

Consequences of genetic erosion on fitness and phenotypic plasticity in European tree frog populations (*Hyla arborea*)

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 inbreeding;
 heterosis.

Abstract

The detrimental effects of genetic erosion on small isolated populations are widely recognized contrary to their interactions with environmental changes. The ability of genotypes to plastically respond to variability is probably essential for the persistence of these populations. Genetic erosion impact may be exacerbated if inbreeding affects plastic responses or if their maintenance were at higher phenotypic costs. To understand the interplay 'genetic erosion–fitness–phenotypic plasticity', we experimentally compared, in different environments, the larval performances and plastic responses to predation of European tree frogs (*Hyla arborea*) from isolated and connected populations. Tadpoles from isolated populations were less performant, but the traits affected were environmental dependant. Heterosis observed in crosses between isolated populations allowed attributing their low fitness to inbreeding. Phenotypic plasticity can be maintained in the face of genetic erosion as inducible defences in response to predator were identical in all populations. However, the higher survival and developmental costs for isolated populations in harsh conditions may lead to an additional fitness loss for isolated populations.

Introduction

Populations characterized by reduced size or isolation resulting from habitat loss and fragmentation generally exhibit a reduced genetic diversity caused by restricted gene flow, genetic drift and inbreeding (Hedrick, 2001; Reed, 2005; Willi *et al.*, 2006). As a consequence, genetic erosion is assumed to decrease the mean fitness of populations (Reed & Frankham, 2003). Numerous studies have observed such a relationship (e.g. Bijlsma *et al.*, 2000; Hooftman *et al.*, 2003; Rowe & Beebee, 2003; Reed & Bryant, 2004; Reed, 2005; Willi *et al.*, 2007b). In these studies, mean fitness was generally assessed through mean survival, fecundity and growth rate in standard

conditions. Adaptive phenotypic plasticity, i.e. favoured by natural selection, has however rarely been considered.

Yet phenotypic plasticity may be of great importance in small isolated populations in which the evolutionary response is expected to be limited by a lowered additive genetic variance (Pertoldi *et al.*, 2007). In such a situation, the persistence of isolated populations may be based on the capacity of few genotypes to react to environmental changes in a plastic way (Sultan, 2000; Pigliucci, 2005; Pertoldi *et al.*, 2007; Schlichting, 2008). However, the effects of genetic erosion may interact with phenotypic plasticity patterns. A recent meta-analysis does not show ample evidence that plasticity is a costly trait in itself, i.e. that individuals pay a large price to carry the option to be plastic (plasticity costs; Van Buskirk & Steiner, 2009); therefore, there is no particular reason to expect that inbreeding and genetic erosion would decrease rather than increase plasticity in general. Yet plastic responses often involves a complex and costly shift (production costs defined by DeWitt *et al.*, 1998;

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Auld *et al.*, 2010; phenotypic costs defined by Callahan *et al.*, 2008) in the developmental trajectory that increases fitness in specific environmental conditions. As any fitness trait, such plasticity can be affected by deleterious mutations whose effect is amplified by inbreeding. The deleterious effects of inbreeding on the expression of phenotypes may be more visible under conditions that reveal more inter-individual variation. In addition, induced phenotypes may become more costly to produce because of the poor general condition of individuals caused by inbreeding. This is suggested by the observation that costs associated with expression of phenotypes seems to be greater or more easily detected under stressful conditions, i.e. when individual condition is lowered for environmental, rather than genetic reasons (Huber *et al.*, 2004; Teplitsky *et al.*, 2005a). Consequently, genetic erosion may impact the expression and the associated costs of plastic responses.

However, such a relationship between plasticity and genetic erosion has not yet been clearly confirmed. Indeed, whereas some studies suggest a reduction in plasticity in genetically eroded populations of plants and animals (Paschke *et al.*, 2003; Pluess & Stocklin, 2004; Auld & Relyea, 2010), Berg *et al.* (2005) did not observe any alteration of plastic response within small isolated populations of plant relative to connected ones. To our knowledge, no study has investigated the costs of inducible phenotypes in relation to the genetic variability of populations.

Anuran amphibians are useful models for the study of genetic erosion and its consequences on fitness and on phenotypic plasticity. Small isolated populations in many species are increasingly common mainly because of habitat fragmentation (Lesbarreres *et al.*, 2003; Cushman, 2006; Gallant *et al.*, 2007). Furthermore, larvae of many species of anurans have been shown to exhibit phenotypic plasticity expressed through different developmental components, adaptive morphology and behaviour (e.g. Van Buskirk, 2002; Laurila *et al.*, 2002; Merila *et al.*, 2004; Relyea, 2004). Inducible defences are adaptive plastic responses increasing survival that result from spatial and temporal variability in predation risk. Their expressions provide a higher survival but are associated with phenotypic costs (reduction in growth and developmental rates), depending on the degree and the temporal variability of predation risk (e.g. Van Buskirk, 2000; Van Buskirk *et al.*, 2002; Teplitsky *et al.*, 2005a,b; Capellan & Nacieza, 2007; Teplitsky & Laurila, 2007).

