is unstable and is likely to be desiccated (Denver *et al.* 1998, Newman 1992).

The northern experimental environment exerted a similar influence, given that significant differences were observed at the beginning of development, with a similar pattern to the one described above, where the transplanted larvae exhibited higher values when compared with their co-specifics native to the environment. However, as development progressed, the differences ceased to be significant, as an advance was detected in the larvae native to the environment. This behavior can be considered a plastic response to the stable environment into which they were transplanted (Newman 1898, 1992, Denver *et al*. 1998, Relyea and Werner 2000).

The effect of the population in function of the rearing environment significantly influenced the parameters studied. The larvae belonging to the southern population exhibited a differential behavior in relation to the rearing context, growing and developing at a faster pace in the transplanted site, although a deceleration in the growth rate could be observed as development progressed. Nevertheless, significant differences were recorded for the variable age at metamorphosis, since the transplanted individuals needed a longer development time than their siblings in the native site, so that an adjustment response to the more stable

environment (such as the transplant site) might be established. This response was reinforced by a higher growth rate for metamorphosed individuals in the native site, which represents a stressing and unstable environment. These aspects are consistent with a plastic response regulated by the environment (Marangoni and Tejedo 2008, Merila *et al.* 2000, Schlichting and Pigliucci 1998)

The larvae belonging to the northern population also exhibited a differential behavior, adjusted to the rearing environment, so that the larvae transplanted into the site of greater instability and hydric stress showed an advance in the developmental stage that was higher than that of their siblings reared in the native environment, and consequently reached metamorphosis earlier than their siblings in the native site.

The responses of the *Rhinella arenarum* populations studied at the rearing context (norms of reaction) evidenced that the genotype of the northern environment exhibited a higher growth rate than the genotype of the southern. Independently from the rearing environment. Thus, we may state that there might be a genetic basis explaining the differences between both populations regarding this trait (Marangoni 2006).

The age, size and growth rate of metamorphosed individuals evidenced the restrictions imposed by the environment, given that the experimental environments influenced these variables, a result similar to what Marangoni (2006) reported for *Bufo calamita*. Worthy of notice were the responses of the individuals to the rearing environment, which differed significantly for both populations. The responses denote phenotypic plasticity as an adaptation to the experimental environment (Newman 1989, 1992, Merila *et al.* 2000) The southern environment the more stressing, presenting an unstable hydroperiod, which can affect the development through changes in the

chemical and water temperature profiles, through greater interaction with other larvae, and through neuroendocrial action (Denver 1995, 1997, Denver *et al.* 1998). In this context, the individuals needed less days for development, with a higher growth rate, independently from the population of origin.

These results are in partial agreement with those of Marangoni & Tejedo (2008), who found differences in the size of metamorphosed individuals as a function of a latitudinal gradient for *Pelobates cultripes*.

Given the results we obtained, we conclude that the divergence in the phenotypic expressions of each genotype studied is a function of the rearing environment, thus evidencing an interaction between genotype and environment (population x environment) in the norms of reaction, and establishing differences between both populations.

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