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Research article

Trade-off between larval development rate and post-metamorphic traits in the frog *Rana latastei*

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Abstract. Development rate early in the ontogeny is believed to correlate positively with fitness. Geographic variation in intrinsic development rate suggests the existence of trade-offs between development rate and other fitness related traits. We investigated whether these trade-offs exist between intrinsic larval development rate and post-metamorphic traits in an organism with a complex life cycle. In laboratory, we measured if the tadpoles of the frog *Rana latastei* with fast intrinsic development rate have a suboptimal post-metamorphic morphology, by comparing froglets from five populations. Then, we evaluated the relationship between age at metamorphosis, hindlimb length and jumping performance for frogs grown in nature in two populations. Under laboratory conditions, froglets with fast intrinsic development had shorter absolute and shorter size-adjusted tibiofibulas. We observed a strong, positive relationship between tibiofibula length and jumping performance. In nature, froglets from the last metamorphosing population had longer absolute and size-adjusted tibiofibulas, and were able to jump further. The cost of fast development could be the shorter legs of early metamorphosing frogs, and their poor jumping performance. Thus, a fast intrinsic development rate may not always be positively related to lifetime fitness, since delayed effects of larval development persist also across life history stages.

Key words: allometry, complex life cycles, developmental trade-offs, jumping performance, local adaptation, locomotor performance, metamorphosis, optimisation

Introduction

Intraspecific variation in the intrinsic development and growth rate has been observed in many animal species. For example, a number of studies on ectotherms observed that populations living in colder climates have the genetic capability to grow and develop faster than conspecifics living in warmer conditions, to counteract the negative effect of low temperature that slows down the developmental rate (Conover and Shultz, 1995). When individuals from populations adapted to different thermal regimes are reared in the same environment, the individuals from the colder environment are the fastest developing. However, since their environment of origin is colder, in nature their development can take a longer time than those of populations living in warmer climates. These phenomena have been frequently described as cases of ‘countergradient variation’ (Conover and Shultz, 1995).

For many organisms, including amphibians and insects, development and growth rate are traits believed to have a strong, positive association with fitness (see Scriber and Slansky, 1981; Semlitsch, 2002 for references). For example, fast growth and development enable amphibian tadpoles to escape death from drying ephemeral ponds and aquatic predators, considerably enhancing their survival (Travis *et al.*, 1985; Banks and Beebee, 1988; Newman, 1988a, 1988b). Moreover, fast larval growth and development can increase growth rate and the chance of survival also after metamorphosis (Pechenik *et al.*, 1998; Altwegg and Reyer, 2003). Thus we expect that natural selection favours the fastest developing genotype in all environments. Given these observations, in some cases intraspecific variation of intrinsic growth and development rate can constitute an enigma to evolutionary biology: it is not clear why some populations develop at rates less than the physiological capacity demonstrated by other, conspecific, populations (Conover and Shultz, 1995; Skelly, 2004).

A few previous studies hypothesized the existence of trade-offs to explain the persistence of this variation: intrinsic growth and development in juveniles may be negatively correlated with other fitness related traits, such as locomotion, feeding, the reproductive output as adults, the metabolism or the ability to withstand predators and environmental changes (Conover and Shultz, 1995; Arendt *et al.*, 2001; Fischer *et al.*, 2004; Skelly, 2004). For example, in the fish *Menidia menidia* the populations with high intrinsic growth rate have lower swimming speed and higher vulnerability to predation than slower growing populations (Billerbeck *et al.*, 2001; Lankford *et al.*, 2001). More generally, there is evidence that growth rate is optimised to local conditions rather than maximised to be as fast as possible (Arendt, 1997, 2003; Morgan and Metcalfe, 2001; Fischer *et al.*, 2004; Stoks *et al.*, 2005).