Here, we proposed to assess in small isolated populations of European tree frogs (*Hyla arborea*) the impact of genetic erosion on (i) the mean fitness of populations, (ii) the expression of inducible defences and (iii) the phenotypic costs of inducible defences. We experimentally compared the performances of *H. arborea* tadpoles and the expression of inducible defences in small isolated populations relative to large connected ones. We additionally used interpopulational crosses to test whether

performances may be restored when inbreeding was suppressed (i.e. to test for a heterotic effect upon outbreeding). We expected tadpoles from isolated populations to show reduced performances and heterosis upon outbreeding. To broaden the range of conditions under which we could detect the effects of genetic erosion on fitness and on inducible defences, we reared tadpoles in two contrasted environments: one was unfavourable because of harsh conditions (in our case, low oxygen concentrations and restricted diet) and the other one was favourable because of benign conditions (i.e. normoxia and *ad libitum* diet). Environmental stress has often been suspected to enhance deleterious effects of inbreeding although the evidence remains mixed (Keller & Waller, 2002; Armbruster & Reed, 2005; Szulkin & Sheldon, 2007; Willi *et al.*, 2007a; Waller *et al.*, 2008).

Material and methods

The European tree frog (*H. arborea*) was chosen for this study because some populations in Europe suffer from genetic erosion as a result of habitat fragmentation (Andersen *et al.*, 2004; Broquet *et al.*, 2010) and are disappearing in several regions of Europe (Fog, 1993). Moreover, tadpoles of this species exhibit a well-known phenotypic plasticity strategy in response to predation risk (i.e. increase in tail fin depth relative to body size; Lardner, 2000; Van Buskirk, 2000, 2002; Teplitsky *et al.*, 2003).

Study area and source populations

We used *H. arborea* populations located in two areas in the same region (i.e. Isère department, South-Eastern France). We surveyed these populations during two consecutive years (Broquet *et al.*, 2010). The first area is the Gresivaudan valley (40 km from Grenoble) and is characterized by high industrialization since the 1960s that has led to landscape fragmentation and isolation of amphibian populations. As a consequence, the two populations sampled in two isolated sites (Cheylas C and Laissaud L geographically distant by 10 km, 45°23'46"N, 5°59'41"E and 45°28'18.02"N, 6°3'51.02"E, respectively) are small (females = 30–50 and 15–25, respectively) and genetically impacted. Broquet *et al.* (2010) analysed these populations with 18 microsatellite loci. They demonstrated the negative consequences of disconnection on allelic richness and gene diversity and that the decrease in population effective size in association to disconnection acts as a genetic bottleneck. The second area (the Isle Cremieu, 40 km from Lyon, 70 km from the first area) is a large plateau (15 km²) where over two hundred ponds and marshes constitute potential amphibian breeding sites. In the two sampled sites (Planches P and Mépieu M geographically distant by 8 km, 45°44'20"N, 5°21'7"E and 45°44'3"N, 5°27'21"E, respectively), tree frog populations are large (> 200

females) and genetically variable relative to the isolated populations (Broquet *et al.*, 2010). In this study, we have contrasted the two small isolated populations against the two large connected ones without separating the effects of population size from those of isolation. Although this question deserves attention, our focus was more on the effects of genetic erosion, which is a common consequence of both small population size and isolation. Moreover, in a conservation perspective, fragmentation of habitats often results in simultaneous decreases in population size and connectivity.

Both isolated and connected areas presented similar elevation (between 250 and 350 m) and climatic conditions during 2007 and 2008 (mean T_{\min} = 6.1 and 8.3 °C, mean T_{\max} = 16 and 16.5 °C, mean precipitations = 74 and 72.3 mm, respectively, for isolated and connected areas; <http://www.meteofrance.com>). Ponds are all permanent, range in surface area from 25 to 80 m², with a bottom in gentle slopes and globally the same maximum depth (< 1.5 m). They are well vegetated and located in terrestrial habitat with a large forest cover. The terrestrial vegetation cover is however slightly more important in the area of connected populations. In terms of predation risk, all four of the sampled ponds had a high density of predatory insects for tadpoles (Odonata larvae) during the study period (Luquet *et al.*, personal observations).

Laboratory experiments

We compared the performances and the ability to express defences of *H. arborea* tadpoles according to their population status (isolated or connected) and the predation risk (with or without predator) in two contrasted environments (harsh and benign conditions). Because of the difficulty of sampling enough individuals in the small isolated populations, we had to conduct the experiments

in harsh and benign conditions over two different years. We are well aware that this is problematic, but such problems are inherent to the study of small populations. However, both years were climatically similar (mean T_{\min} = 7.3 and 7.2 °C, mean T_{\max} = 15 and 16.1 °C, mean precipitation = 69.3 and 77 mm, respectively, in 2007 and 2008; Météo-France data), and tadpoles were collected at similar times of the breeding cycle (from 15th April).

In 2007, we were also not able to obtain enough successful matings in the second pair of populations (L and M populations). We then reared only tadpoles from the C isolated and the P connected populations in harsh conditions to exacerbate the effect of genetic erosion on tadpole performances (Table 1).

In 2008, we reared tadpoles from both isolated (C and L) and both connected (M and P) populations in benign conditions (Table 1). We also set up experimental crosses between populations from the same status (noted inter-population crosses; Table 1). If inbreeding is responsible for fitness losses in small populations, fitness should increase under outbreeding. Because of the very small number of females (15–25) in the L population, we were not able to perform reciprocal crosses (i.e. L females × C males). To follow the same design in connected populations, we also performed asymmetrical interpopulation crosses (only M females × P males; Table 1).

