

## The effects of isolation on fitness and morphometric: *Rana latastei* within the Monza Park

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**Abstract.** We investigated whether long term isolation in a population of *R. latastei* caused inbreeding depression and / or divergence in morphometry if compared with other conspecific populations. We observed reduced embryo survival of the isolated population, suggesting strong inbreeding depression. Moreover, males from this population were significantly larger than those from all other populations. We did not observe a clear morphometric pattern for metamorphs grown under laboratory conditions. Overall, our results suggest that isolation may have strong effects on populations: inbreeding depression is an obvious consequence, but also local adaptation is possible and should be kept into account.

**Keywords.** body size, contemporary evolution, embryo survival, habitat fragmentation, local adaptation, long term isolation.

### INTRODUCTION

Isolation is a major force driving the evolution of populations. Small, isolated populations face the risk of loss of genetic diversity, because of the genetic drift and the increased homozygosity caused by inbreeding. In turn, the loss of genetic diversity can cause a reduced fitness of individuals, because of the so called “inbreeding depression” (Keller & Waller, 2002). For example, in Southern Britain the presence of barriers such as fences and routes caused the isolation of urban populations of *Bufo bufo*: urban populations have higher inbreeding coefficient of individuals, and reduced larval survival, if compared with countryside populations (Hitchlings & Beebee, 1998). However, isolation can also have positive effects on the evolution of populations. It is well known that insular populations can quickly evolve and adapt to the changing environment: isolated populations can evolve also in a few generations, if the populations retain enough genetic variability and the

selection is strong enough to overcome the effects of drift (Keller & Waller, 2002; Stockwell et al., 2003). The expansion of land use subsequent to human population growth results in the fragmentation of natural landscapes (Wilcox & Murphy, 1985). Therefore, in human dominated landscapes the residual patches of natural habitats are frequently small and isolated within an anthropogenic matrix. If the permeability of the matrix is low, the populations living in the patches become isolated, and isolation disrupts the metapopulation systems (Hanski & Gilpin, 1997; Ficetola & De Bernardi, 2004). Thus, there is an increasing concern about the fate of isolated populations, because of the risk of inbreeding depression and stochastic extinctions. Amphibians are vertebrates with low mobility, therefore they are expected to be strongly affected by isolation: the evaluation of the long term effects of isolation on populations can be important for their management. However, the documented cases of long term isolation caused by human modifications of landscapes are scarce. Here we investigate the effects of long term isolation on the populations of *Rana latastei* living in the Monza Park. In 1805, this park has been completely surrounded by a 3m-tall wall. This wall is insurmountable by terrestrial amphibians, thus we can suppose that the *R. latastei* populations living in this area are completely isolated since 200 years. Our population survived under long term isolation, if compared with other populations isolated by human activities (e.g., Hitchlings & Beebee, 1998). We measured some fitness related and morphological traits and we compared them with those of neighbouring *R. latastei* populations. We measured: (1) larval survival, to assess if inbreeding depression causes increased mortality; (2) adult body size in nature and (3) morphometry of metamorphs grown under laboratory conditions, to evaluate whether isolation causes morphological divergences due to local adaptation.

## STUDY AREA AND METHODS

The Monza Park (Western Lombardy, Northern Italy) has a surface area of 740 ha; the wooded area is 400 ha. About 30 wetlands are present in the park, in one of them a *R. latastei* population breeds (thereafter, the population MZ). In the last 5 years, 40-200 *R. latastei* egg masses has been recorded each spring in the park. We performed three independent studies to compare the performances of *R. latastei* population living within the park with those of populations living outside.

(1) We compared the embryo survival of population MZ with those of five populations living along the Ticino and Adda rivers. Both rivers are in western Lombardy, and still retain nearly continuous riparian areas, allowing the persistence of *R. latastei* populations within metapopulations (Ficetola & De Bernardi, 2004). The five sampled populations are: A1 (Adda River, Lodi); A2 (Adda River, Zelo Buon Persico, LO); T1 (Zerbolò, PV); T2 (Beregardo, PV); T3 (Cassolnovo, PV). For each population, we collected 26-32 small portions of clutches, each portion including 15-50 eggs. All eggs were reared in the same environment with outdoor temperature; for each clutch we measured the percentage of hatched eggs and the percentage of embryos surviving from hatching to the free swimming stage (stage 25: Gosner, 1960).

