METAMORPHIC AND SPEED EFFECTS ON HINDLIMB KINEMATICS DURING TERRESTRIAL LOCOMOTION IN THE SALAMANDER *DICAMPTODON TENEBROSUS*

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Summary

The kinematics of the hindlimb during terrestrial treadmill locomotion in *Dicamptodon tenebrosus* were compared between larval and metamorphosed individuals at different speeds. Coordinates of marker points on the salamander's midline, pelvic girdle and left hindlimb were digitized from high-speed videos (200 fields s^{-1}). These yielded kinematic variables describing trunk flexion, pelvic girdle rotation, femoral protraction/retraction and knee flexion/extension. A three-way analysis of variance tested for mean differences among individuals, speeds and metamorphic stages for each variable. No significant overall effects of metamorphosis were found, although several variables showed significant stage $\times$ individual effects. Multivariate analyses revealed that the variance in kinematics of the larvae was significantly greater than that of the metamorphosed salamanders. Several variables showed significant speed effects or strong trends, among them stride length (increases with speed), cycle duration (decreases), contact interval (decreases) and phase variables describing the relative timing between minimum/maximum angles and the beginning of stance/swing phase. Such changes with speed are consistent with those shown for diverse arthropods and tetrapods and suggest that changes in stride length and timing events during a stride represent a general mechanism for effecting an increase in locomotor speed.

Introduction

Amphibian metamorphosis has long been of interest to students of vertebrate and developmental biology because of the pronounced changes in bodily form and habits which occur over a relatively short span of time (Noble, 1931; Duellman and Trueb, 1986). A salamander metamorphosing from an aquatic larva to a terrestrial adult must cope with changes in its diet, anatomy, sensory systems and primary means of locomotion in moving successfully from water to land. In addition to its utility as a window on the mechanisms of changing form and function during ontogeny, salamander metamorphosis is also of interest to the evolutionary biologist in that it represents the closest available experimental model for the tetrapod aquatic-to-terrestrial transition.

Key words: salamander, *Dicamptodon tenebrosus*, locomotion, kinematics, metamorphosis, speed.

Modern amphibians are only distantly related to ancestral tetrapods (Carroll, 1988; Milner, 1988; Panchen and Smithson, 1988). However, urodeles are the most plesiomorphic model available for the study of the requirements for, and consequences of, the aquatic-to-terrestrial shift in tetrapods. Salamanders have been used by many researchers (Schaeffer, 1941; Howell, 1944; Sukhanov, 1974; Edwards, 1977, 1989; Peters and Goslow, 1983) as representative of the primitive method of quadrupedal locomotion. The locomotor system of modern salamanders retains the plesiomorphic traits of a sprawling posture and the generation of undulatory waves by axial muscles. In addition, the skeleton of the pectoral and pelvic girdles and the limbs (especially the tarsus) of salamanders is the closest structural analogue to the condition found in fossils of early tetrapods (Schaeffer, 1941). Finally, the possession of limbs by aquatic salamander larvae may reflect an evolutionary origin as organs for underwater locomotion, as proposed by Eaton (1960) and Edwards (1989). Several lines of evidence suggest that the earliest tetrapods, though possessing tetrapod-type limbs, were either fully aquatic or spent a large portion of their life cycle in water (Eaton, 1960; Ahlberg, 1991; Coates and Clack, 1991), becoming fully terrestrial only in the Permian (Edwards, 1989).