During each year and from 15 April, males and females were caught at night, and random pairs were quickly formed in transparent tanks (17.5 × 33 × 18.5 cm) filled with pond water and vegetation to simulate a natural breeding zone. In each area, breeding tanks were placed close to one pond, and all crosses were performed at the same place (Table 1). When necessary, the animals were quickly transported by car after capture and placed in the tanks. We used each animal only once for reproduction.

Table 1 Description of the experimental design for each year. In 2008, we could not rear within a tank one tadpole from all clutches because each of the 16 partitioned tanks could only receive 48 tadpoles. Therefore, we randomly distributed in a first set of eight tanks, one tadpole from six clutches per population and interpopulation cross. The other sampled tadpoles (from the six others clutches per population and interpopulation cross) were introduced in a second set of eight tanks. In the L population, we used two tadpoles per clutch because only six clutches had been obtained.

Year	2007	2008
Isolated population	C	C and L
Connected population	P	P and M
Intrapop. cross (site of pair realization)	C × C (C site) P × P (P site)	C × C; L × L (C site) P × P; M × M (P site)
Interpop. cross (site of pair realization)	/	C female × L male (C site) M female × P male (P site)
No. clutches per cross	12 (total = 24)	12 (except L × L $n = 6$) (total = 66)
No. tadpoles per clutch	16 (total = 384)	8 (except L × L $n = 16$) (total = 576)
Tanks = replicates	4 tanks without <i>aeshna</i> 4 tanks with 6 <i>aeshna</i> (2 tadpoles per clutch per tank)	8 (4 × 2 set) tanks without <i>aeshna</i> 8 (4 × 2 set) tanks with 6 <i>aeshna</i> (1 tadpole per clutch per tank)
Rearing conditions	Harsh (hypoxia, restricted diet)	Benign (normoxia, <i>ad libitum</i> diet)
Day of measure	31	23

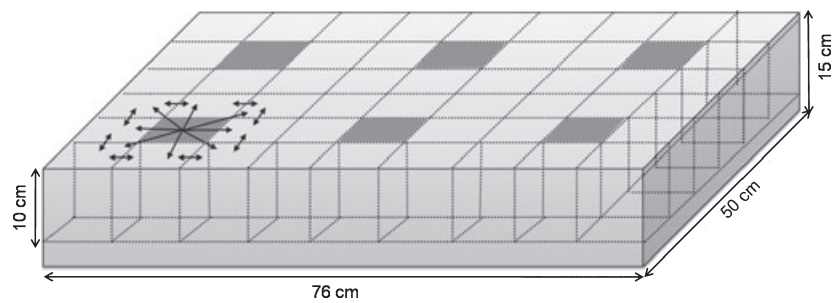


Fig. 1 Partitioned tanks used for individual rearing of tadpoles. The tanks were closed. All the compartments were permeable (wire netting of plastic), allowing free movement of water within the tank and a homogeneous diffusion of predator cues. Depending on the treatment, *aeshna* were (or were not) kept in the shaded compartments; O₂ diffusers were present in the same compartments only in the benign conditions.

Clutches were obtained within the first 24 h. After hatching, tadpoles from 12 pairs per type of cross were transported to the laboratory (Table 1). When tadpoles were 7 days old (stage 24–25, free-swimming stage, Gosner, 1960), they were individually reared in 55-L transparent plastic tanks with 54 permeable compartments (6 × 9 compartments per tank; Fig. 1). Six compartments were empty or contained one predator depending on the treatment. All the compartments were permeable (plastic wire netting), allowing free movement of water within the tank. Before the experiments, we added a drop of dye in each shaded compartment; this confirmed that water diffusion was rapid and relatively homogeneous in all the compartments. Thus, we assume a rapid and homogeneous diffusion of predator cues in the tank during the experiments. Given the position of predators within the tanks, all tadpoles were similarly exposed to predator cues (Fig. 1). We used dragonfly larvae (*Aeshna* sp.) as predators. The experimental treatment with six predators was approximately similar to the ponds with highest Aeshnidae densities in nature (14 m⁻²; Van Buskirk *et al.*, 2003). *Aeshna* larvae were fed with *H. arborea* tadpoles every 2 days as it is known that kairomone (chemical cue released by predators and hunted tadpoles) induces a full defensive response in tadpoles (Van Buskirk & Arioli, 2002). The tanks were randomly distributed in an experimental room that was maintained at 22.5 °C (± 0.8 °C) and with a 12:12 h light–dark cycle. Water in the tanks was renewed every 10 days until the measures of tadpole performances were performed (i.e. at 31 and 23 days in harsh and benign conditions), then three and two times, in harsh and benign conditions, respectively.

Harsh condition experiment

To create harsh conditions, we restricted the tadpole diet with 1 day of starvation every 2 days. The second day, tadpoles were fed with spinach and boiled lettuce *ad libitum*. Moreover, the tanks were covered to limit oxygen exchanges with the atmosphere, and the water was not oxygenated. Consequently, mean water oxygen concentration, checked every day (WTW Oxi 320

oxymeter, WTW, Weilheim, Germany), was low during the entire experiment (mean O₂ concentration = 3.3 ± 0.7 mg O₂ L⁻¹). Although this value can be within the range of oxygen concentrations recorded over nyctemeral cycles in natural ponds, a continuous value across the day and during the entire larval period is rarely observed and is necessarily stressful. This value seems to constitute a good compromise between stressful conditions and the survival of *H. arborea* tadpoles (Luquet *et al.*, personal observations).

