Ecogeographical variation of body size in the newt *Triturus carnifex*: comparing the hypotheses using an information-theoretic approach

Gentile Francesco Ficetola[1*], Stefano Scali[2], Mathieu Denoël[3], Gianpaolo Montinaro[4],
Tanja D. Vukov[5], Marco A. L. Zuffi[6] and Emilio Padoa-Schioppa[1]

[1]*Department of Environmental Sciences, University of Milano-Bicocca. Piazza Della Scienza 1, 20126 Milano, Italy,* [2]*Natural History Museum of Milano, Corso Venezia 55, 20121 Milano, Italy,* [3]*Laboratory of Fish and Amphibian Ethology, Behavioural Biology Unit, University of Liège, 22 Quai van Beneden, 4020 Liège, Belgium,* [4]*Via Lanfranco Maroi, 32, 00148 Roma, Italy,* [5]*Institute for Biological Research Šiniša Stanković, University of Belgrade, Bulevar despota Stefana 142, 11060 Beograd, Serbia,* [6]*Museum of Natural History and Territory, University of Pisa, Via Roma 79, 56011 Calci (Pisa), Italy*

Abstract

Aim Ecogeographical variation of body size in vertebrates (e.g. Bergmann's rule) has long been recognized. However, the patterns and causes of intra-specific eco-geographical variation of body size in ectotherms, and in amphibians in particular, are strongly debated. We identified the relationship between bioclimatic variables and body size predicted a priori by alternative hypotheses (heat balance, endurance, seasonality, starvation resistance, water availability, primary productivity, parental investment) proposed to explain eco-geographical patterns of body size in ectotherms, and we evaluated the relative support of these hypotheses in explaining variation in body size of the Italian crested newt, *Triturus carnifex*.

Location Twenty-three populations covering the whole range of *T. carnifex* (Austria, Croatia, Italy and Slovenia).

Methods We obtained data on body size (snout–vent length, SVL) of 2639 adult newts from direct measurements and the literature; we obtained high-resolution environmental data for the sampled localities. We used an information-theoretic approach to evaluate the support of the data for the different hypotheses. We also integrated information on population genetics in our models.

Results We observed strong geographical variation of body size. The best Akaike information criterion (AIC) models showed that populations with larger body size are associated with cold climates and secondarily with high primary productivity. Furthermore, sexual dimorphism increases in cold climates, as the increase in body size was stronger for females. When taking into account population genetics, we did not find support for relationships with the other variables.

Main conclusion Our results are consistent with three hypotheses proposed to explain ecogeographical variation in amphibians: heat balance, increased parental investment of females and productivity. Information theory provides the framework for comparing hypotheses rather than looking for patterns. We suggest that evaluating the support for mechanisms can provide better insights than simply assessing whether ecogeographical variation is in agreement with some 'rule'.

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Introduction

Several 'ecogeographical rules' have been proposed to explain variation of morphology with geographical features such as latitude, temperature, elevation and isolation (Lomolino et al., 2006; Gaston et al., 2008). Bergmann's rule (Bergmann, 1847), describing the variation of body size, is among the most popular ecogeographical rules. Bergmann's rule, originally formulated at the inter-specific level, states that body size increases in populations at higher latitudes/altitudes, i.e. in cold climates; subsequent studies extended the rule at the intra-specific level (also called the neo-Bergmannian rule; Mayr, 1956; Blackburn et al., 1999). Several studies confirmed that endothermic vertebrates often follow intra-specific patterns consistent with the neo-Bergmannian rule (Ashton, 2002b; Meiri & Dayan, 2003),

particularly in temperate and cold macroclimates (see Rodríguez et al., 2008, for a discussion of inter-specific patterns). The situation is more complex and controversial for ectothermic vertebrates, and for amphibians in particular (Ashton, 2002a; Belk & Houston, 2002; Ashton & Feldmann, 2003; Adams & Church, 2008). In amphibians, a global pattern of larger body sizes in populations living in colder climates seems to be lacking. A recent meta-analysis suggested that Bergmann's rule is not valid at the intra-specific level for amphibians (Adams & Church, 2008; but see also Ashton, 2002a). Because of the lack of a general pattern within both amphibians and reptiles (Ashton & Feldmann, 2003; Adams & Church, 2008), it is unlikely that there will be a single explanation for the ecogeographical variation in body size in vertebrates (Adams & Church, 2008). Nevertheless, body size variation along climatic gradients has been observed in several amphibians. Indeed, some amphibians follow the classical Bergmann's rule, some follow the converse (i.e. larger individuals in warmer climates), while for other species no clear ecogeographical patterns of size have been detected.

