

TRITURUS NEWTS DEFY THE RUNNING-SWIMMING DILEMMA

LUMÍR GVOŽDÍK¹ AND RAOUL VAN DAMME²

¹Department of Population Biology, Institute of Vertebrate Biology AS CR, Studenec 122, 67502 Koněšín, Czech Republic
E-mail: gvozdik@brno.cas.cz

²Department of Biology, University of Antwerp, Universiteitplein 1, 2610 Wilrijk, Belgium
E-mail: raoul.vandamme@ua.ac.be

Abstract.—Conflicts between structural requirements for carrying out different ecologically relevant functions may result in a compromise phenotype that maximizes neither function. Identifying and evaluating functional trade-offs may therefore aid in understanding the evolution of organismal performance. We examined the possibility of an evolutionary trade-off between aquatic and terrestrial locomotion in females of European species of the newt genus *Triturus*. Biomechanical models suggest a conflict between the requirements for aquatic and terrestrial locomotion. For instance, having an elongate, slender body, a large tail, and reduced limbs should benefit undulatory swimming, but at the cost of reduced running capacity. To test the prediction of an evolutionary trade-off between swimming and running capacity, we investigated relationships between size-corrected morphology and maximum locomotor performance in females of ten species of newts. Phylogenetic comparative analyses revealed that an evolutionary trend of body elongation (increasing axilla-groin distance) is associated with a reduction in head width and forelimb length. Body elongation resulted in reduced maximum running speed, but, surprisingly, also led to a reduction in swimming speed. The evolution of longer tails was associated with an increase in maximal swimming speed. We found no evidence for an evolutionary trade-off between aquatic and terrestrial locomotor performance, probably because of the unexpected negative effect of body elongation on swimming speed. We conclude that the idea of a design conflict between aquatic and terrestrial locomotion, mediated through antagonistic effects of body elongation, does not apply to our model system.

Key words.—Amphibians, body elongation, comparative methods, evolutionary trade-off, locomotor performance, semi-aquatic lifestyle.

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The notion of functional trade-offs is central to our understanding of the evolution of whole-organism performance, and it is generally accepted that trade-offs between competing functions may constrain adaptive evolution of individual performance traits (Gould and Lewontin 1979; Rose and Lauder 1996; Orzack and Sober 2001). Selection operating on whole-animal performance typically results in a compromise phenotype that allows for the execution of conflicting functions, but optimizes none. Identifying such conflicts in functional requirements should help us understand limits imposed on the evolution of performance (Garland and Losos 1994).

Trade-offs have received ample consideration in studies of locomotor performance. Biomechanical, physiological, and ecological theory suggests that the evolution of locomotor performance may be constrained because the same functional system (e.g., the limb muscles) often must perform several disparate tasks (e.g., sprinting and long distance running); because a single phenotype cannot simultaneously maximize all performance traits (e.g., running, swimming, and flying; Losos 1990; Garland and Losos 1994; Vanhooydonck et al. 2001; but see Angilletta et al. 2003), or because specialization for locomotor performance in one environment can be achieved only at the cost of compromised performance in other contexts (Levins 1968; Futuyma and Moreno 1988). The empirical evidence is mixed. Studies within species generally find little support for the existence of trade-offs (e.g., Bennett 1980; Garland 1984, 1988; Garland and Else 1987; Sorci et al. 1995; but see Van Damme et al. 2002), and whereas several interspecific comparisons have demonstrated trade-offs between locomotor functions (e.g., Huey et al. 1984; Harper and Blake; 1990; Losos et al. 1993; Fish and Baudinette 1999; Reidy et al. 2000; Vanhooydonck et al.

2001), others have not (e.g., Garland et al. 1988; Vanhooydonck and Van Damme 2001).

Animals that live both in water and on land seem interesting study models in this respect. The survival value of maximum locomotor speed has been demonstrated in both terrestrial and aquatic environments (Christian and Tracy 1981; Jayne and Bennett 1990; Langerhans et al. 2004; Miles 2004). However, the radically different physical properties of air and water create contrasting conditions for locomotion (Denny 1993; Vogel 1994). Aquatic locomotion is primarily governed by drag and buoyancy, whereas terrestrial locomotion entails overcoming gravitational forces (Alexander 2003). This disparity in selection pressures between both environments has produced well-known examples of convergent evolution in several lineages of vertebrates, with distantly related aquatic species (e.g., whales and sharks) resembling each other closely in general body shape and appendages, whereas more closely related species, living in different environments, have strikingly different morphologies (e.g., whales versus boars or peccaries). It seems likely that the conflicting requirements for efficient locomotion in aquatic and terrestrial environments will compromise the overall performance of semiaquatic animals (Fish and Baudinette 1999; Gillis and Blob 2001; Shine and Shetty 2001). That is, morphological adaptations for effective swimming likely will reduce terrestrial locomotor ability and vice versa.

Body elongation may play an important role in this issue. In water, elongate bodies allow large-amplitude propulsive movements, and thus should benefit speed and acceleration capacity. However, on land, relatively stiff, stout bodies seem better suited for generating forward thrust during fast locomotion. In several vertebrate lineages, the evolution of longer

bodies has coincided with a decrease in limb dimensions (Gans 1975; Lande 1978; Greer 1987; Wiens and Slingluff 2001). This appears to be an adaptive trend in aquatic environments, where (nonspecialized) limbs are largely ineffective and merely produce extra drag. In contrast, on land limb length is an important determinant of stride length, which, together with stride frequency, affects running speed in limbed tetrapods (e.g., Barclay 1946; Sukhanov 1968; Alexander 2003). Having a large, stout tail should also promote aquatic locomotion, because this permits the undulation to continue backwards, pushing further against the water. Accordingly, maximum swimming speed in animals that move by axial undulation is strongly affected by tail aspect ratio (the ratio between tail height and tail length/area), tail beat frequency, and amplitude (e.g., Bainbridge 1963; Vogel 1994; Gillis 1996). In contrast, large tails, especially tails with high aspect ratios, may hamper terrestrial locomotion, either because of the extra mass, or by physically obstructing limb movements. These considerations imply a trade-off between swimming and running capacities in animals that use limbs to run and axial undulation to swim.