On 25 May 2007, we randomly sampled 16 tadpoles in each of the 24 clutches (Table 1). We randomly distributed two tadpoles per clutch in each of the eight partitioned tanks. Four tanks were without and four tanks with predators (Table 1).

Benign condition experiment

In the benign conditions, the tadpoles were fed daily *ad libitum* with spinach and boiled lettuce. The tanks were also covered but with six O₂ diffusers in each tank (Fig. 1), mean water oxygen concentration was normoxic during the entire experiment (mean O₂ concentration = 7.4 ± 0.4 mg O₂ L⁻¹).

On 20 May 2008, we randomly sampled eight tadpoles in each of the 60 clutches from C, P and M intrapopulation crosses and from both interpopulation crosses (Table 1). As in the L isolated population, we were able to obtain only six clutches, we sampled 16 tadpoles per clutch. We could not rear one tadpole from all clutches because each of the 16 partitioned tanks could only receive 48 tadpoles (Fig. 1). We then randomly distributed in a first set of eight tanks one tadpole from six clutches per intra- and interpopulation cross. The other sampled tadpoles (from the six others clutches per intra- and interpopulation cross) were introduced into a second set of eight tanks. In the L population, we used two tadpoles per clutch. Eight tanks (four per set) were without *Aeshna* and eight others (four per set) with predators (Table 1).

Plasticity and larval performance measures

Aeshnidae predators are known to cause principally an adaptive change in tail fin depth in many larval anuran

species (e.g. Van Buskirk & McCollum, 2000; Van Buskirk *et al.*, 2003; Teplitsky *et al.*, 2004, 2005b). In this respect, *H. arborea* is the most sensitive species (see Van Buskirk, 2002). Moreover, on the first year (harsh conditions experiment), we measured five morphological traits known to exhibit predator-induced plasticity: tail length, tail fin depth, tail muscle depth, body length and body depth. Tail fin depth was the trait the most modified by the presence of *Aeshna*. Consequently, we have only presented the results referring to this trait (see results for other traits in Table S5 and Fig. S1) and only investigated this trait in the second experiment (benign conditions). The maximum tail fin depth was measured (to the nearest 0.1 mm) using a stereoscopic microscope equipped with an ocular micrometre to assess tadpole investment in inducible defences. As morphological changes in response to predation are usually measured within 20–40 days and approximately at Gosner stage 30–37 (Van Buskirk, 2002), we performed tadpoles measures at 31 and 23 days in harsh and benign conditions, respectively, stages that correspond approximately to the mid-development for *H. arborea*. We also estimated the proportion of surviving tadpoles and measured the growth and developmental performances of all tadpoles (wet body mass to the nearest 0.1 mg and total length to the nearest 0.1 mm; stage according to Gosner, 1960). Results for total length are not presented because of their high redundancy with body mass.

Statistical analyses

The tadpole performances were analysed using mixed-effects models (Pinheiro & Bates, 2000). We performed both analyses of covariances (ANCOVAs) and analyses of variances (ANOVAs) with type III mean squares, using expected means squares method. This method tested significance of effects in a conservative way.

Inducible defences were analysed with tail fin depth as the dependent variable and body mass as the control variable. Tail fin depth and covariate body mass were log transformed to linearize the relationship (Teplitsky & Laurila, 2007).

Larval performances of tadpoles (survival, developmental stage and body mass measured on 31 days in harsh and 23 days in benign conditions) were first analysed with ANOVAs. Because all tadpoles survived in benign conditions, we did not perform an analysis on this survival measure. Secondly, to compare the relative investment within body mass relative to the developmental stage of tadpoles, we introduced body mass as the dependent variable and stage as the control variable.

The results of the two experiments were analysed separately because they were not performed the same year. The tadpole performances in the harsh conditions (except survival) were analysed with predation treatment as the main fixed effect; tank within predation treatment, population and the interaction with predation

treatment were random effects. Tadpole survival was analysed with a generalized linear model using the logit link and a binomial error distribution. This model included however only fixed effects. In the benign conditions experiment, we performed two analyses. First, the differences in the performances of tadpoles from intrapopulation crosses were analysed in the same way as in harsh conditions (analysis detailed earlier). Secondly, to test whether differences in performances following population status are because of inbreeding, we compared the performances of tadpoles from intra- (C and M) and interpopulational (C female \times L male and M female \times P male) crosses. Predation treatment, male population (same or different from the female) and the interaction between these factors were the main fixed effect; tank within predation treatment, female populations, interaction between female, male populations and predation treatment were random effects.

In all the analyses, the nonsignificant interactions were eliminated from the model to obtain the final model. For all the analyses, conditions of variance homogeneity were checked. JMP 8.0 (SAS Institute, Cary, NC, USA) was used for all the statistical analyses.

Results

Effect of genetic erosion on mean population fitness

In benign conditions, tadpoles from crosses within isolated populations (C and L) showed lower performances than those from connected populations (M and P) whatever the predation treatment (Fig. 2, Table 2A). The tadpoles from isolated populations were always less developed and lighter than tadpoles from connected populations in $C < L < P < M$ order (isolated vs. connected contrasts: $t_{332} = -4.72$, $P < 0.001$ and $t_{332} = -4.61$, $P < 0.001$ for stage and body mass, respectively). These significant differences in body mass are explained by differences in the developmental rate of tadpoles (no significant population effect after taking developmental stage into account; Table 3).