In endothermic vertebrates, it is typically assumed that large body size may be favoured in cold climates because a small surface-to-volume ratio increases the efficiency of thermoregulation (heat balance hypothesis: Bergmann, 1847; Mayr, 1956; Meiri & Dayan, 2003; see also Blackburn et al., 1999, and Ashton, 2002b, for different hypotheses). However, the validity of the heat balance hypothesis is debated for ectotherms (Olalla-Tárraga & Rodríguez, 2007). Multiple hypotheses have been proposed to explain the geographical variation in body size occurring at both the intra- and inter-specific levels in ectotherms, and in amphibians in particular. These hypotheses cover different aspects of species life history and physiology, from desiccation tolerance to length of activity period (Ashton, 2002a; Olalla-Tárraga & Rodríguez, 2007; Cvetkovic´ et al., 2009) (Table 1; see below). Such a wide range of hypotheses highlights the difficulty of identifying the ecological or evolutionary causes of the observed body size variation. Explicitly formulated hypotheses have considerable potential to explain the causes of intra-specific body size variation. To be rigorously tested, hypotheses should predict relationships between body size variation and environmental variables. Despite a growing body of literature on body size variation in ectotherms during recent years (e.g. Sacchi et al., 2007; Schutze & Clarke, 2008), most studies have focused on evaluating whether a pattern consistent with Bergmann's rule is present. Only a few studies have explicitly tested the support of hypotheses proposed to explain body size variation (Olalla-Tárraga & Rodríguez, 2007) because hypotheses are all related to geographical variation of climatic features, and climatic features are strongly inter-correlated among them. When analysing ecogeographical variation, attention should be focused on understanding and testing the causal hypotheses of variation, rather than simply describing the patterns (Lomolino et al., 2006).

We considered eight hypotheses formulated to explain intra-specific variation of body size in amphibians; for each hypothesis, we predicted a priori a specific relationship between body size and environmental features (Table 1). Although some of these hypotheses were originally formulated to explain inter-specific patterns (e.g. Olalla-Tárraga & Rodríguez, 2007), their underlying mechanisms can also be effective at the intra-specific level. (1) Heat balance hypothesis: a large body size provides more thermal inertia and is therefore advantageous for thermoregulation in cold environments. For thermoregulating ectotherms, this hypothesis predicts a large body size in cold climate areas (Olalla-Tárraga & Rodríguez, 2007). (2) Endurance hypothesis: a large body size is favoured in areas with high thermal excursion, because it is associated with greater fat reserves (Ashton, 2002a). (3) Seasonality hypothesis: a large body size is expected in areas with a long growing season (Mousseau, 1997; Schutze & Clarke, 2008). (4) Starvation resistance hypothesis: a large body size is associated with seasonal, cold environments where animals are inactive and do not feed for long periods, because large individuals have more reserves and can better survive periods of food shortage (Arnett & Gotelli, 2003; Ashton & Feldmann, 2003). (5) Water availability hypothesis: a large body size is associated with dry climates because of higher desiccation tolerance (Ashton, 2002a). (6) Converse water availability hypothesis: a large body size is associated with areas with greater precipitation because the activity of amphibians is often associated with high water availability or wet periods (Zug et al., 2001). (7) Primary productivity hypothesis: areas with higher productivity allow more food supply and thus permit the attainment of a larger size (Rosenzweig, 1968; Olalla-Tárraga & Rodríguez, 2007). (8) Parental investment hypothesis: larger females can afford a greater parental investment (i.e. larger eggs or larger clutches). This might provides fitness advantages in cold environments (Morrison & Hero, 2003; Angilletta et al., 2004; Ficetola & De Bernardi, 2009). This hypothesis predicts that females are larger in cold climates, that is, sexual dimorphism increases in cold climates. Some of these hypotheses are mutually exclusive as they predict opposite responses to the same environmental variable (seasonality and starvation resistance; water availability and converse water availability), while others consider different environmental variables that might potentially concur in shaping body size pattern.

In this study, we investigated whether these eight hypotheses can explain geographical variation of body size in the Italian crested newt, *Triturus carnifex*. Several species of European newts show a geographical pattern in body size in agreement with the neo-Bergmannian rule. High-altitude populations of *Mesotriton alpestris* and *Lissotriton vulgaris* have a larger average body size than lowland populations (Miaud et al., 2000; Krizmanić et al., 2005). An analysis of several populations of *T. carnifex* did not find a relationship between body size and altitude (Krizmanić et al., 2005). However, this earlier analysis merged individuals from both *T. carnifex carnifex* and *Triturus carnifex macedonicus*, which are currently regarded as distinct species (Arntzen et al., 2007). Furthermore, while the analysis of Krizmanić et al. (2005) embraced a wide range of altitudes, it considered only the eastern distribution range of *T. carnifex*. Here we combine original data and literature data to gather information from more than 2600 individuals covering the whole range of the species, in order to test the support for the different hypotheses proposed to explain ecogeographical variation in body size in amphibians. We used an informationtheoretic approach to evaluate the relative support for the different hypotheses, and we took advantage of high-resolution geographical datasets to use climatic and ecological data instead of proxies such as latitude or elevation. We included also genetic parameters in our analyses because genetic relationships among populations may influence the geographical distribution of body size (Angilletta et al., 2004; Gaston et al., 2008).

Methods

We obtained data on snout–vent length (SVL) representing 2639 individuals from 23 populations of *T. carnifex*, using both the literature and original data. In 13 populations of Italy, Croatia and Slovenia (Appendix 1), adult newts were captured during the breeding season and SVL was measured using a calliper. Some populations were visited multiple times; to avoid repeated measures of the same individuals, individuals were marked using toe clipping or photographed for identification using the ventral pattern. For two populations (Sorisole, Treviglio) data were collected during BSc or MSc thesis fieldwork of students working in our laboratories (D. Ceresoli, O. Milanesi). Furthermore, we searched the published literature to find studies reporting data on body size of *T. carnifex*. We obtained eight further localities, from studies reporting the average SVL of at least nine to ten adults per sex for each population, and providing geographical coordinates or enough details to accurately identify the locality (Appendix 1). Our sampling covered almost completely the altitudinal and geographical range of the species (Fig. 1, Appendix 1). We did not include populations living in areas where *T. carnifex* hybridizes with other taxa. The distance between the two closest localities (Ferriere and Fontanelle, Appendix 1) was c. 1 km. We considered these as different localities because the difference in altitude was 250 m, and they had different climatic features in WorldClim.