Metamorphosing salamanders undergo the shift from an aquatic to a terrestrial existence in a period of a few weeks (Kessel and Kessel, 1944; Chadwick, 1950). No large changes affect limb anatomy across metamorphosis (Ashley *et al.* 1991; Ashley-Ross, 1992); however, the water and air environments impose different locomotor requirements on salamander limbs. In an aquatic situation, the weight of the body is buoyed by the water and, during non-axial locomotion, the limbs may generate propulsion both by gripping the substratum and by pulling or pushing the body (Coghill, 1902; Faber, 1956) and by sculling (acting as paddles). In contrast, on land the limbs must be able to hold the body off the ground while simultaneously balancing the body (much of the time on only two legs) and exerting forward propulsive force (Ashley-Ross, 1994; Gray, 1968; Frolich and Biewener, 1992; Carrier, 1993). In addition, the much lower viscosity of air compared with that of water will provide less opposition to a moving limb and, therefore, terrestrial animals may require finer control of limb movements. This would seem to suggest that terrestrial locomotion is the greater biomechanical challenge for salamanders and might require coordinational skills (e.g. balance) not needed by larvae. However, given the current hypothesis that aquatically evolved tetrapod limbs might have been pre-adapted for terrestrial use, and the fact that few observable changes occur in internal limb morphology across metamorphosis, it is logical to ask whether salamander larvae can walk and/or trot on land. If so, how do their locomotor kinematics compare with those of metamorphosed salamanders?

Two contradictory hypotheses are currently presented in the literature. (1) Ontogenetic changes associated with metamorphosis do not significantly alter behavioural kinematics. This pattern has been found for swimming endurance across metamorphosis in *Ambystoma* (Shaffer *et al.* 1991) and for aquatic feeding (both kinematics and motor pattern) in *Ambystoma* (Lauder and Shaffer, 1988; Shaffer and Lauder, 1988). These studies have shown that, in spite of major morphological changes across metamorphosis, aquatic locomotor endurance and feeding behaviour are strongly

similar in larvae and transformed individuals. Therefore, limb kinematics might also follow this pattern and be identical between the two stages. (2) Ontogenetic changes associated with metamorphosis do affect behavioural kinematics. In contrast to their results for swimming endurance, Shaffer *et al.* (1991) have shown that aquatic burst speed does differ significantly between larval and metamorphosed *Ambystoma*. Frolich and Biewener (1992) have demonstrated differences in kinematic and electromyographic patterns during swimming between larval and metamorphosed *Ambystoma*. Therefore, in terrestrial locomotion, larvae might be expected to show regular differences in kinematics compared with metamorphosed adults, particularly in the distal segments of the limbs, and might even be unable to walk. This hypothesis is further suggested by observations on developing mammals, specifically domestic cats (Peters, 1983), vervet monkeys (Vilensky *et al.* 1990) and humans (Sutherland *et al.* 1980). These authors have all noted differences in locomotor kinematics in their subjects due to neuromuscular immaturity early in ontogeny; maturation is characterized by a cranial-to-caudal, proximal-to-distal progression of coordination. While salamanders tend to be much more reliant on the spinal cord alone for coordinated locomotion than the more cerebrally oriented mammals, it is possible that, because larval salamanders have neither the opportunity (Kessel and Kessel, 1944) nor the need to use their limbs in a terrestrial fashion, they may retain a functionally immature limb locomotor system until metamorphosis.

A substantial body of literature documents that locomotor variables show regular differences not just with ontogeny but also with speed (Hildebrand, 1966; Daan and Belterman, 1968; Goslow *et al.* 1973, 1981; Van De Graaff *et al.* 1982; Biewener, 1983; Halbertsma, 1983; Vilensky and Gehlsen, 1984; Vilensky and Gankiewicz, 1990; Vilensky *et al.* 1990; Full and Tu, 1990, 1991; Full and Weinstein, 1992). Comparisons of salamander limb kinematics at different gaits and speeds, both within and between metamorphic stages, would elucidate possible differences in locomotor capacities in larvae and adults. Furthermore, these results for salamanders may also be compared with known speed effects for other limbed animals to determine what similarities in mechanisms for changing speed exist across a wide range of taxa.

