



The evolution of thermal performance curves in semi-aquatic newts: Thermal specialists on land and thermal generalists in water?

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ARTICLE INFO

Article history:

Received 12 March 2008

Accepted 13 June 2008

Keywords:

Phylogenetic comparative analysis

Reaction norms

Temperature

Co-evolution

Running

Swimming

Ectotherms

Amphibians

ABSTRACT

The position and shape of thermal performance curves (TPCs, the functions relating temperature to physiological performance) for ecologically relevant functions will directly affect the fitness of ectotherms and therefore should be under strong selection. However, thermodynamic considerations predict that relationships between the different components of the TPC will confound its evolutionary optimization. For instance, the “jack-of-all-temperatures” hypothesis predicts a trade-off between the breadth of the TPC and the maximal performance capacity; the “warmer is better” hypothesis suggests that low thermal optima will come with low absolute performances. Semi-aquatic organisms face the additional challenge of having to adjust their TPCs to two environments that are likely to differ in mean temperature and thermal variability. In this paper, we examine how parameters of the TPCs for maximal running and swimming speed have co-evolved in the semi-aquatic newt genus *Triturus*. We consider evolutionary relationships between the width and the height of the TPCs, the optimal temperatures and maximal performance. Phylogenetic comparative analyses reveal that in *Triturus*, swimming and running differ substantially in the (co-)variation of TPC parameters. Whereas evolutionary changes in the TPC for swimming primarily concern the shape of the curve (generalist versus specialist), most interspecific variation in running speed TPCs involves shifts in overall performance across temperatures.

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1. Introduction

Whole-animal performance, the degree to which animals can carry out an integrated, ecologically relevant function (e.g., locomotion, feeding, growth), is generally considered an important determinant of fitness (Huey and Stevenson, 1979; Arnold, 1983). Therefore measuring maximal performance has become an important tool in the study of adaptation (Irschick and Garland, 2001). However, in nature, animals must often perform under sub-optimal environmental conditions (Huey et al., 1989), and therefore their fitness will typically depend not only on their maximal performance capacity, but also on the shape and position of the reaction norm describing the environmental dependence of physiological performance. This realization has prompted broad interest in the factors promoting and constraining the evolution of reaction norms (Gotthard and Nylin, 1995; Via et al., 1995; Schlichting and Pigliucci, 1996; Angilletta et al., 2003; Kingsolver et al., 2007).

Studies of the thermal dependence of performance capacity in ectotherms have played a prominent role in this respect. This is hardly surprising, given the pervasive role of body temperature on virtually all aspects of the behavior and physiology of these animals (Huey and Stevenson, 1979; Huey, 1982; Angilletta et al., 2002). From its early start, two hypotheses have dominated research on the evolution of these special thermal reaction norms, also known as thermal performance curves (TPCs). Both hypotheses emphasize relationships between different characteristics of the curves, and argue that (evolutionary) changes in one of these characteristics must affect the other. The “warmer is better” (or “thermodynamic constraint”) hypothesis maintains that, due to the thermodynamic properties of biochemical and physiological systems, the maximal performance of organisms with high optimal temperatures should be greater than that of organisms with low optimal temperatures (Huey and Kingsolver, 1989; Savage et al., 2004). Most comparative data-sets seem to corroborate this idea (e.g., Eppley, 1972; Bauwens et al., 1995; Frazier et al., 2006; but see Clarke, 2003). The “Jack-of-all-temperatures is a master of none” (Huey and Hertz, 1984) hypothesis rests on the trade-off between flexibility and stability of enzymes (Hochachka and Somero, 2002), and assumes a trade-off between maximal performance and the breadth of the performance curve (Levins, 1968; Huey and Slatkin, 1976). Experimental studies and interspecific comparisons along latitudinal and altitudinal

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gradients provide limited support for this idea (Gilchrist, 1996; Izem and Kingsolver, 2005), and indicate that increased variability of body temperatures typically selects for one phenotypic solution only: thermal generalists (John-Alder et al., 1988; van Berkum, 1988; Navas, 1996; Castañeda et al., 2004). However, the evidence is scant and limited to a few study systems (Angilletta et al., 2002).

The difficulties presumably associated with optimizing TPCs must be especially pertinent to animals that shuttle regularly between different thermal environments. Such animals not only face the constraint of trade-offs among TPC characteristics within a function; in addition they must deal with the difficulty of optimizing different functions to different thermal regimes. For instance, because water has a specific heat capacity 3500 times that of air, and a thermal conductivity 23 times that of air (Dejours, 1987; Denny, 1993), body temperatures in small and medium-sized ectotherms equal temperatures of surrounding environment (Spotila et al., 1992). As a consequence, some aquatic organisms, especially air-breathers, are more likely to experience a more variable body temperature in thermally stratified aquatic environment than active terrestrial organisms, which maintain a relatively stable body temperature by thermoregulatory behavior and/or by shifting their activity times. Increased body temperature variation favors a wider TPC for performance that is relevant for fitness at actual temperature (e.g., burst speed) rather than over longer time period (e.g., growth; Huey and Slatkin, 1976; Gilchrist, 2000; Angilletta et al., 2006). How do semi-aquatic species deal with these divergent selection pressures? Ideally, one would expect that functions acquire the thermal dependence that optimizes the performance in the environment in which they are ecologically relevant. Accordingly, the thermal sensitivity (the slope or derivative of TPC) of crawling capacity is higher than that of swimming in individual species of semi-aquatic snakes (Stevenson et al., 1985; Finkler and Claussen, 1999), some salamanders (Else and Bennett, 1987; Marvin, 2003a,b; Gvoždík et al., 2007; but see Wilson, 2005). On the other hand, one may expect that the potentially conflicting thermal demands set by the two environments may hamper the optimization of TPCs, especially for functions that rely on the same biochemical and physiological systems. However, virtually nothing is known about the magnitude and pattern of co-variation of TPC characteristics of different whole-animal functions in the same species.

Here, we examine, in a phylogenetic context, the direction and magnitude of co-variation among TPC characteristics within and between two whole-animal functions, maximal swimming and running speed, relevant in different thermal contexts in *Triturus* newts. We assume (1) that the general inability to individually optimize model parameters characterizing asymmetric curves will prevent TPC parameters to evolve independently (Gilchrist, 2000; Gilchrist and Kingsolver, 2001); (2) that the evolution of the TPC of one function will be influenced by selection on the other (Huey and Kingsolver, 1989; Angilletta et al., 2003; Frazier et al., 2006) and (3) that the underlying genetic correlations are not constant but change between environments (e.g., Björklund, 2004; Sgrò and Hoffmann, 2004). If the disparate thermal conditions of aquatic and terrestrial environments have modified the functional, selective and/or genetic associations among TPC parameters, interspecific patterns of variation and co-variation in these parameters will differ between running and swimming.

2. Methods

2.1. Study species

The genus *Triturus* comprises four lineages: the small-bodied newts (snout-vent length [SVL] up to 60 mm), the big-bodied

newts (SVL up to 110 mm), *Triturus alpestris* (SVL up to 70 mm), and *Triturus vittatus* (SVL up to 70 mm) (Weisrock et al., 2006; Steinfartz et al., 2007). These newts are usually terrestrial for part of the year (3–8 months, Grossenbacher and Thiesmeier, 2003; Thiesmeier and Grossenbacher, 2004), but they enter still or, more rarely, slow-flowing water in spring to breed. Although females lack both crests and webbing during reproductive period, their aquatic phase usually lasts longer than in males. They feed on a wide variety of small invertebrates, both in and out of the water, and some species occasionally prey on eggs and larvae of other amphibians (Griffiths, 1996). Although the survival value of sprint and swimming speed in the field remains to be demonstrated in these animals, both aquatic and terrestrial locomotor capacity is generally considered ecologically relevant performance traits in newts (e.g., Brodie, 1977; Ashley-Ross and Bechtel, 2004; Brodie et al., 2005; Wilson, 2005).

Newts were collected between 1999 and 2002 at different natural sites in Europe. Specimens of one species (*Triturus boscai*) were obtained from the pet trade. Speed measurements were realized during three consecutive seasons (2000–2002). Because males exhibit a high degree of seasonal plasticity in secondary sexual characteristics (presence/absence of dorsal and caudal crests, webbing), we restricted our analyses to adult, non-reproductive females.