We used WorldClim 1.4 (Hijmans et al., 2005) at a resolution of 30" to obtain several climatic features (temperature and precipitation) for the study populations. We then calculated four climatic variables, related to the different hypotheses formulated to explain body size variation (Table 1). We used the number of months with average temperature > 11 °C as a measure of the length of the activity period as, in spring, *T. carnifex* usually starts its activity when temperature rises above 10.5–11 °C (Galgano, 1944; Fasola & Canova, 1992; G. F. Ficetola, unpublished data). Analyses performed using different threshold temperatures (e.g. 10 °C) yielded the same results. We used annual precipitation and temperature because, in some areas of the range, *T. carnifex* can be active during the whole year (Andreone & Marconi, 2006). We measured productivity by the normalized difference vegetation index (NDVI), obtained from 1-km resolution time-series maps of the vegetation program (http://www.vgt.vito.be). We calculated NDVI as the average of monthly values for the period 2005–07.

To quantify sexual dimorphism, we calculated the sexual dimorphic index (SDI) which is equal to (mean length of larger sex/mean length of smaller sex) - 1 (Lovich & Gibbons, 1992). Some advantages of the SDI are that it produces values that are symmetrical around a central value (i.e. zero), it is properly scaled and it has intuitive values. In *T. carnifex*, it is expressed as positive when females are larger and negative when males are larger (Lovich & Gibbons, 1992).

Statistical analyses

First, we performed an exploratory analysis, and calculated the Pearson's correlation between SVL of each sex or SDI and the environmental variables representing the eight hypotheses (Table 1); we calculated the significance of correlations using geographically effective degrees of freedom (Dutilleul, 1993). Screening of bivariate plots did not detect strong nonlinearity in the relationships.

We used an information-theoretic approach (Burnham & Anderson, 2002), following the procedure detailed in Denoël et al. (2009), to evaluate the relative support for the different hypotheses explaining variation in body size. First, we used maximum likelihood to build linear mixed models, relating SVL to the environmental variables predicted to be important by the a priori hypotheses (Table 1). In all mixed models, we included sex as an independent variable, and population identity as a random factor. We built mixed models representing all possible combinations of environmental variables. We also considered the interaction between sex and climatic variables, as evidence of the parental investment hypothesis which predicts larger females in cold climates. We then calculated Akaike's information criterion (AIC) for each model: models explaining the most variation with the fewest predictors have the lowest AIC values and are considered to be the 'best models'. The use of the AIC corrected for small sample size (AICc) is controversial, and simulation studies suggest that AICc does not perform better than AIC (Richards, 2005; Raffalovich et al., 2008), therefore we report the results obtained using AIC. Because AIC may select overly complex models, we considered a complex model as a candidate model only if it had an AIC less than the AIC of all its sim-

Table 1. Hypotheses formulated to explain ecogeographical variation in body size in ectotherms (see text for references), and Pearson's correlation between body size of *Triturus carnifex* and the corresponding environmental variables.

Hypothesis	Environmental variable	Expected relationship	Males, r	Females, r	
1	Heat balance	Average annual temperature	SVL:	-0.676**	-0.723**
2	Endurance	Annual thermal excursion (Tmax June minus Tmin January)	SVL: +	-0.023	-0.111
3	Seasonality	No. of months with T > 11 °C	SVL: +	-0.653	-0.705
4	Starvation resistance	No. of months with T > 11 °C	SVL:	-0.653**	-0.705**
5	Water availability	Summed annual precipitation	SVL:	+0.431	+0.497
6	Converse water availability	Summed annual precipitation	SVL: +	+0.431†	+0.497*
7	Primary productivity	Normalized difference vegetation index	SVL: +	+0.172	+0.249
8	Parental investment	Average annual temperature	SDI:	-0.405*	