(2) In spring 2000 and 2001, we caught by hand 83 *R. latastei* males and 13 females in the population MZ. For each frog, we measured snout-vent length (SVL) using a calliper; individuals were temporarily marked using plastic labels fixed around the flanks by way of an elastic band, to avoid re-measuring the same frog. Data from frogs of Monza were compared with those in the literature for populations of Lombardy (Pozzi, 1980; Bernini et al., 2000; Gruppo GEV, 2004) and Piedmont (Boano & Sindaco, 1992).

(3) To evaluate morphological differences among populations excluding environmental effects, we reared tadpoles from MZ and from four geographically close populations (T2, A2 and CU, Curone Park, LC; AL, Alserio Lake, CO). Populations T2 and A2 are located south of MZ, AL and CU are northward. No population is more than 50 km far from MZ. From each population, we reared under the same laboratory conditions (20°C, 12:12 h photoperiod) 10 tadpoles from each of five recently laid clutches. For the population CU, we reared tadpoles from only 3 clutches, because of the low fertility of some clutch: overall, we reared 230 tadpoles from 23 clutches. All tadpoles of the same clutch were reared in the same plastic container and fed ad libitum using rabbit chow and lettuce. At metamorphosis, froglets were weighed and photographed; we measured tibiofibula length and head width on the basis of pictures; we also calculated the proportion tibiofibula length / head width, as a measure of the relative tibiofibula length.

## RESULTS

Average hatch percentage ( $\pm$  SE) of the population MZ was  $53 \pm 5.4\%$ ; in all the populations along the rivers Adda and Ticino average hatch percentage was 79% or more (Fig. 1). We observed significant differences in hatching rate between populations (ANOVA on square root-arcsine transformed data:  $F_{5,191} = 20.353$ ,  $P < 0.0001$ ); hatch percentage of MZ was significantly lower than those of all the other populations (Tukey's post hoc: all  $P < 0.0001$ ). In the population MZ  $67 \pm 7.5\%$  of the hatched populations survived until the free swimming stage; in the other populations, average survival from hatch to swimming stage was 89% or more. We observed significant differences in survival rate between populations ( $F_{5,188} = 4.609$ ,  $P = 0.0005$ ); survival percentage of MZ was significantly lower than those of all the other populations (Tukey's test: all  $P < 0.026$ ).

Average ( $\pm$  SE) SVL length of adult frogs from MZ was  $49.7 \pm 0.44$  mm for males and  $57.1 \pm 2.0$  mm for females (Table I). Average SVL of males from MZ was significantly higher than average SVL of all the other populations (Z test:  $P < 0.0001$ ); females of MZ were larger than those of other populations, but differences were not significant (Z test:  $P = 0.147$ ). Males were larger than those of other populations also for the minimum value of SVL (in all other populations, minimum  $SVL \leq 37$  mm: Table I).

At metamorphosis, we did not observe differences among froglets from different populations in body weight ( $F_{4,18} = 0.645$ ,  $P = 0.638$ ), tibiofibula length ( $F_{4,18} = 1.863$ ,  $P = 0.161$ ) or head width ( $F_{4,18} = 1.432$ ,  $P = 0.264$ ). However, we observed marginally non significant differences for the proportion tibiofibula length

/ head width ( $F_{4,18} = 2.446$ ,  $P = 0.08$ ). The simple planned contrasts revealed that froglets from MZ have proportionally shorter tibiofibulas than the populations T2 ( $P = 0.009$ ), A2 ( $P = 0.036$ ) and AL ( $P = 0.059$ ) (Fig. 2). All other pairwise comparisons were not significant (all  $P > 0.27$ ).

## DISCUSSION

Our results suggest that isolation can have strong effects on the evolution of populations. First, the isolated population showed lower embryo survival if compared with populations living within larger metapopulation systems. This result confirms the negative effects of long term isolation on fitness, caused by inbreeding depression and genetic load. Loss of fitness caused by long term isolation could result in an increased extinction risk of populations (Saccheri et al., 1998; Rowe & Beebe, 2003). Moreover, there is some evidence that long term isolation caused by the presence of anthropogenic barriers could have some effect on the evolution of populations.

The analysis of froglets metamorphosed under laboratory did not reveal a clear pattern. We did not observe significant morphometric variation among population; however, it is possible that variation exist for the relative tibiofibula length. The differences among populations were marginally non significant, thus it is possible that this relationship is caused by type-2 statistical error. Moreover, the interpretation of this pattern is complicated by the observation that, in *R. latastei*, relative tibiofibula length is positively related to age at metamorphosis (Ficetola & De Bernardi, 2006). Only a more detailed study, keeping into account the effect of age at metamorphosis, and analysing a larger sample, could shed light on the meaning of this result.