The newts were housed separately or in pairs in plastic boxes (50 × 30 × 25 cm³) holding 15 l of tap water. Female newts are non-territorial, and housing newts in pairs is consequently unlikely to influence their locomotor performance. Each container contained a piece of Styrofoam (10 × 15 cm²) and several clumps of Java moss (*Vesicularia dubyana*) that served as hiding places. Kept in these conditions, the newts did not shift to the terrestrial phase, and remained semi-aquatic. This phenomenon has also been observed in natural conditions (e.g., Hagström, 1979; Fasola and Canova, 1992). The semi-aquatic phase, when newts move in water and on land, poses conflicting demands for optimal performance in both environments, and thus seems more suitable for the purpose of our study than using semi-aquatic and terrestrial newts. To reduce possible effects of prior thermal conditions, the newts were kept in a room at 18–22 °C with a natural photoperiod for at least 3 months before testing (July–August). During the whole of this period, the animals experienced body temperatures within the preferred range of all the species considered (Gvoždík, 2003, 2005, unpublished data). All individuals received similar amounts of food items (earthworms, *Tubifex* worms or fish meat), once or twice a week.

2.2. Traits

A detailed description of the procedure followed for measuring locomotor performance has been published elsewhere (Gvoždík and Van Damme, 2006), so we here restrict ourselves to some basic facts. We measured swimming and running speeds of the newts in a linear racetrack (200 cm long × 10 cm wide) equipped with five pairs of infrared photocells. The sensitivity of the photocells and the positioning of the infrared beams were adjusted to assure that the newts invariably disrupted the beams with the tip of their snout. All performance measurements were carried out in a temperature controlled room, and repeated at 20, 15, 25, 33, 10, and 30 °C. The fastest speed over any 25 cm interval was taken as a measure of maximum running or swimming speed at a given temperature. A previous study, following similar procedures, demonstrated good short-term repeatability of locomotor performance in *Triturus* (Gvoždík et al., 2007). Because species means of the maximal swimming and running speed scales with body size in these species (Gvoždík and Van Damme,

2006) and because body size may confound the effect of speed on escape success (Van Damme and Van Dooren, 1999), relative speed (SVLs^{-1}) rather than absolute speed was used in subsequent analyses.

We describe TPCs for swimming and running speed by parameters estimated using a recently published method, called the Template Mode of Variation (TMV; Izem and Kingsolver, 2005). Although this approach was originally developed for analyzing among-family variation in TPCs in quantitative genetic studies, it can also be used to compare TPCs among species. In short, TMV uses a polynomial function to decompose variation among TPCs into three predetermined modes of variation that are of interest to evolutionary biologists: vertical shift (faster–slower), horizontal shift (hotter–colder), and specialist–generalist trade-offs (Huey and Kingsolver, 1989). Each direction of variation is represented by one parameter, i.e., height, optimal temperature, and width of TPC, obtained using a shape-invariant model:

$$z_i(T) = (1/w_i)z[(1/w_i)(T - T_{\text{opt},i})] + h_i \quad (1)$$

where $z_i(T)$ is performance at temperature T for species i , z represents the common template shape of the curves, h_i is the height of TPC (in units of performance) or overall performance across all temperatures, $T_{\text{opt},i}$ is the optimal temperature (in °C), and w_i is width parameter comprising both the width of TPC and specialist–generalist trade-off (dimensionless).

In addition, we obtained the maximum performance z_{max} from Eq. (1):

$$z_{\text{max},i} = (z(0)/w_i) + h_i \quad (2)$$

where $z(0)$ is the maximum value for the template function $z(T)$. Note that by rearranging Eq. (2), w_i emerges as the inverse of the difference between the maximum and overall performance, standardized by $z(0)$. To enable comparisons with previous studies, we also calculated thermal performance breadths (B_{80} , the range of temperatures at which performance values exceed 80% of the maximum; Huey and Stevenson, 1979), from Eq. (1), using a simple iterative procedure. We assumed that the common template curve (z) was a polynomial of degree 4. Lower-order polynomials generally fail to describe TPCs adequately (e.g., David et al., 1997), and this would hamper meaningful decomposition of the variation (Izem and Kingsolver, 2005). In turn, the fact that we measured performance at six temperatures only precludes the use of higher-order polynomials. Except z_{max} and B_{80} , all computations were realized using the Matlab code by R. Izem (available at <http://www.fas.harvard.edu/~rizem>).

2.3. Phylogeny

The topology of trees used in the comparative analyses follows the results from two recent studies on salamandrid phylogeny based on maximum parsimony and Bayesian analyses of mitochondrial DNA data (Weisrock et al., 2006; Steinfartz et al., 2007; Fig. 1). Concerning the relationships within *Triturus*, nearly all clades were strongly supported in both phylogenies (Bayesian posterior probabilities and parsimony bootstrap values >95%) except positions of *T. alpestris* and members of the *Triturus cristatus* group. We therefore used both phylogenetic hypotheses and considered them as equally probable in our analyses. Unfortunately, information on branch lengths is unavailable for all branches because of incomplete taxon sampling, mtDNA introgression, incomplete lineage sorting and/or the presence of cryptic species. We therefore set all branch lengths equal to one, or used computer-generated branch lengths.

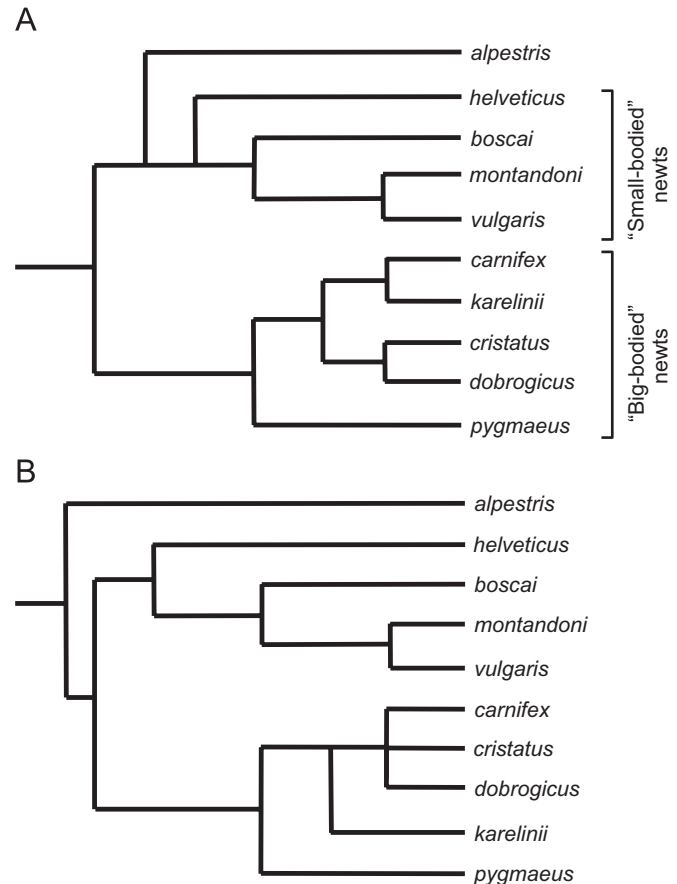


Fig. 1. Hypothesized phylogenetic relationships of *Triturus* species used in this study based on works by Weisrock et al. (2006; A) and Steinfartz et al. (2007; B). Branch lengths are not proportional to actual divergence times.

2.4. Phylogenetic comparative analyses

According to general recommendations (Price, 1997; Garland et al., 1999; Martins et al., 2002; Housworth et al., 2004), we evaluated co-variation among TPC parameters (w , T_{opt} , h , z_{max}) using non-phylogenetic Pearson product-moment correlation of the raw data (TIPS) and using two comparative methods based on different evolutionary assumptions: Felsenstein's independent contrasts method (FIC, Felsenstein, 1985) and the phylogenetic generalized least-squares method (PGLS, Grafen, 1989; Martins and Hansen, 1997). The TIPS analysis is appropriate under the condition of strong stabilizing selection and a negligible influence of genetic drift. The FIC method assumes phenotypic evolution by genetic drift or directional selection with randomly changing optima. The PGLS method considers evolution through genetic drift under various constraints (e.g., stabilizing selection), the strength of which is described by a parameter, α_c . We used the PGLS module in the COMPARE 4.6 package (Martins, 2004) to examine relationships among traits under these assumptions. In order to reduce the number of significance tests, we followed the approach suggested by Ord and Martins (2006) and used only the results of the PGLS method. Simulations indicate that the PGLS method has the best performance under most evolutionary scenarios, especially when sample size is small (Martins et al., 2002). To evaluate the effect of uncertain phylogeny (see below), confidence intervals (CI) were estimated from repeated calculations for 500 possible trees with the same topology but randomly generated branch lengths (i.e., 1000 trees in total; Martins, 1996). The PGLS results were considered significant if 95% CI of

correlation coefficients did not include zero. In addition, we calculated semipartial (part) correlation coefficients (Hair et al., 1998) for PGLS results to obtain information about unique and shared variance associated with correlations between TPC parameters.