†*P* = 0.06, **P* <= 0.05, ***P* <= 0.001.
Asterisks indicate significant association in the direction predicted by the hypothesis (+ positive or – negative); significance was calculated using geographically corrected degrees of freedom (Dutilleul, 1993).
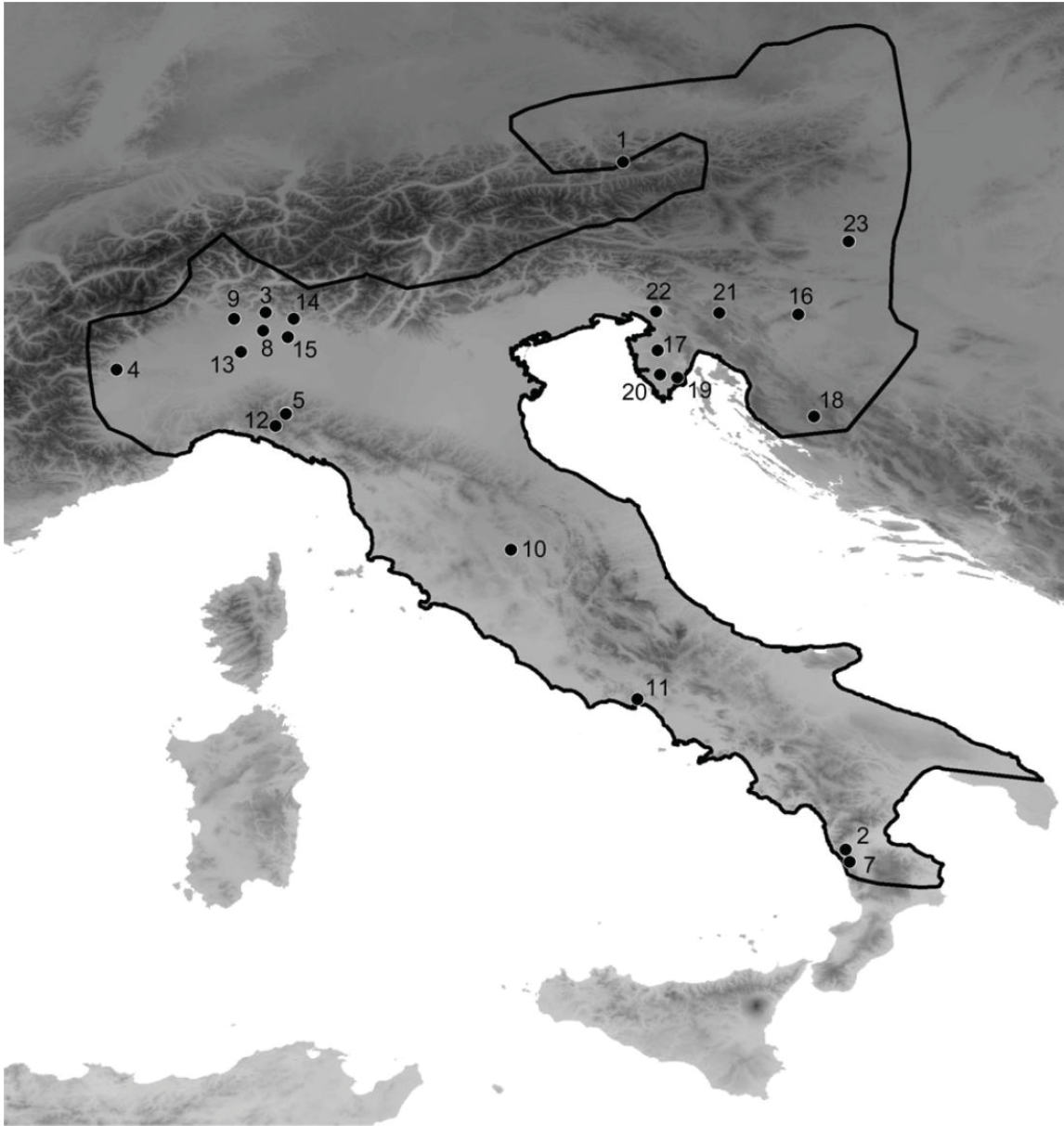SVL, snout–vent length; SDI, sexual dimorphic Index; T, temperature.

Figure 1. Distribution of sampled populations (black dots) within the range of *Triturus carnifex* (bold line). The background map shows the average annual temperature ranging from dark grey (-12 °C) to light grey (18 °C). The species range was redrawn on the basis of maps of the Global Amphibian Assessment (http://www.iucnredlist.org/amphibians). Sampling sites correspond to numbers in Appendix 1.

pler nested models (Richards, 2008); furthermore, we considered as candidate models only the ones performing significantly better than the null model. For each candidate model, we calculated the Akaike weight w_i (AIC weight), representing the probability of the different models given the data (Lukacs et al., 2007). We then used the evidence ratios $E = w_i/w_j$ to compare the relative support for the models representing the different hypotheses by the data (Lukacs et al., 2007). We estimated the amount of variation explained by models using R^2_1, which represents the proportional reduction in errors when a model is compared to the null model (Bickel, 2007). NDVI was log-transformed prior to analysis to improve normality.

A fine-scale intra-specific phylogeny of *T. carnifex* is lacking, therefore it was not possible to perform phylogenetic contrasts (Angilletta et al., 2004). However, information on population genetics, based on allozyme variation, is available for 13 localities through the whole range of *T. carnifex* (Arntzen, 2001). We used this information to evaluate whether intra-specific genetic variation

may have a confounding effect on body size. We used allele frequencies at 40 allozymes to calculate Nei's unbiased genetic distances among the populations investigated by Arntzen (2001). Subsequently, we used principal coordinates analysis (PCoA) to reduce genetic distances to a smaller number of dimensions (Quinn & Keough, 2002). The first two axes of PCoA accounted for 83% of genetic distance among populations; we therefore used the scores of the first two PCoA components as measures of the genetic features of populations. Populations investigated by Arntzen (2001) do not correspond exactly to the ones investigated in the present study. However, in *T. carnifex*, as in most amphibians, genetic diversity has a strong geographical structure, and nearby populations are genetically similar (Arntzen, 2001). We therefore assumed that our 23 populations have the same genetic features (i.e. PCoA scores) as the nearest population for which genetic information is available.