European newts of the genus *Triturus* seem interesting model animals to test the running-swimming conflict hypothesis. Members of this genus differ considerably in the average duration of the aquatic period (between three and eight months per year; Grossenbacher and Thiesmeier 2003; Thiesmeier and Grossenbacher 2004), suggesting variation in selection on aquatic versus terrestrial locomotion abilities among species. Arntzen and Wallis (1999) demonstrated that in a subset of *Triturus* species, the number of trunk vertebrae (i.e., vertebrae bearing ribs) correlates with body elongation (i.e., size-corrected axilla-groin distance), which in turn relates inversely to limb length. Because the more elongate species in their study have longer aquatic periods, Arntzen and Wallis (1999) hypothesized that body elongation is an adaptation that allows higher locomotor performance in water.

In this study we tested the hypothesis of a trade-off between swimming and running performance, mediated by the differential effects of body elongation and its associated morphological changes. We address two hypotheses. First, throughout the whole genus, body elongation is associated with a reduction in limb length. Second, longer bodies increase locomotor performance in the aquatic environment at the expense of running performance on land. We predict that the evolution of longer bodies, larger (longer and higher) tails, and smaller limbs will increase swimming speed, but will decrease running speed. Consequently, we expect a negative relationship between running and swimming speed.

MATERIALS AND METHODS

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), *T. alpestris* (SVL up to 70 mm), and *T. vittatus* (SVL up to 70 mm) (Fig. 1; Steinfartz et al., in press). *Triturus* newts are usually terrestrial for part of the year, but they enter still or, more rarely, slow-flowing water in spring to breed. They feed on a wide variety

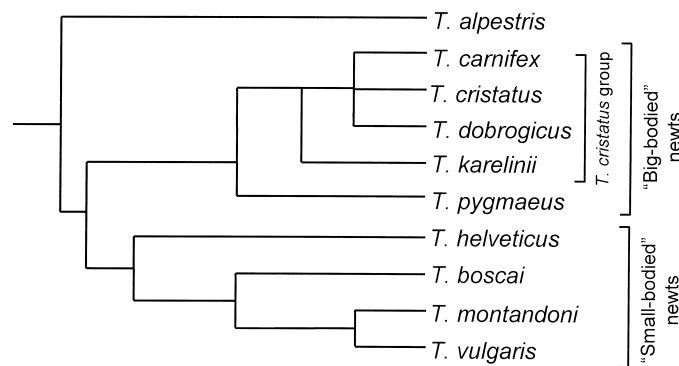


FIG. 1. Hypothesized phylogenetic relationships of *Triturus* species used in this study, as inferred from mitochondrial DNA data (Steinfartz et al., in press). Branch lengths in the graph are not proportional to actual divergence times. The trifurcation in the *T. cristatus* group is considered a hard polytomy.

of small invertebrates, both in and out of the water, and some species occasionally prey on small fish and other amphibians (Arnold and Burton 1992). Although quantitative measurements of locomotor speed in the wild are lacking, newts use fast locomotor bouts to avoid predation both on land (Brodie 1977) and in the water (Griffiths 1996), suggesting that both aquatic and terrestrial locomotor speed are ecologically relevant functions in these animals.

Newts were collected between 1999 and 2002 at different natural sites in Europe. Specimens of one species (*T. boscai*) were obtained from the pet trade. To minimize possible effects of geographical variation, each species was represented by individuals from one population only. 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, nonreproductive females. Mean SVLs of the females used were similar to those reported in the literature (compiled in Malmgren 2001), thus we are confident that, in this respect, our samples are representative for the species studied. Although *Triturus* species exhibit sexual dimorphism in body size and shape, males follow similar (but less pronounced) patterns of among-species variation as females (Malmgren and Tholleson 1999; Malmgren 2001).

The newts were housed separately or in pairs in plastic recipients ($50 \times 30 \times 25$ cm³) holding 15 liter of tap water. Female newts are nonterritorial, and housing newts in pairs is consequently unlikely to influence their locomotor performance. Each container held a piece of styrofoam (10 × 15 cm) and several specimens of Java moss plants (*Vesicularia dubyana*) that served as hiding places. Kept in these conditions, the newts did not shift to the terrestrial phase, and remained semiaquatic. This phenomenon has also been observed in natural conditions (e.g., Andreone and Giacoma 1989). 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 three 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, unpubl. data). All individuals received similar amounts of food items

(earthworms, *Tubifex* worms, or fish meat), once or twice a week.

Morphometrics

We anesthetized the newts by immersing them in 0.08% phenoxyethanol prior to measurement. From each individual, we took the following measurements to the nearest 0.1mm, using digital calipers: SVL (from the tip of the snout to the posterior edge of the cloaca); axilla-groin distance; tail length (from the posterior edge of the cloaca to the tip of the tail) and height (at the highest point); head width (measured as the distance between the corners of the mouth); forelimb length (from the elbow to the tip of the longest finger); hindlimb length (from the groin to the tip of the longest toe). All limb measurements were performed on the left pair of limbs.

Locomotor Performance Measurements

We measured swimming and running speed of the newts in a linear racetrack (200 cm long \times 10 cm wide). The central 100 cm of the racetrack was equipped with four pairs of infrared photocells, placed at 25 cm intervals. 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. The photocells were connected to a computer that recorded the times (precision 0.001 sec) at which the newts passed by. During the swimming trials, the racetrack was filled with 3 cm of tap water; during the running trials, a surface of moist paper towel was used. Because optimal temperatures for locomotion may vary among species (e.g., van Berkum 1986; John-Alder et al. 1988; Navas 1996), all performance measurements were carried out in a temperature controlled room, and repeated at 20, 15, 25, 33, 10, and 30°C. The order in which individuals were subjected to terrestrial and aquatic locomotor trials was randomized. Because newts are mostly nocturnal, all experiments were completed between 18:00 and 24:00 hr. The newts were fed *Tubifex* worms the day following a trial and were allowed to rest for another two days before the next test. Body mass was monitored throughout the performance trials. Three individuals that lost considerable weight ($>15\%$ of their total body mass) were excluded from the analyses.