We used paired t -tests to compare the TPC parameters for swimming and running within species. We evaluated the t -value obtained from an analysis on the actual tip-data against a null distribution generated by paired t -tests on 1000 virtual sets of tip-data, obtained by simulating evolution of the parameters along the candidate phylogenetic trees. Simulations were performed in the PDSIMUL module of the PDAP program (v6.0, Garland et al., 1999; Garland and Ives, 2000). In the simulations, we used both speciation Brownian motion and speciation Ornstein–Uhlenbeck process model, and the wandering traits were kept in bounds equal to the maximal value observed in real +20% and the minimal value observed in real –20%.

A significance level of $\alpha = 0.05$ was used for non-phylogenetic paired t -tests. All means are reported ± 1 standard error. Conventional statistical analyses were performed in Statistica 6.1 (StatSoft, 2000).

3. Results

Mean running and swimming speeds at different temperatures for the ten *Triturus* species considered are presented in Table 1. Applying a three-parameter shape-invariant model (Eq. (1)) to the size-corrected performance data results in two common template curves that provide good approximations of the common shape of the individual species curves (Fig. 2). Accordingly, the models explain 84% of the among-species variation in TPC for running performance, and 92% of the variation for swimming speed. Decomposition of the total variation into the three predetermined directions of variation reveals different patterns in the two locomotor performances (Fig. 3). Most of the interspecific variation in running speed TPCs occurs in the vertical direction (59.8%), followed by the specialist–generalist mode (18.8%), and the horizontal mode (5.1%). In contrast, TPCs for swimming speed vary mostly in the specialist–generalist direction (56.9%), and less in the vertical (25.0%) and horizontal (9.6%) modes.

The common template curves for swimming and running seem to differ in shape (Fig. 2). The species means for the width parameter (w) and the maximal performance (z_{\max}) obtained for running are low compared with those for swimming, but the corresponding t -values are not significant when compared with a null distribution that accounts for the non-independency of the data points (Table 2). We found no indication of a difference in

optimal temperature (T_{opt}) between the two locomotor modes (Table 2). We also found no evidence for correlated evolution between any particular parameter of running TPC and its equivalent for swimming (Table 3).

While the w s for running speed seem homogeneous, w s for swimming speed differ considerably among species (Table 4; Fig. 3). In particular, four species (*T. alpestris*, *Triturus carnifex*, *Triturus dobrogicus*, and *Triturus vulgaris*) seem to have much wider swimming TPCs than the other species (Table 4). A similar picture is obtained when comparing traditional measures of curve width (B_{80} , Table 4).

The patterns of co-variation among TPC parameters (w , z , h , T_{opt}) differ strikingly between swimming and running (Table 5). Comparing TPCs for running among species, w associates positively with T_{opt} and negatively with z_{\max} and h . In contrast,

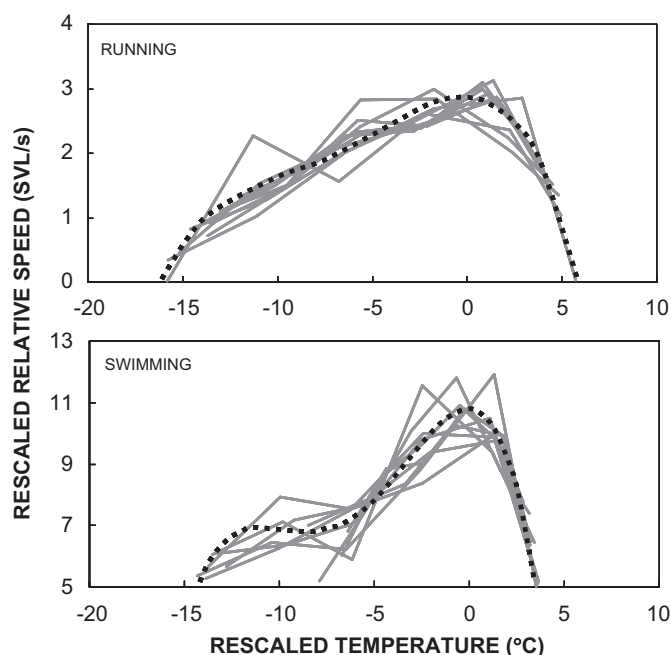


Fig. 2. Rescaled thermal performance curves (TPCs) for running and swimming speed in each species (solid lines) with fitted common template shape $z(T)$ (dashed line). Each TPC of a species (i) and temperature were standardized with respect to the estimates of height (h), location (T_{opt}), and width (w) parameters from the fit to model (Eq. (1)): rescaled $z_i(T) = (z_i(T) - h_i)/w_i$; running $z(T) = 2.88475 - 0.04643T^2 - 0.00522T^3 - 0.00019T^4$; swimming $z(T) = 10.73571 - 0.27228T^2 - 0.03756T^3 - 0.00143T^4$; rescaled $T = (T - T_{\text{opt},i})/w_i$. Rescaled $T_{\text{opt}} = 0$.

Table 1
Maximum running and swimming speeds (mean \pm SE) at six experimental temperatures in *Triturus* species

Species (n)	SVL (mm)	Running speed (cm/s)						Swimming speed (cm/s)					
		10 °C	15 °C	20 °C	25 °C	30 °C	33 °C	10 °C	15 °C	20 °C	25 °C	30 °C	33 °C
<i>T. alpestris</i> (6)	59.0 \pm 1.8	4.1 \pm 0.4	8.0 \pm 1.1	13.3 \pm 0.9	15.4 \pm 1.5	9.8 \pm 1.3	6.9 \pm 1.3	19.6 \pm 2.0	25.8 \pm 2.6	28.1 \pm 2.8	31.7 \pm 4.0	28.3 \pm 4.0	23.6 \pm 2.9
<i>T. boscai</i> (5)	44.4 \pm 1.6	2.7 \pm 0.4	4.5 \pm 1.2	6.5 \pm 1.0	6.7 \pm 1.6	8.3 \pm 1.5	5.8 \pm 1.4	20.7 \pm 1.8	24.5 \pm 2.3	23.7 \pm 2.5	32.4 \pm 3.6	43.3 \pm 3.6	18.1 \pm 2.6
<i>T. carnifex</i> (12)	85.9 \pm 1.0	5.8 \pm 0.4	8.9 \pm 1.0	13.7 \pm 0.8	17.3 \pm 1.4	21.7 \pm 1.2	12.3 \pm 1.2	26.6 \pm 1.2	29.5 \pm 1.5	32.4 \pm 1.6	40.7 \pm 2.3	39.3 \pm 2.3	23.8 \pm 1.7
<i>T. cristatus</i> (8)	77.3 \pm 1.2	4.1 \pm 0.4	7.3 \pm 1.0	11.5 \pm 0.8	12.0 \pm 1.4	15.6 \pm 1.2	11.3 \pm 1.2	21.6 \pm 1.4	29.9 \pm 1.8	32.5 \pm 2.0	41.9 \pm 2.8	44.3 \pm 2.9	18.5 \pm 2.1
<i>T. dobrogicus</i> (12)	84.5 \pm 1.1	3.5 \pm 0.4	5.7 \pm 0.9	8.6 \pm 0.7	9.2 \pm 1.2	13.0 \pm 1.1	8.8 \pm 1.1	25.8 \pm 1.2	32.8 \pm 1.6	33.5 \pm 1.7	39.5 \pm 2.4	36.5 \pm 2.4	26.4 \pm 1.8
<i>T. helveticus</i> (5)	44.7 \pm 1.6	1.7 \pm 0.4	3.7 \pm 1.2	7.0 \pm 1.0	9.0 \pm 1.6	6.4 \pm 1.4	3.9 \pm 1.4	17.9 \pm 1.8	25.5 \pm 2.3	24.1 \pm 2.5	26.8 \pm 3.6	31.9 \pm 3.6	17.1 \pm 2.6
<i>T. karelinii</i> (5)	70.0 \pm 1.6	5.0 \pm 0.4	11.5 \pm 1.2	14.8 \pm 1.0	16.5 \pm 1.6	18.9 \pm 1.5	11.7 \pm 1.4	27.8 \pm 1.8	29.2 \pm 2.3	32.7 \pm 2.5	46.4 \pm 3.6	49.7 \pm 3.6	29.5 \pm 2.6
<i>T. montandoni</i> (6)	45.5 \pm 1.6	1.3 \pm 0.4	4.3 \pm 1.1	8.6 \pm 0.9	11.2 \pm 1.5	11.9 \pm 1.3	0	14.4 \pm 1.8	17.7 \pm 2.3	22.0 \pm 2.5	30.7 \pm 3.6	30.3 \pm 3.6	13.9 \pm 2.6
<i>T. pygmaeus</i> (5)	62.7 \pm 1.6	5.9 \pm 0.4	18.4 \pm 1.2	14.8 \pm 1.0	20.5 \pm 1.6	19.2 \pm 1.5	11.7 \pm 1.4	32.3 \pm 1.8	37.3 \pm 2.3	31.8 \pm 2.5	57.9 \pm 3.6	47.9 \pm 3.6	34.2 \pm 2.6
<i>T. vulgaris</i> (6)	41.9 \pm 1.1	2.2 \pm 0.4	3.4 \pm 1.1	6.5 \pm 0.9	6.2 \pm 1.5	7.6 \pm 1.3	4.5 \pm 1.3	14.2 \pm 1.8	18.8 \pm 2.3	24.2 \pm 2.5	27.8 \pm 3.6	21.5 \pm 3.6	18.7 \pm 2.6