Tadpoles from interpopulational crosses showed significantly different developmental stages depending on the female populations in both predation treatments (significant female pop. \times male pop. interaction, Fig. 2, Table 4A). Tadpoles from crosses between isolated populations showed significantly higher developmental stages than those from intrapopulational crosses ($t_{363} = 4.24$, $P < 0.001$). These differences were not observed for tadpoles from crosses between connected populations ($t_{363} = 1.11$, $P = 0.267$). The body mass of tadpoles from interpopulational crosses was systematically higher than those from intrapopulational crosses in both isolated and connected populations (Fig. 2, Table 4A). These differences in performances were caused by a higher developmental rate of tadpoles (no significant male effect; Table 4B).

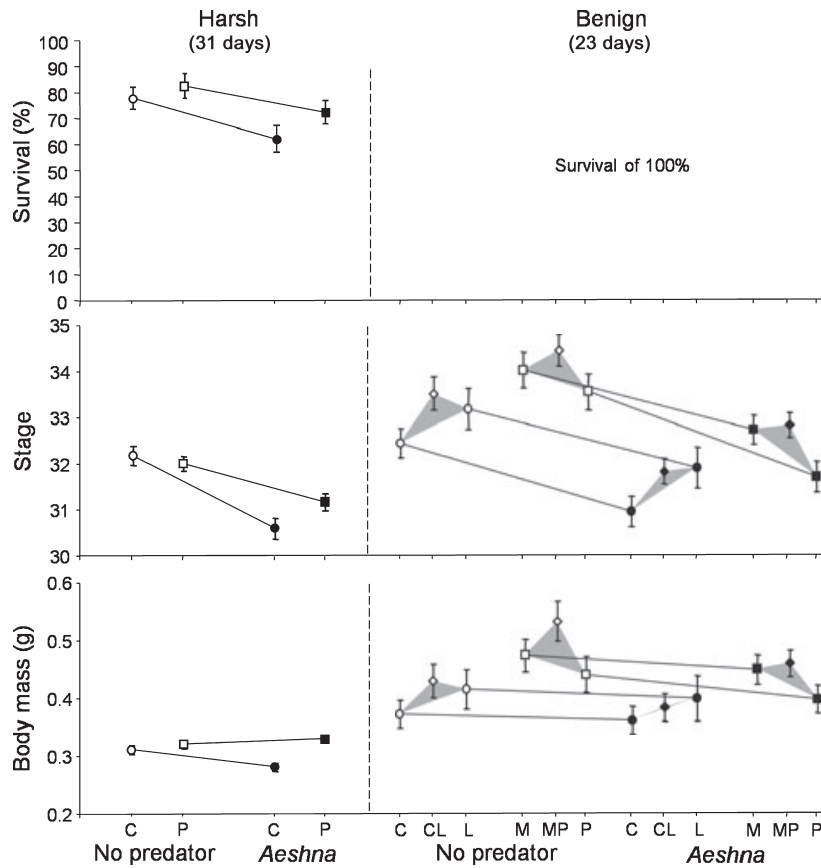


Fig. 2 Survival, developmental stage according to Gosner (1960) and body mass of predator-naïve and predator-induced tadpoles from isolated populations (C and L, circle symbol), connected populations (M and P, square symbol) in harsh and benign environmental conditions and interpopulational crosses (CL and MP, diamond symbol) in benign conditions. For each interpopulational cross, maternal populations are on the left and paternal on the right. Lines represent the reaction norms. Shaded triangles point upwards in case of heterosis. Values are means \pm SE.

Table 2 Mixed-model analyses of variance for developmental performances of tadpoles from isolated and connected populations (intrapopulational crosses) in (A) benign and (B) harsh conditions. The random effects are shown in *italic* (excepted for survival analysis, all effects are fixed). The nonsignificant interactions were all excluded from the final models. Estimates and standard errors are presented in appendix (Table S1).

Source	Stage			Body mass					
	n.d.f., d.d.f.	<i>F</i>	<i>P</i>	n.d.f., d.d.f.	<i>F</i>	<i>P</i>			
(A)									
Predation	1, 14	2.818	0.115	1, 14	0.133	0.721			
<i>Population</i>	3, 332	15.140	< 0.001	3, 332	11.576	< 0.001			
<i>Tank [predation]</i>	14, 332	22.873	< 0.001	14, 332	37.940	< 0.001			
Source	Survival			Stage		Body mass			
	d.f.	χ^2	<i>P</i>	n.d.f., d.d.f.	<i>F</i>	<i>P</i>	n.d.f., d.d.f.	<i>F</i>	<i>P</i>
(B)									
Predation	1	12.320	< 0.001	1, 3	6.440	0.094	1, 2	0.207	0.706
<i>Population</i>	1	4.210	0.04	1, 1	0.355	0.658	1, 1	2.633	0.352
<i>Predation \times population</i>				1, 277	3.433	0.065	1, 277	6.562	0.011
<i>Tank [predation]</i>	6	11.520	0.074	6, 277	3.789	0.001	6, 277	2.611	0.018

Table 3 Mixed-model analyses of covariance for body mass of tadpoles from isolated and connected populations (intrapopulation crosses) in harsh and benign conditions. The random effects are shown in italic. The nonsignificant interactions were all excluded from the final models. Estimates and standard errors are presented in appendix (Table S2).