Conversely, the trade-offs associated to intrinsic development rate remain largely unexplored (but see Fischer *et al.*, 2004). Identifying the costs of rapid intrinsic development is therefore an important next step for understanding life history variation. Moreover, for organisms with complex life cycles, it is not clear whether the intrinsic development rate during larval stages can have a delayed cost after the metamorphosis. For these organisms, the metamorphosis can allow the independent evolution of different life history stages, thus maximising the overall fitness of individuals (Moran, 1994). However, the decoupling between stages may be incomplete, because of the persistence of developmental constraints and carry over effects (Watkins, 2001; Fischer *et al.*, 2004; De Block and Stoks, 2005). A fast intrinsic development rate early in the ontogeny may therefore result in fitness costs that are not exhibited until a later developmental stage. This can make it difficult to detect the cost and benefits associated with the development rate. Amphibians are ideal candidates to explore this hypothesis. Geografic intraspecific variation (such as countergradient variation) for development rate has been demonstrated for several species (Berven *et al.*, 1979; Berven, 1982a, 1982b; Loman, 2002; Laugen *et al.*, 2003; Skelly, 2004). Moreover, the environment that amphibians encounter during the larval development can influence not only the larval growth and development, but also the features of froglets after the metamorphosis, like morphology, survival and jumping performance (Blouin and Brown, 2001; Relyea, 2001; Alvarez and Nicieza, 2002; Relyea and Hoverman, 2003; Vonesh, 2005).

Here we test whether a fast larval development can have a cost after the metamorphosis in the Italian agile frog *Rana latastei* Boulenger 1879. *Rana latastei* is a rare species of brown frog that is endemic of Northern Italy and adjacent countries. It breeds in large wetlands within lowland forest; adults are terrestrial with the only exception of a few days during the breeding season (Pozzi, 1980; Ficetola and De Bernardi, 2004). This frog has limited genetic variability and distribution range (Garner *et al.*, 2004); however, if reared in the same controlled environment, individuals from foothill populations develop ~7% faster than those from lowland populations (Ficetola and De Bernardi, 2005). Conversely, in natural conditions the colder environment causes delayed metamorphosis of foothill populations (Ficetola and De Bernardi, 2005). It has been proposed that these differences are caused by countergradient variation, since foothill populations live in a climate with an average spring temperature 1–2 °C colder; nonetheless, other explanations for the observed differences in intrinsic development rate are indeed possible (see Ficetola and De Bernardi, 2005).

We analysed the relationships between larval development rate, post-metamorphic morphology and jumping ability in *R. latastei*, to evaluate whether differences in intrinsic development rate during larval stages can indirectly affect the frogs also after the metamorphosis. Post metamorphic morphology and jumping performance are traits believed to be important for the survival in anurans (Wassersug and Sperry, 1977; Henein and Hammond, 1997), and we hypothesized that the froglets which develop more slowly can invest more resources in morphological traits that favour terrestrial locomotion. The cost of short larval period can be a suboptimal morphology in the post-metamorphic stage, resulting in decreased locomotion performance (Alvarez and Nicieza, 2002). First, we reared tadpoles under laboratory conditions, to evaluate whether differences in post-metamorphic morphological traits were related to differences in intrinsic development rate. Second, we compared froglets metamorphosed in nature from two different types of populations. These populations have different intrinsic development rate: the population with the fastest intrinsic development rate lives in the colder environment, and in nature it develops more slowly. By comparing the phenotype expressed in these two populations, we explored whether differences in the age at metamorphosis cause differences in morphology comparable to those measured in the laboratory. For these populations we also measured the jumping performance of froglets, to evaluate whether the morphological differences can have a direct effect on locomotory performance.