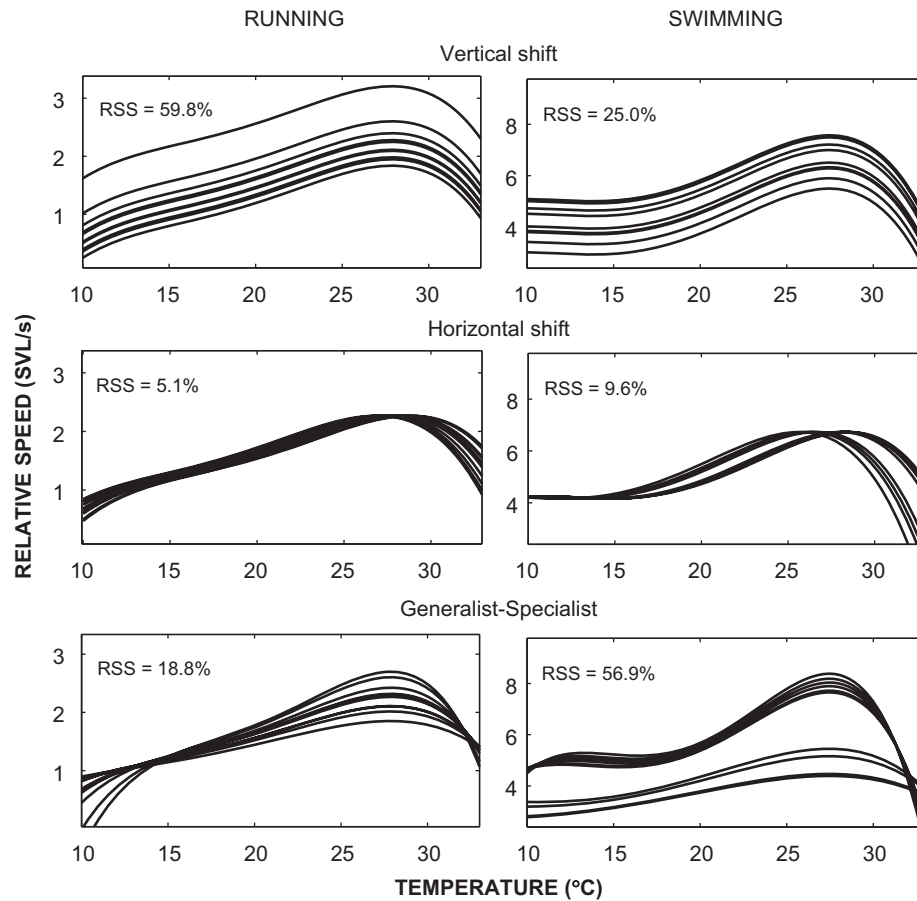


Fig. 3. Decomposition of interspecific variation in thermal performance curves for running and swimming speed into three major directions (see text for details) using Eq. (1). Each solid line represents a species. Ratio of sum of squares (RSS) show relative proportion of total variation explained by the model in a given direction. Each solid line represents a species.

Table 2

Comparisons of parameters of thermal performance curves between running and swimming speed in *Triturus newts*

Trait ^a	Running	Swimming	<i>t</i>	<i>P</i>	Phylogeny A ^b				Phylogeny B			
					BM ^c		OU		BM		OU	
					<i>t</i> _{0.05} ^d	<i>P</i>	<i>t</i> _{0.05}	<i>P</i>	<i>t</i> _{0.05}	<i>P</i>	<i>t</i> _{0.05}	<i>P</i>
<i>h</i> ^e	0 ± 0.13	0 ± 0.23	–	–	–	–	–	–	–	–	–	–
<i>T</i> _{opt}	27.87 ± 0.22	27.45 ± 0.35	0.93	0.38	2.54	0.24	1.11	0.07	2.49	0.25	1.00	0.06
<i>w</i>	1.29 ± 0.04	1.70 ± 0.15	3.05	0.01	9.70	0.97	10.33	0.97	9.46	0.84	10.34	0.94
<i>z</i> _{max}	2.27 ± 0.19	6.73 ± 0.47	10.45	<0.001	21.59	0.87	30.09	0.99	21.97	0.88	29.73	0.99
<i>B</i> ₈₀	8.91 ± 0.19	10.81 ± 1.58	1.19	0.27	6.32	0.85	5.85	0.91	6.18	0.85	6.01	0.92

^a *h*, height (SVL/s); *T*_{opt}, optimal temperature (°C); *w*, width (dimensionless); *z*_{max}, maximum performance (SVL/s); *B*₈₀, thermal performance breadth (°C).

^b Alternative phylogenies (Fig. 1).

^c BM, speciation Brownian motion; OU, speciation Ornstein–Uhlenbeck model of character evolution.

^d Critical value of paired *t*-test distribution based on simulated data along the phylogeny under different evolutionary models.

^e Mean *h* is zero from its definition (Izema and Kingsolver, 2005).

comparison of swimming TPCs yielded negative correlations between *w* and *T*_{opt}, and between *w* and *z*_{max}, and a positive correlation between *T*_{opt} and *z*_{max} (Table 5). Application of different phylogenetic comparative methods resulted in highly similar correlation coefficients, suggesting that these conclusions are robust to various evolutionary assumptions (Table 5). However, due to high intercorrelation between the parameters, the unique variance associated with these relationships is mostly low, which hinders interpretation the importance of these results (running speed: $r^2_{w, T_{opt}(h, z_{max})} = 0.0002$; $r^2_{w, h(T_{opt}, z_{max})} =$

0.04; $r^2_{w, z_{max}(h, T_{opt})} = 0.20$; $r^2_{h, z_{max}(w, T_{opt})} = 0.44$; swimming speed: $r^2_{w, T_{opt}(h, z_{max})} = 0.006$; $r^2_{w, z_{max}(h, T_{opt})} = 0.02$; $r^2_{T_{opt}, z_{max}(w, h)} = 0.001$).

4. Discussion

Because they are exposed to a double set of environmental challenges (water vs. land), semi-aquatic species have received considerable attention from evolutionary biologists since Darwin (e.g., Darwin, 1859; Dawson et al., 1977; Dejours et al., 1987;

Fish, 2000; Gillis and Blob, 2001). However, the implications of a semi-aquatic lifestyle for the evolution of thermal physiology remain largely unexplored. Given the obvious differences in thermal properties of water and air, it seems logical to expect different selection pressures on the thermal dependence of terrestrial and aquatic locomotion (Hochachka and Somero, 2002; Clarke, 2003). On the other hand, since swimming and running are likely to depend at least partly on the same biochemical and physiological machinery, one could also predict that the evolution of their thermal dependence may not be

completely independent. Our results on *Triturus* newts seem to corroborate the former expectation, revealing clear disparity in the interspecific patterns of variation and co-variation of the parameters describing the curves.