Prior to the speed trials, the newts were placed in receptacles containing 2 cm of water that were gradually ($0.5^{\circ}\text{C min}^{-1}$) cooled or heated until they reached the test temperature. After another 30 min, an individual was placed on the racetrack, 10 cm in front of the first photocell. The newt was induced to move down the racetrack by tapping the tip of its tail with a soft paintbrush. Each individual was tested four times at each temperature, allowing a 60 min recovery period between two consecutive runs. Each trial was judged as "good" or "bad" (e.g., van Berkum and Tsuji 1987) and bad trials ($<15\%$) were discarded. The fastest speed over any 25 cm interval was taken as a measure of maximum running or swimming speed. A previous study, following similar proceedings, demonstrated good short-term repeatability of locomotor performance in *Triturus* (L. Gvoždík, M. Puky, and M. Šugerková, unpubl. data). We will assume that the differences in laboratory measures of locomotor performance reliably reflect variation in speed under natural conditions.

We do not actually have field data to support this assertion, but work on lizards (Irschick et al. 2005) has shown that differences between laboratory and field measures of sprint speed are minor in relatively slow species, and all our study species fall into this category. Because the species used in this study vary considerably in body size, and because it has been argued that body size may confound the effect of speed on escape success (Van Damme and Van Dooren 1999), relative speed (SVL s^{-1}) rather than absolute speed was used in the analyses.

Phylogeny

The topology of the tree used in the comparative analyses follows the results from a Bayesian analysis of mitochondrial DNA data (Steinfartz et al., in press, Fig. 1). This phylogenetic hypothesis was preferred because, unlike previous studies (reviewed by Zajc and Arntzen 1999), it is based on complete taxon sampling of all *Triturus* species. It provides fully resolved relationships, except for the *T. cristatus* group, which is considered a hard polytomy (J. W. Arntzen, pers. comm. 2006). Unfortunately, the information on branch lengths provided by Steinfartz et al. is incomplete and/or inconsistent with previously published estimates (e.g., by Oosterbroek and Arntzen 1992; Larson et al. 2003; Babik et al. 2005). We therefore set all branch lengths equal to one, or used computer-generated branch lengths in our comparative analyses (details below).

Phylogenetic Comparative Analyses

Since Felsenstein's (1985) seminal paper, several methods have become available to control for phylogeny in comparative data (Harvey and Pagel 1991; Martins and Hansen 1996). Because they are based on contrasting evolutionary assumptions, different phylogenetic techniques may yield different results, especially when the number of species involved is low (Martins et al. 2002). It has therefore been recommended to use at least two phylogenetic approaches with different assumptions (Martins et al. 2002; Housworth et al. 2004), in addition to a nonphylogenetic analysis (Price 1997; Garland et al. 1999).

Accordingly, we analyzed our data using the raw data (TIPS), with Felsenstein's independent contrasts (FIC, Felsenstein 1985), and with the phylogenetic generalized least squares method (PGLS, Grafen 1989; Martins and Hansen 1997). Felsenstein's independent contrasts method is the most commonly used comparative approach and its assumptions and computational details have been described extensively elsewhere (Felsenstein 1985; Harvey and Pagel 1991; Garland et al. 1992). This method assumes that the data result from an evolutionary process that is similar to Brownian motion, that is, genetic drift or directional selection with randomly changing directions of selection. Phylogenetic generalized least squares is an extension of Felsenstein's method, based on generalized linear model theory (McCullagh and Nelder 1989). We chose to use this method for two reasons. First, it accounts for measurement error, which is important when sample sizes are small (Harmon and Losos 2005). Second, it incorporates the Ornstein-Uhlenbeck model of phenotypic evolution by genetic drift under various constraints

(Lande 1976; Felsenstein 1988; Hansen 1997). The strength of constraints acting on the evolution of a phenotype is described by a parameter, a_c . If $a_c = 0$ and within-species phenotypic variation is negligible, PGLS and FIC yield identical results. However, with larger a_c -values (e.g., $a_c > 15$, indicating strong evolutionary constraints) and/or substantial intraspecific variation, PGLS will yield results that are more similar to raw data analyses. By applying three comparative methods, we cover a broad range of evolutionary mechanisms and assumptions, which should allow us to assess the robustness of the results.

We used the PGLS module in the COMPARE 4.6 package (Martins 2004) to examine relationships among traits, with (1) $a_c = 0$ (i.e., the FIC approach); (2) a_c estimated from the data, using a maximum-likelihood grid search (i.e., the PGLS approach); and (3) a_c set at a large value (i.e., the TIPS approach). The model with the highest log-likelihood value (logL; e.g., Rice 1995) was considered the most appropriate (for details, see Martins and Hansen 1997). Depending on the model selected, we used independent contrasts, PGLS-transformed data (Butler et al. 2000) or raw data to visualize the relationships in plots. To evaluate the effect of unknown branch lengths on results of comparative analyses we repeated calculations for 200 possible trees with the same topology (Fig. 1) but randomly generated branch lengths. We thus obtained 95% confidence intervals for correlation coefficients and regression slopes (Martins 1996) that included sampling variance and variance due to uncertain phylogeny.

Hypotheses on ancestral character states can be useful in understanding evolutionary trajectories. Several methods have been developed to reconstruct ancestral phenotypes (Farris 1970; Maddison 1991; Martins and Hansen 1997; Schlutter et al. 1997; Garland et al. 1999). We chose the approach implemented in the COMPARE package, because it provides standard errors to the estimates, and because it allows estimating ancestral values under different microevolutionary assumptions: Brownian motion (linear PGLS model) or constrained evolution (exponential PGLS model) (Martins and Hansen 1997; Martins 1999).