Materials and methods

Common laboratory experiment

In spring 2003, we collected 23 recently laid clutches from five populations of *R. latastei* in Northern Italy (three foothill populations: AL, CU, MZ; two lowland populations: TC, ZB). We reared five clutches for each of the populations AL, MZ, TC, ZB; due to the low fertility of clutches collected from site CU, we reared only three clutches from this population. See Ficetola and De Bernardi (2005) for further details on the study populations. After hatchling, we haphazardly selected 10 tadpoles from each clutch and put them in the same plastic container filled with 1.5 l of aged tap water. The 23 containers (total: 230 tadpoles) were randomly sorted over the same bench of the laboratory, at constant 20 °C with 12-hour light-dark cycles. We fed tadpoles *ad libitum* with rabbit chow and lettuce; we changed water weekly. Laboratory conditions were apparently more similar to the natural conditions encountered by lowland populations of *R. latastei* than to those encountered by foothill populations. For lowland populations, in 2003, the time from hatch to metamorphosis was similar for laboratory (average: 57.2 days: see 'Results') and natural conditions (most of metamorphs found about 55–65 days after hatching). Conversely, for foothill populations, the average time required from hatch to metamorphosis was 53.4 days under laboratory conditions and about 85–100 days in nature.

After the metamorphosis (Gosner's 1960 stage 45: almost complete tail resorption) froglets were weighed to the nearest 0.1 mg and photographed. Age at metamorphosis was recorded; we released all the froglets in their wetland of origin.

Performance of wild metamorphs

In late winter–spring 2004, we monitored one lowland population (ZB) and one foothill population (MZ). In late February–March, we performed surveys each 2nd–7th day to detect the presence of egg masses. These sites were also periodically visited during spring, to verify the development of tadpoles. When tadpoles approached metamorphosis (late spring–early summer: see below), the populations ZB and MZ were intensively dip-netted to catch metamorphs

(Gosner's stage 39–42). We restricted our sampling to these stages since after stage 39 tadpoles lose feeding structures and cease to gain weigh (Gosner, 1960). We avoided catching later developmental stages, such as froglets living on the terrestrial environment, since it is possible that some of these animals started feeding terrestrial invertebrates, and thus they would be not comparable with just metamorphosed froglets. We caught 13 metamorphs from the population ZB and 26 from the population MZ; the rarity of this species precluded the capture of a larger number of individuals. We transferred metamorphs to the laboratory to standardize the environmental conditions, since differences in environmental temperature cause differences in jumping performance (e.g., Van Buskirk and Saxer, 2001). After the Gosner's stage 45, froglets were weighed to the nearest 0.1 mg and photographed. To measure jumping performance, each froglet was put on a bench and covered by a Petri dish. When the cover was removed, the froglet usually jumped, and we measured the length of the jump. For each froglet we measured three consecutive jumps, since after the 3rd–4th jump some froglet was exhaust and did not jump anymore. Due to a failure to obtain jumps from two of the froglets, the number of jumping froglets was lower than the number of measured froglets. Overall, we obtained jumps from 13 froglets from the population ZB and from 24 froglets from the population MZ. Since tadpoles grew in the field in completely natural conditions, it was not possible to assess the age at metamorphosis of froglets. However, in the population ZB, we observed the first clutches on March 7th, in the population MZ we observed the first clutch on March 9th. In both populations, most of clutches were laid within 10 days from the first ones, thus we assumed that at a given date the tadpoles of both sites had a similar average age.

Data analysis

For each froglet, we measured tibiofibula length on the basis of pictures; for the analyses we used the average of left and right tibiofibulas. In anurans, tibiofibula length is a trait strongly positively related with jumping performance (Zug, 1972; see 'Results'). We used maximum jumping length as a measure of jumping performance, since maximum jumping length seems to be more strongly related to feeding and escape ability than average jumping length; previous studies demonstrated that maximum jumping performance is a measure showing high repeatability in anurans (Henein and Hammond, 1997; Semlitsch *et al.*, 1999; Watkins, 2001). Using average jumping length instead of maximum jump length would not have changed any of our results.