4.1. Differences in TPCs of running and swimming

Because of the high thermal conductance of water, body temperatures of small- to medium-sized aquatic ectotherms closely follow temperatures of surrounding environment. Therefore, aquatic performance should benefit from relatively wide TPCs for locomotor capacity in species inhabiting shallow water bodies, which are characterized by substantial temperature stratification and daily fluctuations (Jacobs et al., 1998). Accordingly, tests on individual species of semi-aquatic ectotherms have revealed that the thermal sensitivity of swimming is low compared with running (Stevenson et al., 1985; Else and Bennett, 1987; Finkler and Claussen, 1999; Marvin, 2003a,b). Our comparative analyses to some extent challenge this idea. First, although our estimates of curve width (w) are higher for swimming than for running in nine out of the 10 species studied, the difference is pronounced in only four of them. The origin of this interspecific variation is unclear. Second, though traditional t -tests suggest a difference in mean width of TPCs between running and swimming ($P = 0.01$), this is not corroborated by more appropriate phylogenetic analyses.

Semi-aquatic animals must cope not only with disparate thermal properties of air and water but also with substantial drag resulting from the relatively high density and viscosity of

Table 3

Correlation coefficients of bivariate relationships between corresponding parameters (species means) of thermal performance curves for running and swimming speed in *Triturus* newts

Trait ^a	TIPS ^b	FIC	PGLS	95% CI ^c	a_c ^d
h	0.20	0.07	0.17	−0.78 to 0.88	5.46 ± 0.19
T_{opt}	−0.19	−0.16	−0.19	−0.99 to 0.99	15.50 ± 0
w	0.45	0.53	0.46	−0.25 to 0.85	11.04 ± 4.47
z_{max}	0.43	0.50	0.45	−0.27 to 0.85	9.81 ± 5.49
B_{80}	−0.06	−0.02	−0.05	−0.66 to 0.60	4.73 ± 0.51

^a h , height (SVL/s); T_{opt} , optimal temperature (°C); w , width (dimensionless); z_{max} , maximum performance (SVL/s); B_{80} , thermal performance breadth (°C).

^b TIPS, non-phylogenetic comparative method; FIC, Felsenstein's independent contrasts; PGLS, phylogenetic generalized least squares.

^c 95% confidence intervals of correlation coefficients obtained using alternative phylogenies. Results were considered significant if 95% CI of correlation coefficients did not include zero. See text for further details.

^d Strength of constraints (mean \pm SE) acting on the correlated evolution of two traits (Martins et al., 2002).

Table 4

Parameters of thermal performance curves for maximum running and swimming speed in *Triturus* newts

Species	Running					Swimming				
	h^a	T_{opt}	w	z_{max}	B_{80}	h	T_{opt}	w	z_{max}	B_{80}
<i>T. alpestris</i>	0.13	27.0	1.24	2.44	9.2	0.80	26.1	2.40	5.26	18.3
<i>T. boscai</i>	−0.18	28.3	1.43	1.84	9.2	0.48	28.3	1.28	8.86	7.6
<i>T. carnifex</i>	0.01	28.3	1.25	2.31	8.9	−0.45	26.7	1.97	5.00	11.5
<i>T. cristatus</i>	−0.16	28.9	1.37	1.95	8.9	−1.23	27.9	1.40	6.41	6.9
<i>T. dobrogicus</i>	−0.43	28.7	1.56	1.42	8.6	0.27	25.6	2.45	4.65	17.9
<i>T. helveticus</i>	−0.32	27.2	1.27	1.94	8.2	−0.22	28.3	1.34	7.81	7.3
<i>T. karelinii</i>	0.33	28.0	1.19	2.75	9.4	−0.40	28.5	1.39	7.31	7.4
<i>T. montandoni</i>	−0.02	26.9	1.07	2.67	8.0	−0.83	28.2	1.31	7.35	6.9
<i>T. pygmaeus</i>	0.93	27.5	1.11	3.54	10.1	0.76	28.4	1.36	8.65	8.2
<i>T. vulgaris</i>	−0.29	27.9	1.37	1.82	8.6	0.84	26.4	2.08	6.00	17.5

^a h , height (SVL/s); T_{opt} , optimal temperature (°C); w , width (dimensionless); z_{max} , maximum performance (SVL/s); B_{80} , thermal performance breadth (°C).

Table 5

Correlation coefficients of bivariate relationships between parameters of thermal performance curves for running and swimming speed in *Triturus* newts

Traits ^a	Running					Swimming				
	TIPS ^b	FIC	PGLS	95% CI ^c	a_c ^d	TIPS	FIC	PGLS	95% CI	a_c
h T_{opt}	−0.31	−0.43	−0.39	−0.92 to 0.65	1.87 ± 0.15	−0.38	−0.60	−0.44	−0.93 to 0.63	9.58 ± 5.92
h w	−0.70	−0.72	−0.70	−0.93 to −0.11	12.88 ± 2.62	0.44	0.64	0.50	−0.89 to 0.99	9.55 ± 5.95
h z_{max}	0.95	0.95	0.95	0.79 to 0.99	11.03 ± 0.62	0.03	−0.25	0.03	−0.64 to 0.67	15.50 ± 0
T_{opt} w	0.72	0.78	0.73	0.19 to 0.93	10.28 ± 5.22	−0.98	−0.99	−0.99	−1.00 to −0.96	8.91 ± 6.59
T_{opt} z_{max}	−0.51	−0.63	−0.60	−0.91 to 0.14	1.50 ± 0.10	0.89	0.91	0.89	0.58 to 0.97	15.50 ± 0
w z_{max}	−0.88	−0.90	−0.88	−0.97 to −0.56	11.36 ± 4.14	−0.87	−0.89	−0.87	−0.97 to −0.53	15.50 ± 0

^a h , height; T_{opt} , optimal temperature; w , width; z_{max} , maximum performance.

^b TIPS, non-phylogenetic comparative method; FIC, Felsenstein's independent contrasts; PGLS, phylogenetic generalized least squares.

^c 95% confidence intervals of correlation coefficients obtained using alternative phylogenies. Significant results (i.e., with 95% CI not including zero) are marked in bold. See text for further details.

^d Strength of constraints (mean \pm SE) acting on the correlated evolution of two traits (Martins et al., 2002).

water. Both physical properties are temperature dependent (Denny, 1993), and thus one might contend that TPC variation between running and swimming merely reflects physical differences between both mediums. If these physical properties matter, consistent differences between TPCs for running and swimming should be detected in all species. However, the fact that marked differences between corresponding TPC parameters for running and swimming speed occurred in four of 10 species only, clearly argues against this hypothesis. Furthermore, in fishes, even artificially high changes in kinematic viscosity affect swimming speed at Reynold's numbers of up to 3600 only (Johnson et al., 1998). Because female newts swim at Reynold's numbers of at least 8600 (Gvoždík, unpublished data), temperature-dependent kinematic viscosity can only have a very minor influence on the shape of TPCs for swimming. Interspecific differences in thermal acclimation to common laboratory conditions may constitute another confounding source of variation in TPCs. Unfortunately, logistic limitations precluded us to further explore this interesting issue, which remains largely unstudied in *Triturus* (see Gvoždík et al., 2007 for an exception). Nonetheless, we feel confident such effects will have played a minor role here, since we kept the laboratory temperature regime within the preferred body temperature range of the examined species (Gvoždík, 2003, 2005, unpublished data). This must have buffered potential species-specific acclimatory responses.