To evaluate whether the relationship between body size and environmental variables might be biased by intra-specific genetic features, we repeated

Tableau 2. Candidate mixed models explaining variation in body size on the basis of environmental features in *Triturus carnifex*

Rank	Fixed variables	Bioclimatic variables	AIC	ΔAIC	*w*
1	Sex	Annual T (-), Sex x Annual T, Annual Prec. (+), NDVI (+)	245.99	0.00	0.387
2	Sex	Annual T (-), Sex x Annual T, Annual Prec. (+)	247.29	1.30	0.202
3	Sex	Annual T (-), Sex x Annual T, NDVI (+)	247.75	1.75	0.161
4	Sex	Annual T (-), Sex x Annual T	247.98	1.98	0.144
5	Sex	Annual T (-), Annual Prec. (+), NDVI (+)	250.94	4.95	0.033
6	Sex	Annual T (-), Annual Prec. (+)	252.24	6.24	0.017
7	Sex	No. of months with T > 11 °C (+), Annual Prec. (+), NDVI (+)	252.66	6.67	0.014
8	Sex	Annual T (-), NDVI (+)	252.69	6.70	0.014
9	Sex	Annual T (-)	252.92	6.93	0.012
10	Sex	No. of months with T > 11 °C (+), Annual Prec. (+)	253.17	7.18	0.011
11	Sex	No. of months with T > 11 °C (+)	254.23	8.24	0.006
12	Sex	Annual Prec. (+), NDVI (+)	263.00	17.01	< 0.001
13	Sex	Annual Prec. (+)	263.26	17.26	< 0.001
14	Sex		267.08	21.09	< 0.001

All models reported here performed significantly better than the null model, and included population identity as a random factor. The sign of the relationships between body size and environmental features is in parentheses (+ positive or - negative). Models are ranked according to their ΔAIC; the model with the lowest ΔAIC is the best AIC model.

AIC, Akaike information criterion; ΔAIC, difference between the AIC of each model and the AIC of the best model. *w*, AIC weight of the model; T, average temperature; Prec, summed precipitation; NDVI, normalized deviance vegetation index.

the information-theoretic analysis described above, by including PCoA scores in all candidate models. An analysis using scores obtained from non-metric multidimensional scaling (Quinn & Keough, 2002) instead than PCoA yielded essentially the same results (not shown).

We performed the analyses in R 2.9 using the packages Nlme and Spdep (http://www.r-project.org). We used sam 3.0 to calculate Moran's *I*, as a measure of spatial autocorrelation of SVL, and to estimate geographically effective degrees of freedom of correlation analysis (Rangel et al., 2006). Furthermore, we used a Monte Carlo permutation test (99 permutations) to assess spatial autocorrelation of residuals (Lichstein et al., 2002); this test allowed us to evaluate whether spatial autocorrelation and the presence of nearby localities could bias the results of analyses (Lichstein et al., 2002).

Results

We observed substantial variation in body size across the 23 populations. Average SVL ranged between 54 and 80 mm in males and between 55 and 88 mm in females. Populations with large SVL were at high altitude and in the north of the species range (Appendix 1). In all populations, females were larger than males; SDI ranged between 0.007 and 0.116. The SVL of females was spatially autocorrelated (Moran's $I = 0.137$, $P = 0.018$), indicating that nearby populations have a similar body size. Spatial autocorrelation was slightly weaker and non-significant for males ($I = 0.103$, $P = 0.28$).

Pearson's correlations showed that, for both sexes, large SVL was significantly associated with cold annual temperature, short activity period and high annual precipitation (Table 1); furthermore, sexual dimorphism increased in cold climates. For both sexes, the correlation between SVL and thermal excursion or normalized difference vegetation index (NDVI) was not significant.

Analysis including environmental features only

Following the best AIC model (Table 2), populations with large body size were associated with cold annual temperature (mixed model: $F_{1,19} = 26.4$, $P < 0.001$; Fig. 2); females were larger than males ($F_{1,21} = 87.5$, $P < 0.001$), but a significant interaction between sex and annual temperature indicated that, in cold climates, females increased body size more than males ($F_{1,21} = 7.1$, $P = 0.015$; Fig. 2). Furthermore, populations with large body size were associated with areas with high annual precipitation and high primary productivity; these relationships were close to significance (precipitation: $F_{1,19} = 2.9$, $P = 0.1$; NDVI: $F_{1,19} = 3.1$, $P = 0.09$) (Table 2). This model explained a substantial proportion of variation ($R^2_1 = 0.644$). The residuals of the best model were not spatially autocorrelated (for both males and females; Monte Carlo permutation test, $P > 0.1$), indicating that spatial autocorrelation did not bias our results.

Simpler models, not including the effect of precipitation or NDVI, had slightly lower support (Table 2). Based on evidence ratios, the model without NDVI was 1.7 times less likely than the model considering NDVI, while the model without precipitation was 2.4 times less likely than the model considering precipitation. Simpler models had much lower support. The model not including the interaction between sex and annual temperature was 12 times less likely than the best model. All the models not including temperature had low support (AIC weight < 0.02; Table 2).

Tableau 3. Candidate mixed models explaining variation in body size on the basis of genetic variation (PCoA scores) and environmental features in *Triturus carnifex*.