Body mass Source	Harsh			Benign		
	n.d.f., d.d.f.	<i>F</i>	<i>P</i>	n.d.f., d.d.f.	<i>F</i>	<i>P</i>
Stage	1, 275	197.258	< 0.001	1, 331	628.680	< 0.001
Predation	1, 1	3.106	0.378	1, 14	3.771	0.072
Stage × predation	1, 275	11.146	0.001			
<i>Population</i>	1, 1	5.794	0.251	3, 331	0.620	0.602
<i>Predation × population</i>	1, 275	4.210	0.041			
<i>Tank [predation]</i>	6, 275	0.357	0.905	14, 331	13.241	< 0.001

Table 4 Mixed-model analyses of (A) variance and (B) covariance for developmental performances of tadpoles from intra- and interpopulation crosses in benign conditions. The random effects are shown in italic. The nonsignificant interactions were all excluded from the final models. Estimates and standard errors are presented in appendix (Table S3).

Source	Stage			Body mass		
	n.d.f., d.d.f.	<i>F</i>	<i>P</i>	n.d.f., d.d.f.	<i>F</i>	<i>P</i>
(A)						
Predation	1, 14	3.372	0.0876	1, 14	0.204	0.658
Male population	1, 1	2.908	0.338	1, 364	8.349	0.004
<i>Female population</i>	1, 1	13.271	0.171	1, 364	63.262	< 0.001
<i>Female population × male population</i>	1, 363	4.934	0.027			
<i>Tank [predation]</i>	14, 363	25.238	< 0.001	14, 364	44.775	< 0.001
(B)						
Source	n.d.f., d.d.f.	Body mass		<i>F</i>	<i>P</i>	
Stage	1, 363			488.405	< 0.001	
Predation	1, 14			2.371	0.1453	
Male population	1, 363			0.004	0.9466	
<i>Female population</i>	1, 363			6.852	0.009	
<i>Tank [predation]</i>	14, 363			14.400	< 0.001	

In harsh conditions, tadpoles from isolated populations had a lower survival than those from connected population although developmental and growth performances were similar in the absence of predator (Fig. 2, Table 2B).

Effects of genetic erosion on phenotypic plasticity

All predator-exposed tadpoles developed relatively deeper tails than predator-naïve tadpoles, whatever the populations and the environmental conditions (nonsignificant population × predation interaction in all conditions, Fig. 3, Table 5). Similarly, interpopulation crosses did not affect tail fin depth (Table 5).

In benign conditions, all tadpoles survived until the measurements of performances (23 days). Predators seem to cause a slower developmental rate (Fig. 2). However, because the random tank effect was high, the main effect of predation was not or marginally significant for this trait (Tables 2A and 4A). Moreover, in the benign

conditions (*ad libitum* diet particularly), tadpole body mass was not affected by the presence of predators (Fig. 2, Tables 2A and 4A).

In harsh conditions, all predator-exposed tadpoles exhibited in contrast a lower survival and a delayed developmental rate (Fig. 2, Table 2B). However, exposure to predators clearly altered more strongly the development and growth of tadpoles from the isolated population in these conditions (significant predation × population interaction, Fig. 2, Table 2B). Predator-exposed tadpoles from the isolated population had a lower developmental stage (marginally significant) and body mass than those from connected population. The same pattern exists for survival but is not significant (Fig. 2, Table 2B). Moreover, despite correction on developmental stage, in the predation treatment, tadpoles from isolated population had a significantly lower body mass than those from connected populations (significant predation × population interaction, Table 3).

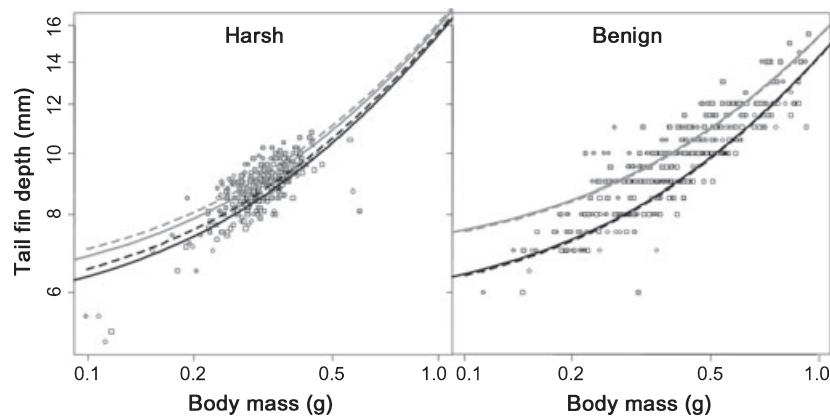


Fig. 3 Tail fin depth responses (corrected on body mass; log-log scale) of predator-induced (grey) and predator-naïve (black) tadpoles from isolated (dashed line) and connected (solid line) populations in harsh and benign conditions. Open and filled symbols show the naïve and the induced tadpoles, respectively. For the sake of clarity, in benign conditions both populations within population status are not distinguished and interpopulational crosses are not represented.