In *Triturus*, the optimal temperatures for running and swimming are remarkably similar and show relatively little interspecific variation given the split of major lineages within this genus is dated to 35–55 mya (Steinfartz et al., 2007). Consistently with the earlier T_{opt} data presented for frogs and lizards (Huey and Bennett, 1987; John-Alder et al., 1988; Huey et al., 1989; Bauwens et al., 1995; but see van Berkum, 1986), this finding may indicate that T_{opt} is evolutionary conservative also in *Triturus* newts (Hertz et al., 1983). However, because other relevant information, such as environmental temperatures and field body temperatures, or TPCs for other ecologically relevant functions is lacking, future studies will need to evaluate whether the rigidity of T_{opt} is due to the absence of heritable variation in optimal temperatures, the weak relationship between locomotor speed and fitness (Angilletta et al., 2002), high effectiveness of behavioral thermoregulation (Bogert, 1949; Huey et al., 2003), strong internal stabilizing selection (Schwenk and Wagner, 2001) or antagonistic genetic correlations with other traits (e.g., Arnold, 1987). In addition, we would like to caution that the interspecific differences noted here could be both enhanced or masked by the fact that we restricted our study to one population for each species. Another limitation of the current study is that we considered female performance only. It is not impossible that some of the interspecific differences in swimming speed observed in females originate through shared genetic architecture with males and have no particular adaptive reason in females. Males of different newt species to various degrees develop seasonal tail crests, a feature that may alter swimming speed (e.g., Janzen and Brodie, 1989). Future studies should investigate the role of such variation in the interspecific patterns described here.

4.2. Co-variation of swimming and running TPC

Our results provide no evidence for the joint co-variation of the individual TPC characteristics (w , T_{opt} , h , z_{max}) with the equivalent characteristic of the other locomotory function. Hence, the idea that the requirement to perform well in the thermal conditions of one medium (e.g., water) would constrain the tuning of the TPC to the thermal particularities of the other medium (e.g., air) remains unsupported by our data. However, TPCs for terrestrial and

aquatic locomotor capacity of *Triturus* newts show substantially different interspecific patterns of variation. Whereas TPCs for running speed evolved mainly in the vertical direction (overall performance), TPCs for swimming speed show major evolutionary shifts in the specialist–generalist direction (shape). Both patterns of TPC variation are considered alternative evolutionary responses by which ectotherms can improve their performance at sub-optimal temperatures (Autumn et al., 1999; Angilletta et al., 2002). The two alternative responses differ in their effect on maximal performance: shifts in the specialist–generalist direction come at the expense of a lowered maximal performance; vertical shifts leave the maximal performance untouched, but may require substantial morphological changes. This may offer an explanation of why vertical shifts are more pronounced in running while specialist–generalist shifts are prevalent in swimming. Because running performance is already relatively poor in *Triturus*, evolutionary changes in TPC that further lower maximal running performance may not be endurable. However, other explanations are possible. For instance, the interspecific pattern of TPC variation may merely reflect different lines of least genetic resistance (Angilletta et al., 2003). Explaining why TPCs of running and swimming show disparate patterns of interspecific variation will require information on the variability of the thermal environment (aquatic and terrestrial) in the respective species, the selection pressures on locomotion and swimming capacity, and the variation in genetic architecture underlying these performance measures.

4.3. Co-variation of TPC traits within swimming and running

While the evolution of TPC characteristics of swimming seems uncoupled from those of running, there is ample evidence for the correlated evolution of characteristics within TPCs of running and swimming (i.e., h , T_{opt} , w , and z_{max} ; see Table 5). This result is in agreement with various functional, biochemical, thermodynamic, and ecological considerations (see Introduction). The evolutionary association among TPC characteristics is especially intricate for running speed. All four parameters show significant coupling, implying that shifts in the specialist–generalist direction are linked to horizontal as well as vertical shifts in TPCs. In swimming, the evolution of one parameter, h , seems relatively independent of that of the other three (T_{opt} , w , z_{max}), which are tightly interconnected. Alas, the low unique variance associated with the relationships among TPC characteristics hinders the functional interpretation of the bivariate interactions.

4.4. The TMV method

As far as we know, this study is the first to employ the TMV method proposed by Izem and Kingsolver (2005) to describe interspecific variation in TPCs. The results seem promising. The model explained an important part of the total variation and in this regard performed better than when describing among-family variation in TPC for relative growth rate in *Pieris* butterflies (the dataset for which the method was applied initially). The difference may not be surprising, as interspecific data tend to have higher “signal-to-noise ratios” than intraspecific data. It also suggests that species-level TPCs for running and swimming speed are well captured by the common template curve, which is a key assumption of the method. One potential drawback of the TMV method in its current version is that it does not take phylogeny into account (Felsenstein, 1985; Harvey and Pagel, 1991; Martins and Hansen, 1996). Fortunately, our conclusions on the co-variation patterns among TPC characteristics seem robust with respect to the assumptions of different evolutionary models, and

the high a_c values obtained by the PGLS method indicate that correlated evolution is impeded by the presence of strong evolutionary constraints (Martins et al., 2002). This result reinforces the likelihood that parameters generated by the TMV method draw a reliable picture of the interspecific variation in TPCs. Still, a version of TMV that acknowledges the interdependence of data groups (species) would be a most welcome tool for exploring the evolution of TPC on an interspecific level.

5. Conclusions

We believe that our study generates several insights that may contribute to a general understanding of the evolution of thermal biology (Levins, 1968; Huey and Slatkin, 1976; Lynch and Gabriel, 1987; Huey and Kingsolver, 1993; Gilchrist, 1995, 2000; Angilletta et al., 2006). First, the prediction that the difference between TPCs relevant to the aquatic and the terrestrial environment could be captured in a simple specialization/generalization dichotomy proved too simple. Most models of TPC evolution emphasize shifts in the specialist–generalist or horizontal direction, but our results on *Triturus* newts suggest that vertical shifts may also play an important role. Accordingly, studies of TPCs for various functions in a range of ectotherms have detected sufficient genetic variation to allow such evolutionary shifts (Kingsolver et al., 2004; Izem and Kingsolver, 2005). Hence, future models of thermal evolution should permit changes in all three major directions (see also Angilletta et al., 2003). Second, patterns of evolutionary co-variation detected for one whole-organism function cannot be readily extrapolated to others. This conclusion follows from our finding that interrelations between TPC characteristics differ widely between swimming and running. In our study system, the disparity may originate from differences in selection between the terrestrial and aquatic environment, but differences in the intrinsic properties of the thermal physiology (e.g., heritabilities) underlying different functions may also produce divergent evolution in TPCs of functions employed under similar circumstances. Future models in evolutionary thermal biology should appreciate this possibility and should not rely on empirical TPC parameters obtained for a single “whole-animal” function (see also Huey, 1982; Stevenson et al., 1985). Third, the TMV method developed by Izem and Kingsolver (2005) may prove an excellent tool to tackle questions regarding preferred direction and constraints on the evolution of TPCs.

Acknowledgments

This manuscript was improved by comments from several anonymous reviewers. We thank J.W. Arntzen, P. Kotlík, P. Mikulíček, J. Piálek, K. Poboljsaj, R. Rozínek, M. Šugerková, R. Valíčková, and V. Zavadil for their help with obtaining newts. LG wishes to thank Monika for psychical support; J. Piálek for logistic support. Permits to capture newts were issued by the Ministry of the Environment of the Czech Republic (OOP/1709/99-V492), the Ministry of the Environment of the Slovak Republic (900/382/98-4.1), the Ministry of the Environment, Spatial Planning and Energy, Ljubljana, Slovenia (354-12-47/99) and by the Institute of Nature Conservation, Lisboa, Portugal. This work was financially supported by grants from the Czech Science Foundation (206/00/D046, 206/01/0695, and 206/06/0953), and the Czech Ministry of Education (LC06073).

References

Angilletta, M.J., Niewiarowski, P.H., Navas, C.A., 2002. The evolution of thermal physiology in ectotherms. *J. Therm. Biol.* 27, 249–268.