Statistical Analyses

Because the newt species vary considerable in body size (Malmgren 2001), we corrected all morphological variables for size prior to further analyses, using Mosimann's (1970) multivariate method. This method avoids problems associated with the use of residuals from ordinary least squares regression and with other methods controlling for body size effects (Klingenberg 1996; Darlington and Smulders 2001; Butler and Losos 2002). Mosimann's (1970) method removes the effects of size for each observation using a directly measured index of individual size (here SIZE, the geometric mean of the seven morphometric traits measured). Each individual was adjusted for size by taking the difference of each log-variable with logSIZE.

Species means for size-corrected (i.e., relative) variables and independent contrasts were checked for normality (normal quantile plot). Because no substantial deviations from normality were detected, we used Pearson product-moment correlations to test for associations between variables. The

relationships between relative axilla-groin distance and forelimb and hindlimb length were estimated using ordinary least squares regression. We used backward stepwise multiple regression (with P for removing a variable = 0.1) to evaluate associations between relative axilla-groin distance, tail length, and tail height on relative swimming speed. Because multicollinearity may confound results of multiple regression (Hair et al. 1998), we first checked for intercorrelations among predictor variables. All regressions with independent contrasts were forced through the origin (Garland et al. 1992). A significance level of $\alpha = 0.05$ was used for all statistical tests. All means are reported \pm standard error. Statistical analyses were performed in Statistica 6.1 (StatSoft 2000).

RESULTS

Table 1 summarizes the morphological and performance data for females of ten species of *Triturus*. Models of evolution under strong evolutionary constraints showed the best fit in all analyses (comparison of maximum logL-values, $a_c \geq 15.5$, Table 2). Accordingly, uncertainties in the phylogeny did not affect the results of hypothesis tests (Tables 2 and 3).

Consistent with the body elongation hypothesis, axilla-groin distance is negatively correlated with both forelimb length and head width when controlling for the effect of body size (Fig. 2A, C; Table 2). The relationship between relative axilla-groin distance and hindlimb length is statistically non-significant, but a negative trend similar to that with relative forelimb length can be observed (Fig. 2B; Table 2). Contrary to our expectations, we found no evidence for an association between relative axilla-groin distance and tail length or tail height (Fig. 2D,E; Table 2).

The associations between axilla-groin distance, forelimb length, and head width remain significant when only "big-bodied" newts are considered (axilla-groin distance vs. forelimb length: $r = -0.97$, $n = 5$, $P = 0.006$; axilla-groin distance vs. head width: $r = -0.95$, $P = 0.014$). In "small-bodied" newts, the association between relative axilla-groin length and head width is near to statistical significance ($r = -0.92$, $n = 4$, $P = 0.08$), but there is no evidence for a correlation between relative axilla-groin length and fore limb length ($r = -0.03$, $n = 4$, $P = 0.49$).

According to the logL-values, the effects of relative axilla-groin distance, tail length, and tail height on relative swimming speed are best tested using a model that assumes evolution under strong constraints (PGLS: $a_c = 15.5$; logL = 24.01; FIC: logL = 21.37; TIPS: logL = 24.02). Therefore, we used the raw data to select the best predictor variables for relative swimming speed (for comparative purposes, we also present results of an analysis using independent contrasts, see Table 3). Species with longer tails and shorter axilla-groin distances (relative to body size) swim faster (backward stepwise regression, $F_{2,7} = 5.62$, $r^2 = 0.51$, $P = 0.04$, Fig. 3; Table 3).

Similarly, the effects of relative axilla-groin distance, tail length, and tail height on relative swimming speed are best tested using a model that assumes evolution under strong constraints ($a_c = 13.05$; PGLS: logL = 23.39; FIC: logL = 21.52; TIPS: logL = 23.39). Therefore, we used the raw data

TABLE 1. Summary statistics (mean \pm SE) of morphological traits (in mm) and maximum locomotor performance (in cm s^{-1}) for ten species of *Triturus* newts. Species are ordered with respect to their phylogeny (Fig. 1).

Species (n)	Snout-vent length	Axilla-groin distance	Head width	Forelimb length	Hindlimb length	Tail length	Tail height	Swimming speed	Running speed
<i>T. alpestris</i> (6)	59.0 \pm 1.8	31.2 \pm 1.1	9.7 \pm 0.3	18.9 \pm 0.6	17.2 \pm 0.6	46.5 \pm 1.6	6.8 \pm 0.5	34.4 \pm 4.0	15.3 \pm 1.4
<i>T. carnifex</i> (12)	85.9 \pm 1.0	46.8 \pm 0.6	14.2 \pm 0.1	26.9 \pm 0.3	25.4 \pm 0.3	65.9 \pm 0.9	13.0 \pm 0.3	44.0 \pm 2.3	21.8 \pm 0.8
<i>T. cristatus</i> (8)	77.3 \pm 1.2	43.9 \pm 0.8	11.2 \pm 0.2	20.4 \pm 0.4	19.8 \pm 0.4	57.2 \pm 1.2	10.3 \pm 0.3	47.2 \pm 2.8	15.9 \pm 1.0
<i>T. dobrogicus</i> (12)	84.5 \pm 1.1	52.6 \pm 0.6	10.1 \pm 0.2	19.1 \pm 0.3	19.8 \pm 0.3	64.0 \pm 1.0	9.6 \pm 0.3	45.0 \pm 2.4	13.0 \pm 0.8
<i>T. karelinii</i> (5)	70.0 \pm 1.6	36.4 \pm 1.0	11.5 \pm 0.2	23.1 \pm 0.5	22.3 \pm 0.5	57.2 \pm 1.5	9.6 \pm 0.4	51.6 \pm 3.6	21.3 \pm 1.3
<i>T. pygmaeus</i> (5)	62.7 \pm 1.6	33.3 \pm 1.0	10.7 \pm 0.2	19.0 \pm 0.5	18.3 \pm 0.5	51.4 \pm 1.5	9.2 \pm 0.4	59.7 \pm 3.6	23.7 \pm 1.3
<i>T. helveticus</i> (5)	44.7 \pm 1.6	23.9 \pm 1.0	7.8 \pm 0.2	15.0 \pm 0.5	13.9 \pm 0.5	40.1 \pm 1.5	4.5 \pm 0.4	33.0 \pm 3.6	9.0 \pm 1.3
<i>T. boscai</i> (5)	44.4 \pm 1.6	24.4 \pm 1.0	8.1 \pm 0.2	13.3 \pm 0.5	12.0 \pm 0.5	45.4 \pm 1.5	5.3 \pm 0.4	44.1 \pm 3.6	8.5 \pm 1.3
<i>T. montandoni</i> (6)	45.5 \pm 1.6	24.2 \pm 1.0	7.3 \pm 0.2	16.4 \pm 0.5	15.2 \pm 0.5	38.4 \pm 1.5	3.6 \pm 0.4	32.7 \pm 3.6	11.7 \pm 1.3
<i>T. vulgaris</i> (5)	42.7 \pm 1.6	23.9 \pm 1.0	6.5 \pm 0.2	12.9 \pm 0.5	11.8 \pm 0.5	37.1 \pm 1.5	4.4 \pm 0.4	28.8 \pm 3.6	8.6 \pm 1.3