Rank	Fixed variables	Bioclimatic variables	AIC	ΔAIC	*w*
1	Sex; PCoAscores	AnnualT(-), Sex x Annual T, NDVI (+)	242.44	0.00	0.822
2	Sex; PCoAscores	AnnualT(-), Sex x Annual T	246.68	4.24	0.099
3	Sex; PCoAscores	AnnualT(-), NDVI (+)	247.44	5.00	0.068
4	Sex; PCoAscores	AnnualT(-)	251.68	9.24	0.008
5	Sex; PCoAscores	AnnualT(-), Annual Prec. (+)	253.53	11.09	0.003
6	Sex; PCoA scores	Annual Prec. (+), no. of months with T > 11 °C (+), NDVI (+)	257.25	14.81	0.001
7	Sex; PCoA scores	Annual Prec. (+), no. of months with T > 11 °C (+)	259.85	17.40	< 0.001
8	Sex; PCoA scores	No. of months with T > 11 °C (+)	260.81	18.37	< 0.001
9	Sex; PCoA scores	Annual Prec. (+), NDVI (+)	262.64	20.19	< 0.001
10	Sex; PCoA scores	Annual Prec. (+), annual excursion (-)	262.65	20.21	< 0.001
11	Sex; PCoA scores	NDVI (+)	265.03	22.59	< 0.001
12	Sex; PCoA scores	Annual Prec. (+)	265.64	23.20	< 0.001
13	Sex; PCoA scores		267.67	25.22	< 0.001

All models reported here performed significantly better than the null model, and included population identity as a random factor. The sign of the relationships between body size and environmental features is in parentheses (+ positive or - negative). Models are ranked according to their ΔAIC; the model with the lowest ΔAIC is the best AIC model.
AIC, Akaike information criterion; ΔAIC, difference between the AIC of each model and the AIC of the best model. *w*, AIC weight of the model; PCoA, principal coordinates analysis; T, average temperature; Prec, summed precipitation; NDVI, normalized deviance vegetation index.
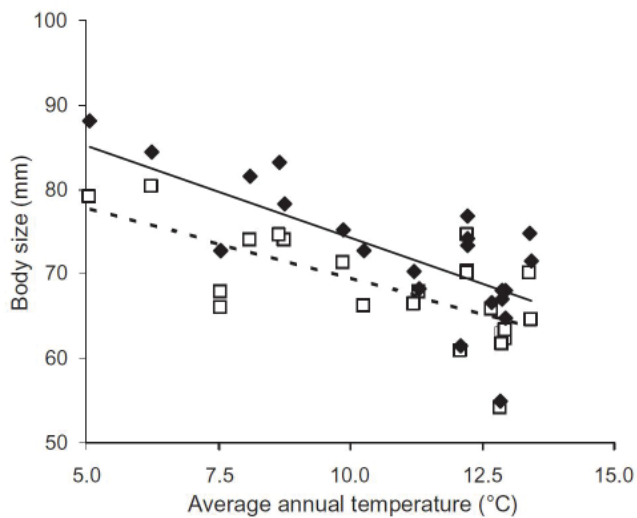

Figure 2. Relationship between body size (snout–vent length) and average annual temperature in 23 *Triturus carnifex* populations. Empty squares, broken line: males; filled diamonds, continuous line: females.

Analysis including genetic parameters

Including the genetic features of populations (PCoA scores) slightly improved the fit of models. Some models including both environmental and genetic features showed lower AIC scores than the corresponding models without PCoA scores (compare Tables 2 & 3). When genetic features were taken into account, the best AIC model suggested that populations with large body size were associated with cold annual temperature (mixed model: $F_{1,18}$ = 30.3, $P <$ 0.001) and high NDVI ($F_{1,18}$ = 6.1, $P = 0.02$); females were larger than males ($F_{1,21}$ = 85.2, $P < 0.001$). Also in this analysis, a significant interaction between sex and annual temperature indicated that, in cold climates, females increased body size more than males ($F_{1,21}$ = 6.9, $P = 0.016$). Furthermore, there was a significant relationship between PCoA scores (dimension 1) and SVL ($F_{1,18}$ = 8.7, $P = 0.009$). This model explained a substantial proportion of variation ($R^2_1 = 0.715$). The residuals were not spatially autocorrelated (for both males and females; Monte Carlo permutation test, $P >= 0.1$).

A simpler model not including NDVI had lower support; based on evidence ratios, the model without NDVI was 12 times less likely than the model considering NDVI. All models with weight > 0.07 included both temperature and the sex x temperature interaction; models not including temperature and the interaction had very low support (Table 3). When taking into account genetic features, annual precipitation was not included in models with high support (Table 3).

Discussion

A correct evaluation of mechanisms underlying eco-geographical variation should require the proposal, comparison and disproval of multiple hypotheses, as remarked by Mayr (1956) more than 50 years ago. However, it has often been difficult to explicitly evaluate the value of the multiple hypotheses proposed since then. The analysis of ecogeographical intra-specific variation is usually focused on detecting the presence/absence of patterns, and subsequently formulating hypotheses to explain these patterns. Information theory provides a framework for the analysis of multiple scientific hypotheses, allows the direct quantification of their evidence and gives direct measures of the support of alternative models (Lukacs et al., 2007). Our analysis showed that the combination of an information-theoretic approach with high-resolution climatic data can help to disentangle the mechanisms of body size variation in the

newt *T. carnifex*. Furthermore, our analysis shows that it is likely that multiple mechanisms concur to the overall pattern, as the best model provides support for a multi-factorial explanation of body size.

The comparison among alternative models supports three hypotheses. Populations with large body size were associated with cold climates (Table 1; Fig. 2), in agreement with the predictions of the heat balance hypothesis for thermoregulators (Olalla-Tárraga & Rodríguez, 2007). Furthermore, the increase in body size was particularly strong for females (Fig. 2), as predicted by the parental investment hypothesis (Morrison & Hero, 2003; Angilletta et al., 2004). Finally, when taking into account differences in temperature, body size was larger in populations living in areas with high primary productivity. The analysis including environmental features only was in agreement with the converse water availability hypothesis (Table 2), but this was not confirmed when taking into account genetic differentiation (Table 3). One hypothesis (starvation resistance) predicted a pattern similar to the one observed in our data (Table 1); however, the support for this hypothesis by the data was low when compared with the alternative ones (Tables 2 & 3). Finally, three hypotheses (endurance, water availability and seasonality) predicted patterns opposed to the one observed in our data (Table 1).