Table 5 Mixed-model analyses of covariance for log (tail fin depth) in harsh and benign conditions. The first analysis (intrapopulational crosses) compared tadpoles from isolated and connected populations. The second analysis compared tadpoles from intra- and interpopulational crosses to determine a heterotic effect. The random effects are shown in *italic*. The nonsignificant interactions were all excluded from the final models. Estimates and standard errors are presented in appendix (Table S4).

Log (tail fin depth)	Source	Harsh			Benign		
		n.d.f., d.d.f.	<i>F</i>	<i>P</i>	n.d.f., d.d.f.	<i>F</i>	<i>P</i>
Intrapop	Log (body mass)	1, 277	639.097	< 0.001	1, 331	1124.864	< 0.001
	Predation	1, 6	53.080	< 0.001	1, 14	116.725	< 0.001
	<i>Population</i>	1, 277	14.419	< 0.001	3, 331	0.778	0.507
	<i>Tank [predation]</i>	6, 277	1.363	0.229	6, 277	2.454	0.003
Interpop	Log (body mass)				1, 363	586.056	< 0.001
	Predation				1, 14	146.046	< 0.001
	Male population				1, 363	0.231	0.631
	<i>Female population</i>				1, 363	1.881	0.171
	<i>Tank [predation]</i>				14, 363	1.785	0.039

Discussion

We investigated the impact of genetic erosion on developmental performances and more specifically on phenotypic plastic responses and provide the first evidence that the expression of phenotypic plasticity can be maintained in populations submitted to genetic erosion but at the expense of higher costs (reduced survival, lower developmental and growth rates). We have also confirmed an effect of genetic erosion on fitness in reducing tadpole performances but with variable intensity depending on environmental conditions. These two points will be discussed.

Effect of genetic erosion on mean population fitness

As we expected, tadpoles from isolated populations performed significantly worse than those from connected

populations. Even under benign conditions (normoxia, *ad libitum* diet) and in the absence of predators, tadpoles from isolated populations exhibited a slower development leading to a reduction in body mass. These modifications have potentially important consequences on individual fitness because these individuals are expected to metamorphose later and/or with higher mortality (Wilbur, 1984). Late metamorphosis may be highly detrimental to future fitness through its impact on adult survival and reproduction success (Altwegg & Reyer, 2003).

Two causes could explain this pattern. Delayed development and reduced growth rate could be consequences of small population size and genetic erosion. Theory predicts that genetic erosion in small isolated populations reduces mean fitness through accumulated inbreeding and genetic load, increasing the risk of population extinction (Hedrick, 2001; Frankham *et al.*, 2002; Keller

& Waller, 2002; Reed, 2005). Alternatively, differences in the population environments may lead, under a scenario of local adaptation, to quick divergence in some variables related to ecological performances because of micro-evolutionary events (see Beebee, 2005 for review; Johansson *et al.*, 2007; Richter-Boix *et al.*, 2010). Indeed, although all the ponds are in the same region with homogeneous climatic conditions and are ecologically similar in terms of hydroperiod, vegetation and the presence of predators (Luquet *et al.*, personal observation), we could not totally exclude that populations experience different environmental conditions. Moreover, variation in the availability of resources with worse terrestrial and pond conditions for isolated populations could decrease female physiological condition and ability to invest in their progeny, leading to reduced individual performances in offspring.

Our results on interpopulational crosses support the idea that the low performances of tadpoles from isolated populations are largely a consequence of genetic load and not of maternal effects. In the two isolated populations, the maternal population was not the best parental population (lower performances in intrapopulational crosses than the paternal populations). Consequently, maternal effects would tend to generate negative, rather than positive heterosis. Yet, offspring from crosses between isolated populations were phenotypically superior not only to the midparent values, but also to the best parental population (the paternal population, L). This provides evidence that heterosis is manifest in these interpopulational crosses and that inbreeding exists in our isolated populations, contributing to reduce performances for some larval traits. These results are consistent with a majority of studies that detect a positive relationship between genetic diversity and mean fitness across populations (e.g. Reed & Frankham, 2003 and references cited therein; Rowe & Beebee, 2003).

Although our experimental results suggest that genetic erosion in our isolated populations may lead to a reduced fitness in favourable environmental conditions, surprisingly, developmental and growth performances were similar in isolated and connected populations in harsh conditions (C and P). However and consistently with our expectations, we found that tadpoles from isolated population (C) had a lower survival in these conditions. Halverson *et al.* (2006) observed a similar effect of inbreeding on the performances of wood frog (*Rana sylvatica*) larvae in the field. The authors showed a significant negative relationship between inbreeding and survival in the wild, but there was no evidence that inbreeding influenced growth or development. Exposition to such severe stress (i.e. restricted diet and permanent low oxygen concentration in our case or wild conditions) could lead individuals to mobilize resources to survive and minimize allocation to development and growth, concealing the effects of inbreeding on these two performances (Kalinowski & Hedrick, 1999). Finally,

these contrasted results confirm that environmental stress may increase the effect of inbreeding on fitness (Armbruster & Reed, 2005), but that such effects, which are not consistently observed, should be not generalized (Keller & Waller, 2002; Szulkin & Sheldon, 2007; Willi *et al.*, 2007a; Waller *et al.*, 2008).