The males of population MZ were larger than those from all the other analysed populations. Interesting, differences among populations were strong for males, but not significant for females. Since both average and minimum length were larger for MZ males, it is likely that larger size of MZ males was not related to older individuals. Several explanations could be hypothesized for differences in body size. First, it is possible that within the park favourable environmental conditions (such as more food availability) allow a faster growth, or that the population is well adapted to the environment and the frogs can grow quickly. However, females were not larger than those from other populations, thus these explanations do not have strong support. It is possible that some breeding related mechanism promoted larger size in only one sex. For example, since almost all the population breed in only one small ditch, it is possible that only the largest males have access to reproduction. It is possible that we caught only large males because we sampled frogs mainly during the breeding season, or that sexual selection promoted larger size on males, since the breeding wetland have a limited surface, thus enhancing the strength of interactions among males. Further studies are required to explore this hypothesis, including skeletochronological analyses evaluating the age of individuals.

In conclusion, we observed that isolation can cause loss of fitness, but also local adaptation is possible. Thus, conservation plans aimed to the management of isolated populations should avoid the risks of inbreeding depression, but should also keep into account the ongoing evolutionary processes, avoiding the loss of local adaptation caused by genetic homogenisation (Stockwell et al., 2003; Ficetola & De Bernardi, 2005).

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**FIGURES AND TABLES**

Population	Males		Females	
	Average	Min-Max	Average	Min-Max
Monza	49.7 ± 0.44	41.7-57.5	57.1 ± 2.0	42.5-64.1
Brianza (Pozzi, 1980)	48.5	37.0-48.5	49.5	42.0-57.0
Sartirana Lake (Gruppo GEV, 2004)	41.8 ± 0.71	35-50	56.4 ± 0.73	46-66
Ticino River (Bernini et al., 2000)	42.8 ± 0.48	35.1-54.9	46.3 ± 1.05	35.3-60.0
Piedmont (Boano & Sindaco, 1990)	43.8 ± 0.86	NA	51.3 ± 1.67	NA-66.5

Table I. SVL length of *R. latastei* from the population MZ and from four populations of Lombardy and Piedmont. If data are available, average values are ± SE; all measures are mm. NA: data not available.

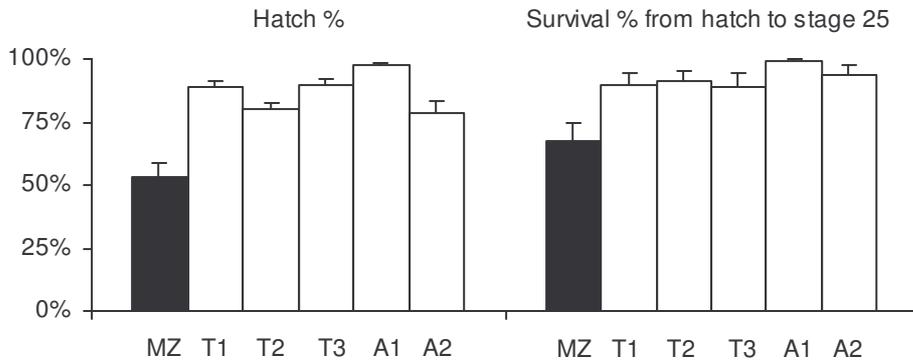


Fig. 1. Hatch percentage and survival from hatch to stage 25 in clutches from the population MZ (black bar) and in populations along rivers (white bars). Error bars equal 1 SE.

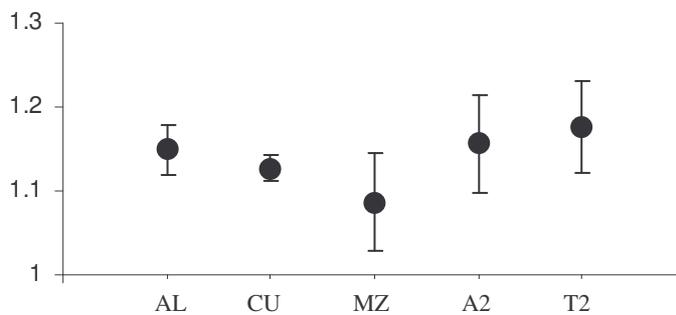


Fig. 2. Relative tibiofibula length in metamorphs from five *R. latastei* populations. Error bars equal 1 SE.