in the backward stepwise multiple regression. Only axilla-groin distance was retained in the final model (Fig. 4; Table 3). Because the predictor variables in this model were inter-correlated, we checked the amount of unique and shared variance in running speed explained by axilla-groin distance. Judged by the semipartial correlation coefficients, axilla-groin distance uniquely explained 52% and shared only 1% of the variance explained by the variation in limb length. Relative axilla-groin length is therefore a reliable predictor of running speed. Species with shorter axilla-groin distances run faster.

Contrary to our expectation, relative swimming and running speeds showed no negative correlation (Fig. 2F; Table 2).

The linear and exponential PGLS models yielded similar estimates of ancestral states and their standard errors (Table 4), suggesting that these estimates were robust to various microevolutionary assumptions. However, the high a_y -values (Table 4) indicate that all traits were subjected to constrained evolution. Because both PGLS methods produce poor estimates of ancestral states under this condition (Martins 1999), we feel reluctant to use these results in the reconstruction of the evolution of performance in *Triturus*.

DISCUSSION

In this study, we used morphometric and locomotor performance data on female newts to test hypotheses concerning (1) correlates of body elongation evolution, and (2) their role in the putative conflict between aquatic and terrestrial locomotion in semiaquatic vertebrates. Our results support the idea of body elongation evolution occurring concomitantly with a reduction of head width and limb length. In contrast, predictions on causal relationships between morphological and locomotor performance traits were only partially confirmed. Surprisingly, we found no evidence for a trade-off between relative swimming and running speed at the interspecific level. Below, we discuss how these findings may affect our understanding of the evolution of body elongation and the relationship between aquatic and terrestrial locomotion.

Body Elongation

The present study reveals considerable interspecific variation in body elongation among female *Triturus* newts. Body elongation in newts is associated with the number of trunk vertebrae (Arntzen and Wallis 1999), a trait that responds plastically to developmental temperature in a variety of vertebrate taxa (e.g., Barlow 1961; Lecyk 1965; Osgood 1978), including salamanders (Orska and Imiolek 1962; Peabody and Brodie 1975; Jockusch 1997). Hence, genotype-environment interactions might affect the interspecific variation in relative axilla-groin length observed in this study. However, it seems unlikely that such effect would be strong enough to confound our results. First, the plastic response in vertebrate number is generally small, even when a wide range of experimental temperatures is applied (see references above). Second, female newts actively select oviposition sites with favorable water temperatures (Gvoždík 2005), thus reducing the variation in thermal conditions during egg development

TABLE 2. Correlations of log-transformed relative running (Run) and swimming (Swim) speeds (in SVL s⁻¹), and log size-corrected morphological traits in newts using nonphylogenetic, Felsenstein's independent contrasts (FIC), and phylogenetic generalized least squares (PGLS) approaches. Significant results are marked in bold.

Traits ^a	Nonphylogenetic			FIC			PGLS		
	<i>r</i>	<i>P</i>	logL ^b	<i>r</i>	<i>P</i>	logL	<i>r</i>	95% CI ^c	logL
AGD HW	-0.86	0.002	33.58	-0.89	0.002	32.04	-0.86	-0.97 to -0.50	33.58
AGD FLL	-0.66	0.04	29.82	-0.68	0.04	28.79	-0.66	-0.91 to -0.06	29.82
AGD HLL	-0.47	0.18	28.22	-0.43	0.25	25.27	-0.47	-0.84 to 0.23	28.22
AGD TL	0.10	0.78	27.02	0.56	0.18	24.24	0.10	-0.55 to 0.70	27.01
AGD TH	0.08	0.82	27.00	-0.05	0.90	22.46	0.08	-0.58 to 0.67	26.99
Run Swim	0.32	0.36	17.84	0.59	0.10	15.96	0.32	-0.38 to 0.79	17.84

^a AGD, axilla-groin distance; HW, head width; FLL, forelimb length; HLL, hindlimb length; TL, tail length; TH, tail height.

^b log-likelihood value of given model. The highest row value indicates the best fit.

^c 95% confidence intervals of correlation coefficients obtained using alternative phylogenies, see text for details.

(Huey 1991) and, hence, the variation in trunk vertebrate number. Finally, within-species variation in the number of trunk vertebrae is small (Arntzen and Wallis 1999). Therefore we assume that the effect of developmental plasticity on axilla-groin distance variation is minor relative to the interspecific, genetic variation in this trait.