- Angilletta, M.J., Wilson, R.S., Navas, C.A., James, R.S., 2003. Tradeoffs and the evolution of thermal reaction norms. *Trends Ecol. Evol.* 18, 234–240.
- Angilletta, M.J., Bennett, A.F., Guderley, H., Navas, C.A., Seebacher, F., Wilson, R.S., 2006. Coadaptation: a unifying principle in evolutionary thermal biology. *Physiol. Biochem. Zool.* 79, 282–294.
- Arnold, S.J., 1983. Morphology, performance and fitness. *Am. Zool.* 23, 347–361.
- Arnold, S.J., 1987. Genetic correlation and the evolution of physiology. In: Feder, M.E., Bennett, A.F., Burggren, W.W., Huey, R.B. (Eds.), *New Directions in Ecological Physiology*. Cambridge University Press, Cambridge, pp. 189–212.
- Ashley-Ross, M.A., Bechtel, B.F., 2004. Kinematics of the transition between aquatic and terrestrial locomotion in the newt *Taricha torosa*. *J. Exp. Biol.* 207, 461–474.
- Autumn, K., Jindrich, D., DeNardo, D., Mueller, R., 1999. Locomotor performance at low temperature and the evolution of nocturnality in geckos. *Evolution* 53, 580–599.
- Bauwens, D., Garland Jr., T., Castilla, A.M., Van Damme, R., 1995. Evolution of sprint speed in lacertid lizards: morphological, physiological, and behavioral covariation. *Evolution* 49, 848–863.
- Björklund, M., 2004. Constancy of the G matrix in ecological time. *Evolution* 58, 1157–1164.
- Bogert, C.M., 1949. Thermoregulation in reptiles, a factor in evolution. *Evolution* 3, 195–211.
- Brodie Jr., E.D., 1977. Salamander antipredator postures. *Copeia* 1977, 523–535.
- Brodie III, E.D., Feldman, C.R., Hanifin, C.T., Motychak, J.E., Mulcahy, D.G., Williams, B.L., Brodie Jr., E.D., 2005. Parallel arms races between garter snakes and newts involving tetrodotoxin as the phenotypic interface of coevolution. *J. Chem. Ecol.* 31, 343–356.
- Castañeda, L.E., Lardies, M.A., Bozinovic, F., 2004. Adaptive latitudinal shifts in the thermal physiology of a terrestrial isopod. *Evol. Ecol. Res.* 6, 579–593.
- Clarke, A., 2003. Costs and consequences of evolutionary temperature adaptation. *Trends Ecol. Evol.* 18, 573–581.
- Darwin, C., 1859. *On the Origin of Species by Means of Natural Selection or, the Preservation of Favoured Races in the Struggle for Life*. John Murray, London.
- David, J.R., Gibert, P., Gravot, E., Petavy, G., Morin, J.P., Karan, D., Moreteau, B., 1997. Phenotypic plasticity and developmental temperature in *Drosophila*: analysis and significance of reaction norms of morphometrical traits. *J. Therm. Biol.* 22, 441–451.
- Dawson, W.R., Bartholomew, G.A., Bennett, A.F., 1977. A reappraisal of the aquatic specializations of the Galapagos marine iguana (*Amblyrhynchus cristatus*). *Evolution* 31, 891–897.
- Dejours, P., 1987. Water and air physical characteristics and their physiological consequences. In: Dejours, P., Bolis, L., Taylor, C.R., Weibel, E.R. (Eds.), *Comparative Physiology: Life in Water and on Land*. IX-Liviana Press, Padova, pp. 3–11.
- Dejours, P., Bolis, L., Taylor, C.R., Weibel, E.R., 1987. *Comparative Physiology: Life in Water and on Land*. IX-Liviana Press, Padova.
- Denny, M.W., 1993. *Air and Water*. Princeton University Press, Princeton, NJ.
- Else, P.L., Bennett, A.F., 1987. The thermal dependence of locomotor performance and muscle contractile function in the salamander *Ambystoma tigrinum nebulosum*. *J. Exp. Biol.* 128, 219–233.
- Eppley, R.W., 1972. Temperature and phytoplankton growth in the sea. *Fish. Bull.* 70, 1063–1085.
- Fasola, M., Canova, L., 1992. Residence in water by the newts *Triturus vulgaris*, *T. cristatus* and *T. alpestris* in a pond in northern Italy. *Amphibia-Reptilia* 13, 227–233.
- Felsenstein, J., 1985. Phylogenies and the comparative method. *Am. Nat.* 125, 1–15.
- Finkler, M.S., Claussen, D.L., 1999. Influence of temperature, body size, and inter-individual variation on forced and voluntary swimming and crawling speeds in *Nerodia sipedon* and *Regina septemvittata*. *J. Herpetol.* 33, 62–71.
- Fish, F.E., 2000. Biomechanics and energetics in aquatic and semiaquatic mammals: platypus to whale. *Physiol. Biochem. Zool.* 73, 683–698.
- Frazier, M.R., Huey, R.B., Berrigan, D., 2006. Thermodynamics constrains the evolution of insect population growth rates: “warmer is better”. *Am. Nat.* 168, 512–520.
- Garland Jr., T., Ives, A.R., 2000. Using the past to predict the present: confidence intervals for regression equations in phylogenetic comparative methods. *Am. Nat.* 155, 346–364.
- Garland Jr., T., Midford, P.E., Ives, A.R., 1999. An introduction to phylogenetically based statistical methods, with a new method for confidence intervals on ancestral values. *Am. Zool.* 39, 374–388.
- Gilchrist, G.W., 1995. Specialists and generalists in changing environments. 1. Fitness landscapes of thermal sensitivity. *Am. Nat.* 146, 252–270.
- Gilchrist, G.W., 1996. A quantitative genetic analysis of thermal sensitivity in the locomotor performance curve of *Aphidius ervi*. *Evolution* 50, 1560–1572.
- Gilchrist, G.W., 2000. The evolution of thermal sensitivity in changing environments. In: Storey, K.B., Storey, J. (Eds.), *Environmental Stressors and Gene Responses*. Elsevier Science, Amsterdam, pp. 55–70.
- Gilchrist, G.W., Kingsolver, J.G., 2001. Is optimality over the hill? In: Orzack, S.H., Sober, E. (Eds.), *Adaptationism and Optimality*. Cambridge University Press, Cambridge, pp. 219–241.
- Gillis, G.B., Blob, R.W., 2001. How muscles accommodate movement in different physical environments: aquatic vs. terrestrial locomotion in vertebrates. *Comp. Biochem. Physiol. A* 131, 61–75.
- Gotthard, K., Nylin, S., 1995. Adaptive plasticity and plasticity as an adaptation—a selective review of plasticity in animal morphology and life-history. *Oikos* 74, 3–17.