We used linear models to evaluate the relationships between population of origin, length of larval development, weight at metamorphosis, tibiofibula length and jumping performance. Because we were interested in both absolute and size-independent performance, we used both ANOVA and ANCOVA models, using body weight (hereafter weight) and tibiofibula length as covariates. The tadpoles reared in the same container cannot be treated as independent, therefore, for froglets reared under laboratory conditions, we used mixed models with a nested design. We considered geographic position of origin (lowland/foothill) as a fixed factor and experimental container as a random factor nested within geographic position. All the tadpoles within the same container hold from the same clutch, therefore this factor is intended to take into account both the variability between experimental units and the variability among families within population. The complete model structure would include geographic position, population nested within geographic position and container within population within geographic position. However, population did not have a significant effect in any one of our models (all $p > 0.4$), thus this factor was removed from the models for simplicity and to increase power (Sokal and Rohlf, 1995). Including population within the models would not change qualitatively any of our results. Sample size was not homogeneous among groups, therefore if necessary degrees of freedom were approximated (Satterthwaite, 1946).

For the froglets metamorphosed in nature, no data about the age at metamorphosis of each individual is available. However, all the froglets from the locality ZB that were caught metamorphosed between June 7 and June 18, while all the froglets from the locality MZ metamorphosed between June 23 and July 6. Given that the eggs of both populations were laid almost contemporarily, but no overlap for date of metamorphosis was observed, we assumed that the froglets from ZB metamorphosed at a younger age than the froglets from MZ. The date of metamorphosis cannot be used to estimate the age at metamorphosis, since it is possible that, within population, the first metamorphs did not metamorphose at a younger age, but simply hatched earlier. Thus, in our analysis we used population of origin as a factor; we considered ZB (the lowland population) as an early metamorphosis population in natural conditions, and MZ (foothill) as a later metamorphosing population. This assumption is confirmed by several years of field observations, during which lowland tadpoles always metamorphosed earlier than foothill tadpoles (G.F.F., unpublished).

Interaction terms were tested in preliminary analysis, however they were not included in the final models since they were always non significant (all $p > 0.1$). Bivariate plots were examined to evaluate the presence of non-linearity. We did not observe strong evidence of non-linearity for the relationships between continuous variables within the interval of variation that we examined. Means are reported ± standard errors; when descriptive statistics are reported for variables with higher level in the nested design of laboratory study, means and standard error are based on the values on the lower hierarchical level (i.e., the average of a population is based on the average values of five containers). We did not observe strong violations of model assumptions (normality, homoscedasticity or non-autocorrelation); no cases had an undue influence on the models (all Cook's distances < 1) (Bowerman and O'Connell, 1990).

Results

Laboratory study

Since some tadpoles died prior to metamorphosis, we obtained a total of 180 froglets. Survival was not different between lowland and foothill clutches ($F_{1,22} = 0.061, p = 0.808$). The tadpoles from foothill populations grew faster and metamorphosed earlier than those from the lowland populations ($F_{1,23,751} = 11.953$, $p = 0.002$; Fig. 1; see also Ficetola and De Bernardi, 2005). The average age at metamorphosis of foothill populations (±SE) was 53.4 ± 0.5 days; the average age of lowland populations was 57.2 ± 1.3 days. At metamorphosis, average tibiofibula length was 5.41 ± 0.084 mm for foothill populations and 5.69 ± 0.010 for lowland populations; average weight at metamorphosis was 196 ± 6 mg for foothill populations and 210 ± 2 mg for lowland populations. Froglets from foothill populations had significantly shorter tibiofibulas (Table 1a; Fig. 1a). Despite the positive relationship between body weight and tibiofibula length (see below), keeping equal body weight, foothill populations had proportionally shorter tibiofibulas (Table 1b).

The ANCOVA showed that, within a given container, the last metamorphosing froglets had the longest tibiofibulas (Table 1c); after taking into account the effect of age at metamorphosis, the differences in tibiofibula length between the lowland and foothill groups were not anymore significant, suggesting that they were mainly caused by the differences in age at metamorphosis (Table 1c). The relationship between age at metamorphosis and tibiofibula length was strong also among populations, using the average values of each population for these variables (linear regression: $F_{1,3} = 11.5$, $p = 0.042$, $r^2 = 0.795$; Fig. 1a). Within container, the last metamorphosing froglets were the heaviest; at a given age, the differences in weight between the lowland and foothill groups were not significant (Table 1d). Tibiofibula length was strongly positively related to body weight (Pearson's correlation: $r = 0.823, n = 180, p < 0.0001$); however, keeping equal body weight, the last metamorphosing froglets of each container had proportionally longer tibiofibulas (Table 1e). After taking into account the effect of age at metamorphosis and body size, we did not find differences in tibiofibula length between foothill and lowland populations (Table 1e). The relationship between age at metamorphosis and relative tibiofibula length was strong also among populations,