Effects of genetic erosion on phenotypic plasticity

We expected genetic erosion to alter phenotypic plasticity patterns by (i) a reduced expression of inducible defences; (ii) increased phenotypic costs in some fitness-related traits. Our results showed the expression of inducible defences by tadpoles from isolated populations has been maintained to a similar level than in connected populations despite a slower development. *Hyla arborea* tadpoles from isolated populations increased their tail fin depth in the presence of predator just as tadpoles from large connected populations did, regardless of the environmental conditions in the experiments. Similarly, interpopulational crosses did not change plastic response of tadpoles, whereas they did increase basic performances in isolated populations. Thus, induced defences do not seem to be affected by recessive deleterious mutations as are fitness traits. There are several possible explanations to this result. First, predation may be such a strong selective force acting against the drift effect in isolated populations that anti-predator defences are maintained (Lardner, 2000).

Indeed, in environments where predation risk is variable and unpredictable, inducible defences are crucial for the survival of anuran tadpoles (Van Buskirk *et al.*, 2003; Teplitsky *et al.*, 2005c). Second, the genetic architecture of plasticity may differ from that of basic fitness traits. The main origin of inbreeding load is directional dominance variance caused by detrimental, partially recessive mutations (Charlesworth & Charlesworth, 1999). These mutations tend to decrease growth, developmental rates and physiological performances (Houle *et al.*, 1996; Houle, 1998), but the number of genes involved in these traits is probably higher than the number of genes involved in inducible defences. Consequently, the probability of having some genes involved in plasticity with deleterious mutation increase with the number of deleterious mutations in the genome and then with the degree of inbreeding. This may be supported by Auld & Relyea (2010) study that compared inducible defences of a hermaphroditic snails *Physa acuta* in the presence and absence of chemical cues from predatory crayfish. The highly inbred snails from self-crosses exhibited reduced inducible defences relative to out-crossed lineages. In our isolated populations, inbreeding may be not strong enough to lead to deleterious mutations in genetic architecture of plasticity.

In benign conditions, the expression of inducible defences lead to phenotypic costs for all tadpoles in terms of growth and developmental rates, as consistently

observed in the literature (McCollum & Leimberger, 1997; Van Buskirk *et al.*, 1997; Van Buskirk & Relyea, 1998; Relyea & Werner, 2000; Teplitsky *et al.*, 2005c). These costs were similar for tadpoles from isolated and connected populations.

Our results obtained in harsh environmental conditions show, however, that when they are physiologically challenged to cope with hypoxic conditions and starvation, tadpoles from an isolated population pay a higher fitness cost for inducible defences than do tadpoles from a connected population. All tadpoles allocated resources in inducible defences, but in tadpoles from the isolated population this allocation was at the expense of survival, growth and developmental rates. We can reasonably expect that these higher costs of the induced phenotypes paid during the larval stage will extend to the metamorphosis and ultimately result in a substantial reduction in total fitness. Although we do not have performances at metamorphosis because of very high mortality (> 80%) because of harsh conditions, we still observed the same patterns at forelimbs emergence (Gosner stage 42) with a trend for reduced survival and a longer larval period for predator-induced tadpoles from isolated populations (Table S6; Fig. S2). These results support the hypothesis that inbreeding, although it does not compromise inducible defences themselves, decreases the overall energy budget of developing tadpoles in such a way that the allocation of resources towards defence comes at a higher cost. This cost remains unexpressed when food and oxygen are abundant enough, but becomes important when total metabolic fluxes are limited by starvation and low oxygen concentrations.

Conclusions

Our study confirmed that genetic erosion and inbreeding in small isolated populations have a negative effect on mean fitness in natural amphibian populations. Second, phenotypic plasticity patterns (i.e. inducible defences) are maintained in the face of genetic erosion, even in adverse conditions, while most basic fitness traits decrease; yet, when facing several physiological challenges at the same time (i.e. predator cues and metabolic stress), tadpoles from a small isolated population pay a higher fitness cost for their inducible defences. This may lead to an important additional loss of fitness in small fragmented populations. Consequently, as phenotypic plasticity represents a central component of adaptation for many species, it deserves more empirical attention in the framework of genetic erosion.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Tail and body morphological responses (corrected on body mass; log-log scale) of predator-induced (grey) and predator-naïve (black) tadpoles from isolated (dashed line) and connected (solid line) populations in harsh conditions.

Figure S2 Survival and larval period at Gosner stage 42 (emergence of forelimbs) of predator-naïve and predator-induced from the C isolated population (circle symbol) and P connected population (square symbol) in harsh environmental conditions.

Table S1 Estimates and standard errors of mixed-model analyses of variance for developmental performances of tadpoles from isolated and connected populations (intra-population crosses) in (A) benign and (B) harsh conditions (Table 2).

Table S2 Estimates and standard errors of mixed-model analyses of covariance for body mass of tadpoles from isolated and connected populations (intrapopulation crosses) in harsh and benign conditions (Table 3).

Table S3 Estimates and standard errors of mixed-model analyses of (A) variance and (B) covariance for developmental performances of tadpoles from intra- and inter-population crosses in benign conditions (Table 4).

Table S4 Estimates and standard errors of mixed-model analyses of covariance for log (tail fin depth) in harsh and benign conditions (Table 5).

Table S5 Mixed-model analyses of covariance for log (tail length), log (tail muscle depth), log (body length) and log (body depth) in harsh conditions.

Table S6 Mixed-model analyses of variance for performances of froglets from isolated and connected populations at Gosner stage 42 in harsh conditions.

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