Some authors discarded the role of heat, assuming that ectotherms are usually in thermal equilibrium with the environment, and therefore large body size does not constitute an advantage (reviewed by Blackburn et al., 1999). However, several amphibians, including some species of aquatic salamanders, are able to maintain a body temperature consistently above the environmental temperature, particularly by using behavioural mechanisms such as control of activity period, basking and selection of the warmest patches in the thermal landscape (Heath, 1975; Zug et al., 2001). As the rate of heat gain or loss decreases with increasing body size, large body size can provide advantages during thermoregulation (Zug et al., 2001, p. 188; Meiri & Dayan, 2003). Of course, this mechanism can be valid only for thermoregulators; it has been therefore proposed that only those amphibians that thermoregulate should follow the Bergmann cline (Olalla-Tárraga & Rodríguez, 2007). In this respect, it should be remarked that anurans seem to have stronger thermoregulation capabilities than urodeles (Brattstrom, 1963). Nevertheless, thermoregulation mechanisms have been proposed at least for some species of salamanders (Spotila, 1972; Heath, 1975), and studies on thermoregulation of urodeles remain scarce and limited to a few taxa (Hutchinson & Dupré, 1992). For instance, we are not aware of studies on thermoregulation in *T. carnifex* or in related species. Our results are in agreement with the hypothesis that heat balance is a key mechanism determining body size in *T. carnifex*. However, it should be kept in mind that this does not constitute evidence that thermoregulatory advantages are the ultimate cause of body size variation. Our analysis showed that the prediction of the heat balance hypothesis is better supported by the data than the alternative mechanisms proposed; nonetheless other hypotheses, such as the temperature–size rule (Adolph & Porter, 1996; Angilletta & Dunham, 2003), cannot be completely discarded (discussed below).

Although both males and females showed larger body size in cold climates, the response of females to climatic conditions was stronger (Fig. 2), and sexual dimorphism increased as predicted by the parental investment hypothesis. In crested newts, large females have more oocytes and heavier ovaries, i.e. they have higher fecundity than smaller females (Cvetkovic´ et al., 1996). This suggests that the increased sexual dimorphism can be related to an increase of parental investment (more eggs, larger eggs or both). A larger clutch volume is often observed in amphibian populations living in cold climates (Morrison & Hero, 2003). Cold temperatures slow down development rate, and parental investment can be extremely important for the fitness of offspring in stressful environments. For example, larvae which hatched from large eggs can have faster development or better survival than those hatched from small eggs (Räsänen et al., 2005; Ficetola & De Bernardi, 2009). Furthermore, in cold climates amphibians attain maturity later (see below); a large number of eggs might increase the number of offspring reaching maturity. The study of parental investment is a major research field in ecology and evolutionary biology. Nevertheless, the possibility that parental allocation explains ecogeographical variation of body size has been little explored (Angilletta et al., 2004). Our analysis suggests that variation in parental investment might be an important factor explaining body size variation.

Finally, we observed larger body size in areas with high primary productivity. Productivity and food availability are considered major causes of intraspecific body size variation in mammals (Rosenzweig, 1968; Meiri et al., 2007), but have received little attention in large-scale analyses of amphibians (but see Olalla-Tárraga & Rodríguez, 2007, and Olalla-Tárraga et al., 2009, for analyses at the interspecific level). Nevertheless, it should be noted that NDVI increases only slightly the variation explained by the model (the difference in R^2_1 between model 1 and model 2 = 0.08), and that the model including NDVI only (model 11 in Table 3) had very low support, suggesting that temperature and the temperature x sex interaction are the major drivers of body size variation in *T. carnifex*.

The analyses with and without genetic information yielded different results: the role of precipitation (converse water availability hypothesis) was supported only by the analysis not considering population genetics. This occurred because populations in the eastern range of the species (i.e. Croatia and Slovenia) are genetically related (Arntzen, 2001), have large body size (Table 1) and live in areas with relatively high precipitation. Analyses including phylogeny often revealed weaker ecogeographical patterns (Blackburn et al., 1999; Angilletta et al., 2004). Phylogenetic history can have an important role in determining body size, and therefore has confounding effects in the analysis of body size variation (Blackburn et al., 1999; Angilletta et al., 2004). Our analysis confirms that the pattern of body size variation is partially related to genetic relationships among populations: the best models including environmental features only showed a higher AIC than models that also include genetic information (Tables 2 & 3). Nevertheless, our measure of genetic relationship among populations (variation at 40 allozymes) was not able to explain the overall pattern of body size, as the support of the model including genetic features but not considering environmental parameters (model 13 in Table 3) was nearly zero.