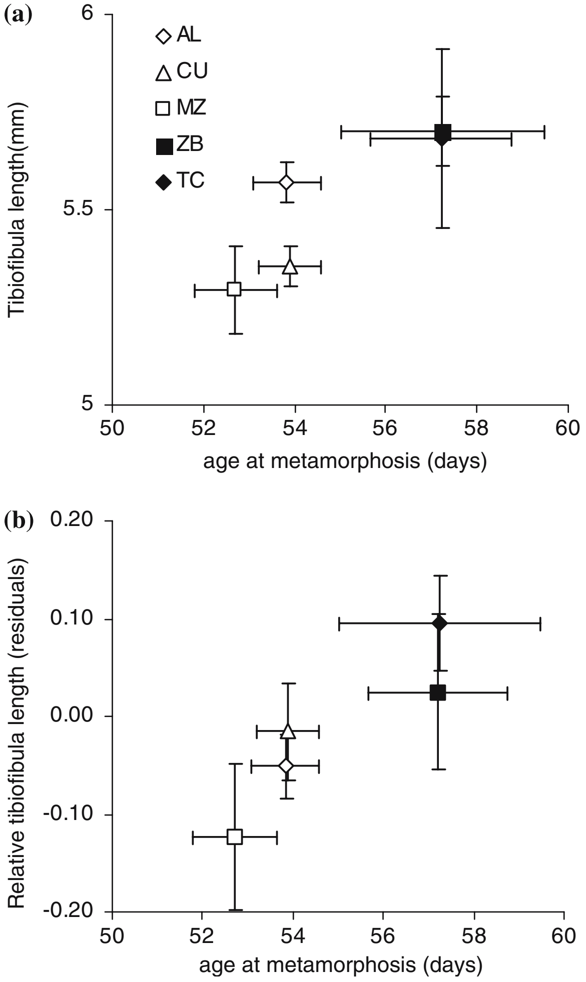

Figure 1. Relationship between age at metamorphosis and tibiofibula length in *R. latastei* from five populations. (a) Relationship between the average values of populations; (b) relationship between the average age and the average residuals of the relationship between tibiofibula length and body weight. Error bars equal standard errors of the mean. Filled symbols: lowland populations: empty symbols: foothill populations.

using the average age at metamorphosis of populations and the residuals of the relationship between tibiofibula length and weight (linear regression: $F_{1,3} = 13.3$, $p = 0.035$, $r^2 = 0.818$; Fig. 1b).

Performance of wild metamorphs

In the field conditions, the froglets from the early metamorphosing population (ZB, lowland) had shorter absolute tibiofibula length and lower jumping per-

Table 1. Effect of geographic position (foothill/lowland) and age at metamorphosis on post-metamorphic traits of froglets raised under laboratory conditions

Dependent	Independent	*F*	d.f.	*p*
(a) Tibiofibula length	Geographic position	5.988	1, 24.435	**0.022**
	Experimental container	1.790	21, 157	**0.024**
(b) Tibiofibula length	Body size	307.741	1, 156	**<0.0001**
	Geographic position	4.775	1, 26.490	**0.038**
	Experimental container	1.303	21, 156	0.180
(c) Tibiofibula length	Age at metamorphosis	139.866	1, 156	**<0.0001**
	Geographic position	0.049	1, 24.809	0.826
	Experimental container	3.606	21, 156	**<0.0001**
(d) Weight	Age at metamorphosis	180.012	1, 156	**<0.0001**
	Geographic position	0.866	1, 23.035	0.362
	Experimental container	6.637	21, 156	**<0.0001**
(e) Tibiofibula length	Age at metamorphosis	9.053	1, 155	**0.003**
	Body size	102.961	1, 155	**<0.0001**
	Geographic position	1.194	1, 32.795	0.282
	Experimental container	1.279	21, 155	0.197