Consistent with our prediction and with previous findings (Arntzen and Wallis 1999), the evolution of body elongation concurred with a reduction of head width and limb length in female newts. A similar trend has been described in a variety of fossil and extant groups of vertebrates (Gans 1975; Greer 1987, 1991; Wiens and Slingluff 2001; Adriaens et al. 2002; Bejder and Hall 2002; Caldwell 2003). Surprisingly however, the trend varied between *Triturus* lineages. Whereas "big-bodied" newts follow the general trend of body elongation with concurring head width and limb reduction, head width in "small-bodied" newts was only marginally associated with axilla-groin length, and limb length was not. The disparity in elongation trends between the two *Triturus* lineages echoes earlier findings in other vertebrate groups (Gans 1975; Lande 1978; Caldwell 2003; but see Wiens and Slingluff 2001), in which the evolution towards limblessness proceed-

ed through two stages of elongation (Gans 1986): (1) a change in body diameter relative to body length and (2) an ongoing reduction of relative body diameter with a concomitant reduction in limb dimensions. The difference in body elongation between the two *Triturus* lineages suggests that they may be representative of these two stages of evolution toward extreme elongation and perhaps limblessness. Species with intermediate morphologies are lacking from modern groups of elongated, obligatorily aquatic salamanders (*Amphiuma*, *Proteus*, and *Siren*), and the fossil record of these groups is currently poor (Rieppel and Grande 1998). *Triturus* newts may provide a valuable alternative system to study the developmental mechanisms and adaptive significance of body elongation, and its role in the aquatic lifestyle of caudate amphibians.

Although these studies represent a major trend in various vertebrate groups (see references above), little is known about the factors driving the evolution of body elongation. From a biomechanical perspective, body elongation and its concurrent morphological changes seem advantageous to animals moving in confined places (e.g., burrows, crevices, dense vegetation) or to animals that specialize in undulatory lo-

TABLE 3. Results of the backward procedure of stepwise multiple regression testing the effect of size-corrected morphological predictors on relative swimming and running speed (in SVLs⁻¹). Results based on independent contrasts (regression through the origin) and correlation coefficients (*P*s are the same as for regression coefficients) are given for comparative purposes. Please note that the use of either raw data or phylogenetic generalized least squares transformed data gives identical results in this case.

Effect ^a	Parameter	95% CI	<i>t</i>	df	<i>P</i>	<i>r</i>
Swimming speed						
Raw data						
Intercept	0.48	0.08 to 0.95 ^b	2.00	7	0.085	
AGD	-1.29	-2.21 to -0.02 ^b	2.57	7	0.037	-0.67 ^c
TL	1.63	0.25 to 3.07 ^b	2.40	7	0.047	0.70 ^c
Independent contrasts						
AGD	-1.29	-2.70 to 0.12	2.17	7	0.07	-0.63 ^c
TL	2.10	-0.10 to 4.31	2.26	7	0.06	0.63 ^c
Running speed						
Raw data						
Intercept	0.72	0.55 to 0.87 ^b	5.95	8	< 0.001	
AGD	-1.92	-3.16 to -0.86 ^b	3.02	8	0.016	-0.73
Independent contrasts						
AGD	-1.98	-3.22 to -0.87	3.66	8	0.006	-0.79

^a AGD, axilla-groin distance; TL, tail length.

^b 95% confidence intervals including sampling variance and variance due to unknown phylogeny.

^c partial correlation.