Amphibians have indeterminate growth, and increased longevity/reduced mortality might be a further hypothesis for explaining large body size in ectotherms living in cold climates (Angilletta et al., 2004; Cvetković et al., 2009). Unfortunately, estimating age in amphibians is particularly complex and labour intensive. Adolph & Porter (1996) predicted

that, in cold climates, ectotherms mature slowly but have delayed maturity. This model predicts smaller size at age, larger size at maturity, but invariant average adult body size across climates (Adolph & Porter, 1996; Belk & Houston, 2002). The possibility that age variation at the range scale may explain ecogeographical variation has rarely been investigated in amphibians. In the toad *Bufo bufo*, high-latitude/altitude populations can attain greater longevity (Cvetković et al., 2009). However, variation in longevity is not enough to determine large body size in cold climates, because of the reduced growth rate. Indeed, climatic features remain the major factors determining body size in *B. bufo* (Cvetković et al., 2009). Similarly, in the newt *Triturus cristatus*, a species closely related to *T. carnifex*, age variation does not explain body size variation, because populations living in colder climates have an older age at maturity but also a slower growth rate, and do not necessarily attain larger sizes (Dolmen, 1983). Broadly speaking, models that predict Bergmann's clines in ectothermic vertebrates as a mere by-product of thermal constraints on growth and maturation are poorly supported (Angilletta et al., 2004), suggesting that simple variations in age cannot be the ultimate explanation of variation in body size (Belk & Houston, 2002; Angilletta et al., 2004).

The analysis of large-scale geographical variation is based essentially on a correlative approach, but determining the exact mechanisms of body size variation (e.g. phenotypic plasticity, local adaptation) would require experimental approaches (Guillaumet et al., 2008). Unfortunately, experiments are often unfeasible at macroscales (Diniz-Filho, 2008), particularly for vertebrates (but see Arnett & Gotelli, 2003, and Schutze & Clarke, 2008, for experiments in insects). Nevertheless, the evaluation of the support of multiple a priori hypotheses, and the inclusion of potential confounding factors such as population genetics, can help to establish the relationships between macroecological patterns and processes (Diniz-Filho, 2008; Gaston et al., 2008).

Strong debate is ongoing on the generality of ecogeographical rules. Assessing whether rules are general requires the analysis of a sufficient (i.e. very large) number of species. However, the number of species investigated with a good coverage of the whole range remains invariably small, making generalizations difficult (Gaston et al., 2008). Evaluating the different hypotheses explaining ecogeographical variation may be a different, but more effective, approach. Instead of simply assessing whether a pattern is present or not, this can allow the identification of mechanisms most likely to explain variation. In turn, the knowledge of mechanisms, combined with information on the features of the organisms, may help to assess the conditions under which explanations are valid.

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Biosketches

G. Francesco Ficetola is a post-doc combining multiple approaches (landscape ecology, macroecology, evolutionary ecology) to improve knowledge of the ecology and conservation of amphibians and reptiles.

Correspondence: Gentile Francesco Ficetola, Department of Environmental Sciences, University of Milano-Bicocca, Piazza Della Scienza 1, 20126 Milano, Italy.
E-mail: francesco.ficetola [a] unimi.it

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Appendix 1. Populations of *Triturus carnifex* analysed in Austria (AU), Italy (IT), Croatia (HR) and Slovenia (SLO): location, snout–vent length (SVL), sample size (*n*) and data source

| | Locality | Latitude | Longitude | Altitude | Males | | Females | | Source |
					SVL	*n*	SVL	*n*	
1	Ameisensee Lake (AU)	47.55	13.45	1282	79.2	24	88.1	27	Schabetsberger et al. (2004)
2	Crello (IT)	39.57	16.04	672	63.4	171	68.1	209	Giacoma (1988)
3	Cesana Brianza (IT)	45.81	9.30	280	60.9	49	61.4	53	This study
4	Druento (IT)	45.14	7.58	262	70.3	53	73.4	51	Andreone & Giacoma (1989)
5	Ferriere (IT)	44.63	9.54	1150	65.9	79	72.7	128	Fasola & Canova (1992)
6	Fontanelle (IT)	44.63	9.54	950	67.9	119	72.7	37	This study
7	Laghicello (IT)	39.43	16.08	1100	66.1	60	72.8	51	Joly & Giacoma (1992)
8	Monza (IT)	45.59	9.28	175	65.9	49	66.7	45	This study
9	Parco Pineta (IT)	45.73	8.94	366	66.4	7	70.2	11	This study
10	Perugia (IT)	43.05	12.15	240	63	170	67	169	Zaffaroni et al. (1992)
11	Pornito, Maranola (IT)	41.32	13.62	750	67.8	50	68.3	129	This study
12	Rezzoaglio (IT)	44.49	9.42	1330	80.4	43	84.4	55	Cavallotto et al. (1992)
13	Rosate (IT)	45.35	9.02	100	54	118	55	105	Zaffaroni et al. (1992)
14	Sorisole, Pertosino (IT)	45.73	9.63	295	70.1	19	74.1	40	D. Ceresoli (unpublished)
15	Treviglio (IT)	45.52	9.57	121	62.3	142	64.8	110	O. Milanesi (unpublished)
16	Budinjak-Žumberak (HR)	45.78	15.48	770	73.9	14	81.6	20	This study
17	Istarske Toplice (HR)	45.37	13.85	20	70.2	26	74.8	18	This study
18	Lički Osik (HR)	44.60	15.67	580	73.9	10	78.3	9	This study
19	Salakovci (HR)	45.05	14.08	320	64.5	20	71.5	15	This study
20	Goče, Štanjel (SLO)	45.08	13.88	316	61.7	19	68.1	16	This study
21	Podstrmec (SLO)	45.80	14.57	560	74.6	26	83.3	19	This study
22	Goče, Štanjel (SLO)	45.82	13.83	250	74.7	13	76.9	18	This study
23	Turjanci (SLO)	46.63	16.07	195	71.3	11	75.2	13	This study