Results of ANOVA and ANCOVA models.

formance than the froglets from the later metamorphosing population (MZ, foothill) (Table 2; Fig. 2). Differences in body weight were not significant, despite lowland froglets tended to have lower average body weight (Table 2). Maximum jump was positively related to both weight ($F_{1,35}$ = 14.128, p = 0.0006, r^2 = 0.288) and tibiofibula length ($F_{1,35}$ = 44.150, p < 0.0001, r^2 = 0.558) (Fig. 2b, c). However, after taking into account the effect of tibiofibula length, the effect of body size was not significant (multiple regression model: effect of tibiofibula length $F_{1,34}$ = 21.870, p < 0.0001; effect of body weight $F_{1,34}$ = 0.678, p = 0.416, r^2 = 0.566). The froglets from the early metamorphosing population had also shorter size-independent tibiofibula length (Table 3a; Fig. 2a); shorter size-independent maximum jump (Table 3b; Fig. 2b) and shorter tibiofibula-independent maximum jump (Table 3c; Fig. 2c).

Table 2. Post-metamorphic traits of froglets grown in nature in early and later metamorphosis populations: descriptive statistics and results of one-way ANOVA

Variable	Mean ± SE		*F*	d.f.	*p*
	ZB	MZ			
Weight (mg)	277.8 ± 18.3	292.3 ± 7.50	0.755	1, 37	0.400
Tibiofibula (mm)	6.65 ± 0.253	7.59 ± 0.095	15.602	1, 37	0.0003
Max jump (mm)	167 ± 10.6	245 ± 10.2	23.466	1, 35	<0.0001

Population ZB: lowland, early metamorphosis in nature; population MZ: foothill, later metamorphosis in nature.

Table 3. Differences in post-metamorphic traits among froglets grown in nature, in early and later metamorphosis populations

Dependent	Independent	*F*	d.f.	*p*
(a) Tibiofibula length	Population	39.368	1, 36	**< 0.0001**
	Weight	109.952	1, 36	**< 0.0001**
(b) Maximum jump	Population	27.006	1, 34	**< 0.0001**
	Weight	17.261	1, 34	**0.0002**
(c) Maximum jump	Population	5.675	1, 34	**0.023**
	Tibiofibula length	19.711	1, 34	**< 0.0001**

Results of ANCOVA models.

Discussion

Our results can be summarised as (1) both absolute and size-adjusted tibiofibula length were higher in the later metamorphosing froglets. This result has been confirmed for froglets grown under laboratory conditions as well as in the field. The differences among froglets in age at metamorphosis had completely different causes in the two data sets (that is, in laboratory the later metamorphosing were the froglets with slower intrinsic development rate, while in nature the later metamorphosing were those living in the colder environment), suggesting that this result is robust. (2) There was a strong, positive relationship between tibiofibula length and jumping performance. (3) Both absolute and size-adjusted jumping performance was higher in the later metamorphosing froglets; moreover, the froglets from the later metamorphosing population jump longer also keeping equal tibiofibula length.

Altogether, these results support the idea that the later metamorphosing froglets have a strong advantage in terms of locomotor performance if compared to the earlier metamorphosing froglets. This study did not directly measure the effect of jumping performance on survival; however, jumping performance is positively related to the ability to catch food and/or escape predators both in frogs of the genus *Rana* and in other anurans (Zug, 1972; Wassersug and Sperry, 1977; Heinen and Hammond, 1997). This suggests that the shorter tibiofibulas and smaller leaps can have negative effects on survival and/or feeding ability of faster developing froglets. That is, the advantages in terms of survival of earlier metamorphosing tadpoles (i.e., lower risk of predation in the water, lower risk of drying) seem to be balanced by a disadvantage in terms of locomotion after metamorphosis. This trade-off could hinder the spread of the fast development genotype in all the populations, and explains the persistence of geographic variation for intrinsic development rate (Conover and Shultz, 1995). A tadpole from foothill populations (i.e., a tadpole with fast intrinsic development rate), if transplanted in a lowland wetland where the warm temperature fasten development, could metamorphose very early and thus have short legs and a poor jumping performance, with possible negative effects on their survival.