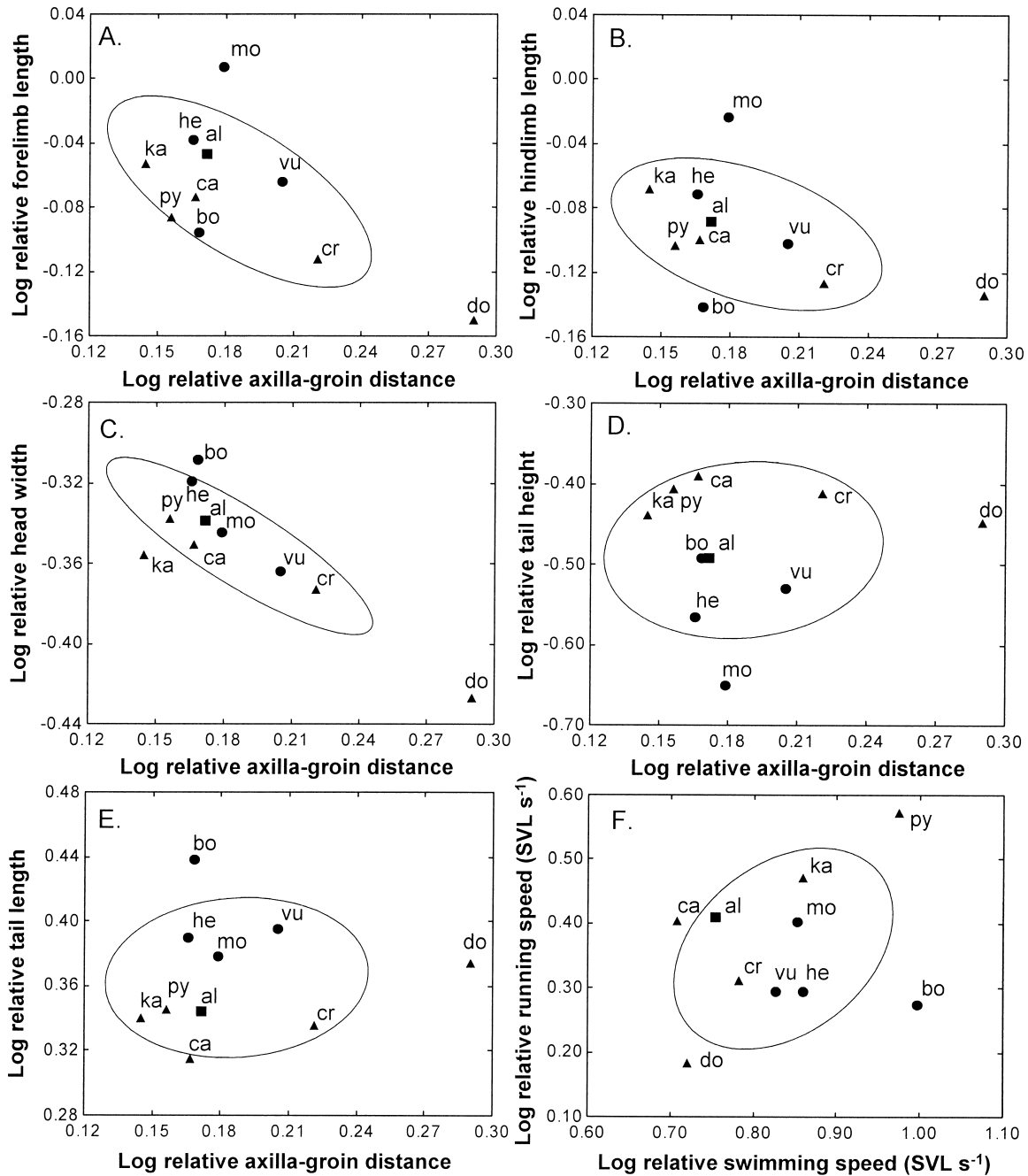


FIG. 2. Bivariate relationships between size-corrected morphological and performance traits with 50% confidence ellipses. See Table 2 for statistical details. Circles denote “small-bodied” newts and triangles “big-bodied” newts. Point labels refer to the species (first two letters of species name, Table 1).

comotion (Gans 1975). In undulatory swimmers, more elongate animals can recruit greater proportions of their body to propel themselves through the water (Gillis 1996). Accordingly, body elongation in members of the *T. cristatus* group with prolonged aquatic phases has been considered an adaptation enhancing swimming capacity (Arntzen and Wallis 1999). However, our performance measurements demonstrate that body elongation reduces, rather than aids, maximal swimming speed in female *Triturus*. This surprising result

suggests that our expectations of how body shape should influence swimming speed in newts were naive, and beckons for information on the kinematics and mechanics of aquatic locomotion in these animals. More specifically, we need to know to what extent body undulations contribute to useful forward thrust. If it turns out that the tail, rather than the body, drives swimming, then this could explain the inverse relationship between relative axilla-groin length and maximal swimming speed. Indeed, having a rather short trunk with

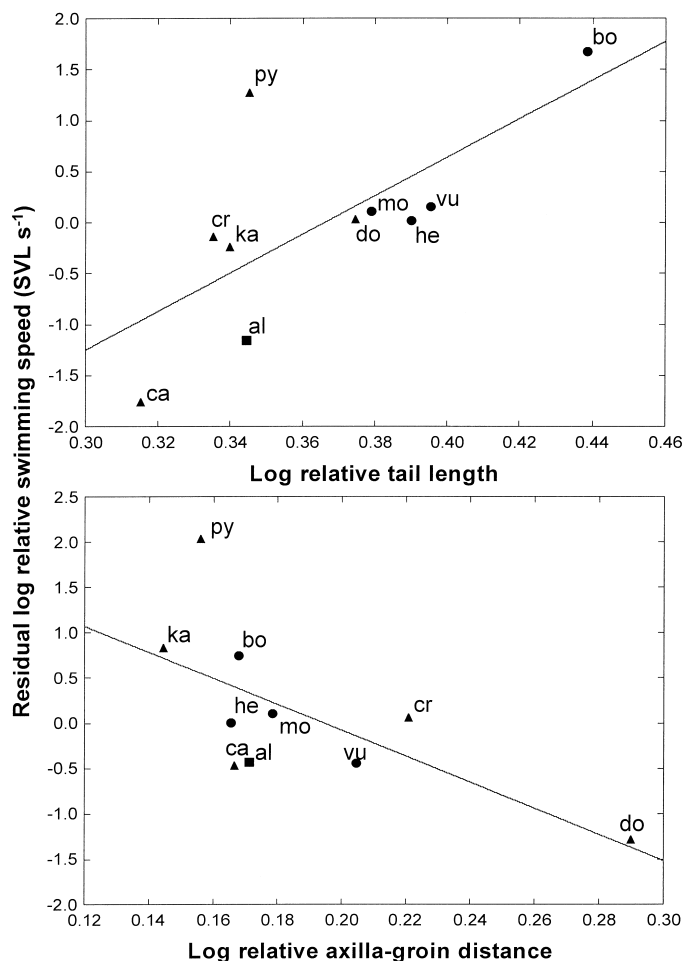


FIG. 3. Size-corrected tail length (top) and axilla-groin distance (bottom) affect residual relative swimming speed (SVL s^{-1}) of *Triturus* newt species. Swimming speeds were corrected for effects of axilla-groin distance (top) or tail length (bottom) to portray unique effects for tail length and axilla-groin length, respectively. See Table 3 and text for statistical details. Circles denote “small bodied” newts and triangles “big-bodied” newts. Point labels refer to the species (first two letters of species name, Table 1).

few vertebrae may increase body stiffness (Long and Nipper 1996) and this would help reduce the recoil of the anterior part of the body and, ultimately, decrease the amount of drag experienced during swimming (Webb 1988; Domenici and Blake 1997). Our results corroborate the idea of an important role for the tail in swimming (for theoretical considerations, see also Bainbridge 1963; Vogel 1994; Gillis 1996 and for similar empirical results, see Bennett et al. 1989; Gillis 1997).

If body elongation is an adaptation to aquatic life, it must benefit the animals through some function other than swimming speed. Possibly, body elongation aids aquatic acceleration capacity (Webb 1984; Domenici and Blake 1997; Brainerd and Patek 1998). However, fast acceleration requires a relatively high body and tail, in addition to a flexible body. In *Triturus* newts, body elongation may enhance flexibility through increased vertebrae numbers, but it is not associated with increased tail dimensions. This suggests that a need for high acceleration capacity or swimming speed has