- Grafen, A., 1989. The phylogenetic regression. *Phil. Trans. R. Soc. London B* 326, 119–157.
- Griffiths, R.A., 1996. *Newts and Salamanders of Europe*. Academic Press, London.
- Grossenbacher, K., Thiesmeier, B., 2003. *Handbuch der Reptilien und Amphibien Europas. Schwanzlurche IIA*. AULA-Verlag, Wiebelsheim.
- Gvoždík, L., 2003. Postprandial thermophily in the Danube crested newt, *Triturus dobrogicus*. *J. Therm. Biol.* 28, 545–550.
- Gvoždík, L., 2005. Does reproduction influence temperature preferences in newts? *Can. J. Zool.* 83, 1038–1044.
- Gvoždík, L., Van Damme, R., 2006. *Triturus* newts defy the running-swimming dilemma. *Evolution* 60, 2110–2121.
- Gvoždík, L., Puky, M., Šugerková, M., 2007. Acclimation is beneficial at extreme test temperatures in the Danube crested newt, *Triturus dobrogicus* (Caudata, Salamandridae). *Biol. J. Linn. Soc.* 90, 627–636.
- Hagström, T., 1979. Population ecology of *Triturus cristatus* and *T. vulgaris* (Urodela) in SW Sweden. *Holarctic Ecol.* 2, 108–114.
- Hair Jr., J.F., Anderson, R.E., Tatham, R.L., Black, W.C., 1998. *Multivariate Data Analysis*. Prentice-Hall, Upper Saddle River, NJ.
- Harvey, P.H., Pagel, M.D., 1991. *The Comparative Method in Evolutionary Biology*. Oxford University Press, Oxford.
- Hertz, P.E., Huey, R.B., Nevo, E., 1983. Homage to Santa Anita: thermal sensitivity of sprint speed in agamid lizards. *Evolution* 37, 1075–1084.
- Hochachka, P.W., Somero, G.N., 2002. *Biochemical Adaptation*. Oxford University Press, New York.
- Housworth, E.A., Martins, E.P., Lynch, M., 2004. The phylogenetic mixed model. *Am. Nat.* 163, 84–96.
- Huey, R.B., 1982. Temperature, physiology, and the ecology of reptiles. In: Gans, C., Pough, F.H. (Eds.), *Biology of the Reptilia*. Vol. 12. Physiology C, Physiological Ecology. Academic Press, London, pp. 25–91.
- Huey, R.B., Bennett, A.F., 1987. Phylogenetic studies of coadaptation: preferred temperatures versus optimal performance temperatures of lizards. *Evolution* 41, 1098–1115.
- Huey, R.B., Hertz, P.E., 1984. Is a jack-of-all-temperatures a master of none? *Evolution* 38, 441–444.
- Huey, R.B., Kingsolver, J.G., 1989. Evolution of thermal sensitivity of ectotherm performance. *Trends Ecol. Evol.* 4, 131–135.
- Huey, R.B., Kingsolver, J.G., 1993. Evolution of resistance to high temperature in ectotherms. *Am. Nat.* 142, S21–S46.
- Huey, R.B., Slatkin, M., 1976. Cost and benefits of lizard thermoregulation. *Q. Rev. Biol.* 51, 363–384.
- Huey, R.B., Stevenson, R.D., 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *Am. Zool.* 19, 357–366.
- Huey, R.B., Niewiarowski, P.H., Kaufmann, J., Herron, J.C., 1989. Thermal biology of nocturnal ectotherms: is sprint performance of geckos maximal at low body temperatures? *Physiol. Zool.* 62, 488–504.
- Huey, R.B., Hertz, P.E., Sinervo, B., 2003. Behavioral drive versus behavioral inertia in evolution: a null model approach. *Am. Nat.* 161, 357–366.
- Irschick, D.J., Garland Jr., T., 2001. Integrating function and ecology in studies of adaptation: investigations of locomotor capacity as a model system. *Ann. Rev. Ecol. Syst.* 32, 367–396.
- Izem, R., Kingsolver, J.G., 2005. Variation in continuous reaction norms: quantifying directions of biological interest. *Am. Nat.* 166, 277–289.
- Jacobs, A.F.G., Heusinkveld, B.G., Nieveen, J.P., 1998. Temperature behavior of a natural shallow water body during a summer period. *Theor. Appl. Climatol.* 59, 121–127.
- Janzen, F.J., Brodie, E.D., 1989. Tall tails and sexy males—sexual behaviour of rough-skinned newts (*Taricha granulosa*) in a natural breeding pond. *Copeia* 1989, 1068–1071.
- John-Alder, H.B., Morin, P.J., Lawler, S., 1988. Thermal physiology, phenology, and distribution of tree frogs. *Am. Nat.* 132, 506–520.
- Johnson, T.P., Cullum, A.J., Bennett, A.F., 1998. Partitioning the effects of temperature and kinematic viscosity on the c-start performance of adult fishes. *J. Exp. Biol.* 201, 2045–2051.
- Kingsolver, J.G., Ragland, G.J., Shlichta, J.G., 2004. Quantitative genetics of continuous reaction norms: thermal sensitivity of caterpillar growth rates. *Evolution* 58, 1521–1529.
- Kingsolver, J.G., Massie, K.R., Shlichta, J.G., Smith, M.H., Ragland, G.J., Gomulkiewicz, R., 2007. Relating environmental variation to selection on reaction norms: an experimental test. *Am. Nat.* 169, 163–174.
- Levins, R., 1968. *Evolution in Changing Environments*. Princeton University Press, Princeton, NJ.
- Lynch, M., Gabriel, W., 1987. Environmental tolerance. *Am. Nat.* 129, 283–303.
- Martins, E.P., 1996. Conducting phylogenetic comparative studies when the phylogeny is not known. *Evolution* 50, 12–22.
- Martins, E.P., 2004. COMPARE, Version 4.6b. Computer Programs for the Statistical Analysis of Comparative Data. Indiana University, Bloomington, IN.
- Martins, E.P., Hansen, T.F., 1996. The statistical analysis of interspecific data: a review and evaluation of phylogenetic comparative methods. In: Martins, E.P. (Ed.), *Phylogenies and the Comparative Method in Animal Behavior*. Oxford University Press, Oxford, pp. 22–75.
- Martins, E.P., Hansen, T.F., 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *Am. Nat.* 149, 646–667.
- Martins, E.P., Diniz, J.A.F., Housworth, E.A., 2002. Adaptive constraints and the phylogenetic comparative method: a computer simulation test. *Evolution* 56, 1–13.
- Marvin, G.A., 2003a. Effects of acute temperature and thermal acclimation on aquatic and terrestrial locomotor performance of the three-lined salamander, *Eurycea guttolineata*. *J. Therm. Biol.* 28, 251–259.
- Marvin, G.A., 2003b. Aquatic and terrestrial locomotor performance in a semiaquatic plethodontid salamander (*Pseudotriton ruber*): influence of acute temperature, thermal acclimation, and body size. *Copeia* 2003, 704–713.
- Navas, C.A., 1996. Metabolic physiology, locomotor performance, and thermal niche breadth in neotropical anurans. *Physiol. Zool.* 69, 1481–1501.
- Ord, T.J., Martins, E.P., 2006. Tracing the origins of signal diversity in anole lizards: phylogenetic approaches to inferring the evolution of complex behaviour. *Anim. Behav.* 71, 1411–1429.
- Price, T., 1997. Correlated evolution and independent contrasts. *Phil. Trans. R. Soc. London B* 352, 519–529.
- Savage, V.M., Gillooly, J.F., Brown, J.H., West, G.B., Charnov, E.L., 2004. Effects of body size and temperature on population growth. *Am. Nat.* 163, 429–441.
- Schlichting, C.D., Pigliucci, M., 1996. *Phenotypic Evolution: A Reaction Norm Perspective*. Sinauer, Sunderland, MA.
- Schwenk, K., Wagner, G.P., 2001. Function and the evolution of phenotypic stability: connecting pattern to process. *Am. Zool.* 41, 552–563.
- Sgrò, C.M., Hoffmann, A.A., 2004. Genetic correlations, tradeoffs and environmental variation. *Heredity* 93, 241–248.
- Spotila, J.R., O'Connor, M.P., Bakken, G.S., 1992. Biophysics of heat and mass transfer. In: Feder, M.E., Burggren, W.W. (Eds.), *Environmental Physiology of the Amphibians*. Chicago University Press, Chicago, pp. 59–80.
- StatSoft Inc., 2000. STATISTICA for Windows (Computer Program Manual). StatSoft, Inc., Tulsa, OK.
- Steinfartz, S., Vicario, S., Arntzen, J.W., Caccone, A., 2007. A Bayesian approach on molecules and behavior: reconsidering phylogenetic and evolutionary patterns of the Salamandridae with emphasis on *Triturus* newts. *J. Exp. Zool. B* 308, 139–162.
- Stevenson, R.D., Peterson, C.R., Tsuji, J.S., 1985. The thermal dependence of locomotion, tongue flicking, digestion, and oxygen consumption in the wandering garter snake. *Physiol. Zool.* 58, 46–57.
- Thiesmeier, B., Grossenbacher, K., 2004. *Handbuch der Reptilien und Amphibien Europas. Schwanzlurche IIB*. AULA-Verlag, Wiebelsheim.
- van Berkum, F.H., 1986. Evolutionary patterns of the thermal sensitivity of sprint speed in *Anolis* lizards. *Evolution* 40, 594–604.
- van Berkum, F.H., 1988. Latitudinal patterns of the thermal sensitivity of sprint speed in lizards. *Am. Nat.* 132, 327–343.
- Van Damme, R., Van Dooren, T.J.M., 1999. Absolute versus per unit body length speed of prey as an estimator of vulnerability to predation. *Anim. Behav.* 57, 347–352.
- Via, S., Gomulkiewicz, R., de Jong, G., Scheiner, S., Schlichting, C.D., van Tienderen, P.H., 1995. Adaptive phenotypic plasticity: consensus and controversy. *Trends Ecol. Evol.* 5, 212–217.
- Weisrock, D.W., Papenfuss, T.J., Macey, J.R., Litvinchuk, S.N., Polymeni, R., Ugurtas, I.H., Zhao, E., Jowkar, H., Larson, A., 2006. A molecular assessment of phylogenetic relationships and lineage accumulation rates within the family Salamandridae (Amphibia, Caudata). *Mol. Phylog. Evol.* 41, 368–383.
- Wilson, R.S., 2005. Consequences of metamorphosis for the locomotor performance and thermal physiology of the newt *Triturus cristatus*. *Physiol. Biochem. Zool.* 78, 967–975.