This conclusion is supported by the observation that under laboratory conditions the froglets from foothill populations metamorphosed earlier and had shorter tibiofibula; conversely, in nature the froglets from foothill population metamorphosed later and had longer tibiofibula. Thus, tibiofibula length at metamorphosis, and at least partially jumping performance, seems to be mainly related to the age at metamorphosis, irrespective from the population of origin. We did not measure jumping performance for the froglets reared under laboratory conditions; therefore, a potential criticism to our conclusion might be that, in nature, the froglets from the later metamorphosing population (MZ) could have better jumping ability than those from the early metamorphosing population for factors others than their age at metamorphosis. However, we showed that both relative and absolute tibiofibula length increase with increasing age at metamorphosis also under laboratory conditions (Fig. 1), and that maximum jump is strongly related to tibiofibula length in both populations (Fig. 2; Zug, 1972). It is therefore likely that, at least partially, jumping performance is positively related to the time necessary to metamorphosis (see Relyea, 2001; Alvarez and Nicieza, 2002).

Several mechanisms may explain the trade-off between larval development rate and post metamorphic traits such as tibiofibula length and jumping performance. In amphibians, tadpoles should reach a minimum size before they can metamorphose (Wilbur and Collins, 1973). If environmental conditions are not limiting for factors such as food availability or density, tadpoles with a delayed metamorphosis can feed for a prolonged period. In turn, these additional resources could be allocated in traits important for post-metamorphic life, such as longer hindlimbs. Moreover, in anurans thyroid hormones controlling the metamorphosis are the same controlling hindlimb development. It is possible that a prolonged exposure to these hormones in the last metamorphosing tadpoles caused the observed allometry (Emerson, 1986). Finally, in the field study, froglets from the later metamorphosing population had longer maximum jump also after taking into account the effect of longer legs. This effect could be due to differences other than age at metamorphosis between these two sites, such as differences in food availability. However, Alvarez and Nicieza (2002) showed that froglets reared at lower temperature had more energetic reservoirs. Thus, it is possible that differences in energetic reservoirs caused at least partially the differences in jumping performance. A further possible cause of longer jumps in the froglets with slow development is the increase of muscle fibres per muscle unit mass with increasing size or age (Emerson, 1978).

It is difficult to find any general pattern relating larval development and post-metamorphic traits in amphibians. A number of studies tried to evaluate