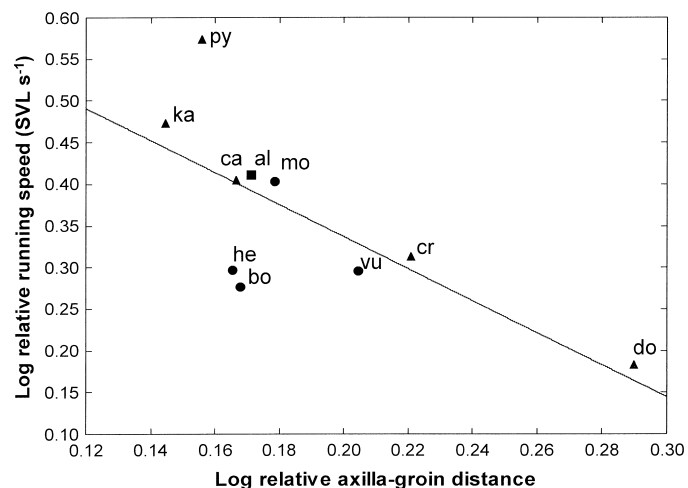


FIG. 4. Size-corrected axilla-groin distance affects relative running speed (SVL s^{-1}) of *Triturus* newt species. See text and Table 3 for statistical details. Circles denote “small bodied” newts and triangles “big-bodied” newts. Point labels refer to the species (first two letters of species name, Table 1).

not been a primary selective agent in the evolution of body elongation in female newts. Increased maneuverability seems a more likely asset of long bodies and one that may be of vital importance to species living in aquatic habitats with high structural complexity (dense vegetation). Also, one cannot rule out the possibility that body elongation serves a function outside locomotion (e.g., increased space for storing clutches).

The observed differences in relative body length between more aquatic and more terrestrial members of a clade may also result from more stringent selection for shorter bodies on land than in the water. In our study system, relative axilla-groin length (and not relative limb length) is the principle structural component explaining variation in running speed. The fact that species with relatively short trunks run faster seems to contradict the idea that longer bodies should allow more lateral flexion, larger stride lengths, and hence higher running speeds (Sukhanov 1968). Perhaps having a short, stiff trunk reduces excessive flexion and torsion of the body,

TABLE 4. Ancestral state estimates (\pm SE) of morphological and performance traits at the root of the newt phylogeny (Fig. 1). The ancestral states were calculated using both linear and exponential models assuming both Brownian motion evolution and the evolution under a strong constraint, respectively. The values of the restraining force (a_y) acting on a trait were estimated by the least squares model.

Trait	State		a_y
	Linear model	Exponential model	
Axilla-groin distance	0.17 ± 0.61	0.19 ± 0.62	3.70
Head width	-0.34 ± 0.66	-0.35 ± 0.66	3.74
Forelimb length	-0.05 ± 0.57	-0.07 ± 0.58	3.70
Hindlimb length	-0.09 ± 0.58	-0.10 ± 0.59	3.73
Tail length	0.35 ± 0.66	0.37 ± 0.67	3.63
Tail height	-0.49 ± 0.63	-0.48 ± 0.64	3.47
Swimming speed	0.80 ± 0.28	0.83 ± 0.89	3.79
Running speed	0.41 ± 0.68	0.36 ± 0.67	3.74

thereby decreasing the internal work necessary during locomotion, and hence allowing higher speeds (Van Damme and Vanhooydonck 2002).

No Trade-Off between Swimming and Running Speed

We found no evidence for a trade-off between maximum swimming and running speed in female newts. This is consistent with results obtained from intraspecific studies on semiaquatic frogs, salamanders, and snakes (Bennett et al. 1989; Navas et al. 1999; Shine and Shetty 2001; Nauwelaerts et al. 2005). A failure to detect a negative association between putatively conflicting performance functions in intraspecific studies is typically ascribed to low interindividual variation, confounding effects of “overall quality” differences among individuals, or lack of motivation of the study animals (Vanhooydonck et al. 2001; Van Damme et al. 2002). These explanations seem less likely in interspecific comparisons, where differences in morphology, performance, and ecology are generally more pronounced.

In this case, the hypothesis of a functional trade-off seems an invalid assumption, originating from an incomplete understanding of the kinematics and mechanics of two putatively conflicting functions. Clearly, more work is needed on the question of exactly how musculoskeletal systems can accommodate to cope with different environments (Biewener and Gillis 1999; Ashley-Ross and Bechtel 2004). The unpredicted antagonistic effects of body elongation and tail length on swimming speed, combined with the weak association between these two traits, may provide a functional explanation for the absence of a trade-off between swimming and running capacity in female *Triturus* newts.

It has been argued that the evolution of two performance traits is rarely compromised if the traits are functionally decoupled, that is, propelled by different structures (Domenici and Blake 1997; Blake 2004). The decoupling of aquatic locomotion (driven by the tail) from terrestrial locomotion (driven by the body/limbs), may help explain how newts succeeded in maximizing both types of performances. In addition, the antagonistic effects of a particular feature on different functions may be “swamped” by effects of other traits that influence the two functions in the same direction (Garland 1994; Van Damme et al. 2002). Many biochemical, physiological, and morphological characters (e.g., enzyme activity, muscle and blood buffering capacity, glycogen storage, respiratory functions, relative muscle mass) may enhance (or decrease) both running and swimming capacity (Biewener and Gillis 1999; Brainerd and Simons 2000; for a similar reasoning on the endurance-speed trade-off, see Garland 1994).

Alternatively, the idea of a trade-off between aquatic and terrestrial speed may be valid, but difficult to demonstrate with the current dataset. A possible factor is the limited amount of variation among species in the relative use of aquatic and terrestrial habitats. All species in the study can be considered “generalists” because they make use of both environments. Detecting trade-offs in generalists is deemed more difficult because selection regimes may not be sufficiently different to invoke extreme adaptations in one or the other direction (Vanhooydonck and Van Damme 2001).

In conclusion, our study reveals several unexpected results regarding the evolution of form and locomotory function in newts and exposes shortcomings in our understanding of how a single musculo-skeletal system may cope with different environmental conditions. Understanding the selection pressures that acted during evolutionary transitions between major environments will require a combination of biomechanical and kinematic analyses, and measurements of different types of performance as well as their ecological relevance. In this respect, and because of their resemblance with the first tetrapods (Edwards 1989), caudate amphibians seem excellent models to study the water-land transition (Clack 2002).

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