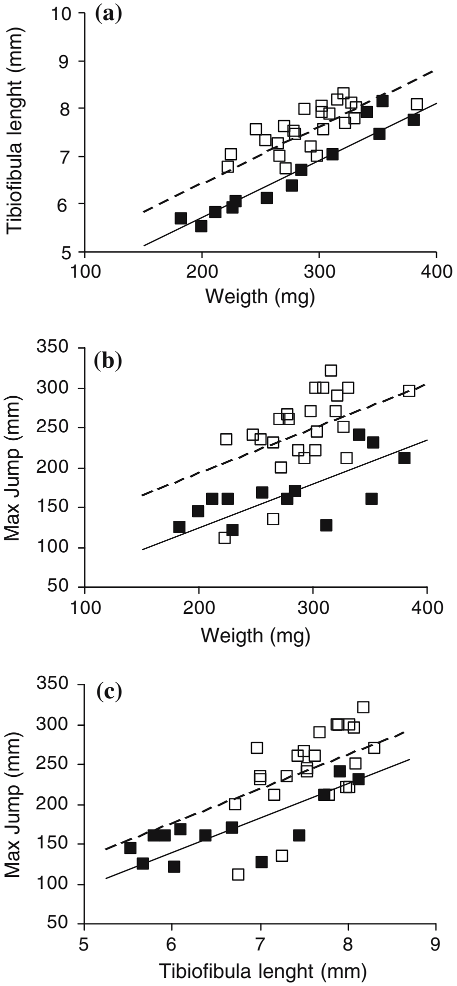

Figure 2. Relationship between post-metamorphic traits (tibiofibula length, body weight and maximum jump length) in froglets grown in nature. Filled squares and solid line: population ZB (lowland, early metamorphosis in nature); empty squares and broken line: population MZ (foothill, later metamorphosis in nature). Regression lines are fitted on the basis of ANCOVA models.

whether the conditions that larval anurans encounter have consequences on morphology and jumping performance after metamorphosis. However, only a subsample of these studies observed a relationship, and there is poor concordance across studies. For example, Relyea (2001) observed that *Rana sylvatica* tadpoles reared with presence of predators have a prolonged larva period and longer legs after the metamorphosis. Conversely, this relationship was not observed for *R. ridibunda* and *Hyla versicolor* tadpoles (Van Buskirk and Saxer, 2001; Relyea and Hoverman, 2003), despite the use of similar experimental protocols. More generally, some studies confirm our results, observing that a prolonged larval period causes longer hindlimbs in metamorphs (Emerson, 1986; Relyea, 2001; Alvarez and Niciezia, 2002), some studies did not observe any significant relationship (Blouin and Loeb, 1991; Van Buskirk and Saxer, 2001; Relyea and Hoverman, 2003), and some studies observed an apparently opposite pattern, the later metamorphosing froglets having shorter hindlimbs (Blouin and Brown, 2001). Indeed, Altwegg and Reyer (2003) showed that in the frogs *R. esculenta* and *R. lessonae* development rate is positively related with post-metamorphic growth rate and survival, suggesting that larval development rate can be positively related with post-metamorphic fitness. We can hypothesize that different species of anurans are subjected to different selective pressures, and thus optimisation and covariance between traits can be very different across species and habitats (Schmidt and Van Buskirk, 2005). Moreover, it is possible that tadpoles reared at cold temperatures close to the lower thermal tolerance limit have strong physiological disadvantages as a result of developmental stress (Smith-Gill, 1983).

Intrinsic development rate has often been used as a surrogate for fitness, for example in studies relating genetic diversity or maternal influence to fitness (see Mousseau and Fox, 1998; Rowe *et al.*, 1999; Rowe and Beebee, 2001). Sometimes, these studies dealt with populations that are tens or hundreds of km apart, but adaptive variation for intrinsic development rate has been demonstrated also at smaller interpopulation distances (e.g., Skelly, 2004; Van Buskirk and Arioli, 2005). Our results suggest that a fast intrinsic development rate can be not equally adaptive in different environments, thus, intrinsic development rate may not be a very good surrogate measure for fitness. The possibility of differences among populations in their optimal development rate will have to be considered carefully in future studies using differences in larval performance across populations as a surrogate for fitness.

We showed that the cost of fast larval development can be evident after the metamorphosis: for species having a complex life history, performance during each stage must be considered to fully understand lifetime fitness (Semlitsch et al, 1999; De Block and Stoks, 2005; Vonesh, 2005). In complex life cycles, metamorphosis is frequently viewed as a response to selection for developmental independence on different life cycle stages (the adaptive decoupling hypothesis), because it allows the pre- and post-metamorphic stages to adapt independently to their respective environments, without correlated negative effects (reviewed by Moran, 1994). However, our results confirm that larval and adult traits are not entirely evolutionarily independent in amphibians (Watkins, 2001), and suggest that trade-offs could persist also across life history stages, despite the presence of metamorphic events. At least in some cases, these trade-offs can explain the persistence of geographic variation in intrinsic development rate; however, the relationship between age at metamorphosis and post metamorphic traits could be not consistent across studies and species.

Therefore, more work is required to fully understand the relationship between metamorphic events and trade-offs.

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