In this study, the Pacific giant salamander, *Dicamptodon tenebrosus*, was used to examine metamorphic and speed effects on terrestrial locomotion. *Dicamptodon* was chosen for several reasons. This salamander has a distinct aquatic larval phase, during which it does not venture onto land (Kessel and Kessel, 1944), and then undergoes complete metamorphosis into the terrestrial form. *Dicamptodon* also possesses robust limbs and reasonable endurance and behaves relatively tractably on a treadmill. The experimental design, contrasting kinematics for the same individual salamanders as both larvae and metamorphs at different speeds, allows determination of metamorphic stage effects (and any interaction with speed) while controlling for individual differences across metamorphosis. A separate analysis of speed effects for metamorphs, which included an additional individual, provided more statistical power to determine kinematic changes with speed. The primary objectives of this paper are (1) to compare statistically the kinematics of locomotion across metamorphic stages and speeds; and (2) to compare these kinematics with published values for mammalian hindlimb kinematics in order to

examine similarities and differences in strategies for changing speed in these divergent tetrapod groups. A detailed analysis of the hindlimb movements is presented in the accompanying paper (Ashley-Ross, 1994). Some of this material has been reported previously in abstract form (Ashley-Ross, 1991).

Materials and methods

Animals

Four *Dicamptodon tenebrosus* (Good, 1989) collected as larvae in Mendocino County, CA (California scientific collector permits no. 7058 and no. 7614), were videotaped as both larvae and metamorphs. Additional footage was obtained from a fifth metamorphosed animal. Larvae were maintained in 37.9 l aquaria. After being videotaped as larvae, salamanders either metamorphosed on their own or were induced to metamorphose by the addition of thyroxin to their tank water. After metamorphosis, individuals were kept in modified aquaria fitted with moss-covered platforms to allow the animals access to both land and water. Metamorphosed salamanders were kept for a minimum of 1 month before being videotaped. Snout–vent length (SVL; measured from the tip of the snout to the anterior angle of the vent) of each specimen was measured with calipers after the animal had been anaesthetized with MS-222. The position of the acetabulum was determined by palpation of the hip region, and the distance that the acetabulum lay cranially of the ilium on the left side of the body was also measured (see variables description, below). The SVLs of the specimens used were as follows: animal 1, larval, 8.29 cm, metamorphosed, 9.51 cm; animal 2, larval and metamorphosed, 9.62 cm; animal 3, larval, 8.23 cm, metamorphosed, 8.14 cm; animal 4, larval, 7.86 cm, metamorphosed, 8.51 cm; animal 5, metamorphosed, 8.09 cm.

Video recording

Both larval and metamorphosed Pacific giant salamanders were filmed during locomotion at several speeds on a variable-speed motor-driven treadmill. Salamanders either walked voluntarily towards a simulated burrow (a section of black polyvinylchloride tubing suspended at the far end of the treadmill) or were encouraged to walk by touching or gently squeezing the base of the tail. Animals were always placed on the treadmill with their left side facing the cameras. Further details of video recording methods are given in Ashley-Ross (1994).

Video analysis

Three strides were analysed for each animal in each of three speed categories: 'slow walk', <0.5 SVL s^{-1}; 'medium walk', 0.5–1.0 SVL s^{-1}; and 'trot', >1.0 SVL s^{-1}. Only stride sequences in which the animal was in steady-speed motion were analyzed; these did not include either starts or stops. In most cases, particularly for the lower speed categories, more than three strides were recorded for each animal. Therefore, in order to try to maximize differences between the contiguous speed categories, the three strides chosen for analysis from each animal were as closely speed-matched as possible among individuals. For each stride chosen, a minimum of 20 frames, equally spaced in time,

were transferred from video tape to magnetic or optical media for digitizing. The coordinates of the painted marker points (see Ashley-Ross, 1994) on the salamander were digitized using a custom video-analysis program (MeasurementTV, DataCrunch Software, San Clemente, CA), and these coordinate files were subsequently imported into a spreadsheet program. The spreadsheet was used to compute the angle variables defined below. To reduce the effect of digitizing error on the calculation of statistical variables, curves produced by plotting these angle values over the duration of a stride were smoothed by Gaussian filtering using the curve-fitting and analysis program Igor (WaveMetrics, Lake Oswego, OR). These smoothed variables were then used for determination of minimum, maximum and range values for each kinematic variable (defined below), which were then compared among groups by analysis of variance (see below). Timing of footfalls during a stride was determined by examining all video fields (200 fields s^{-1} sampling rate).

Definition of variables

The variables used in this study have been described in the accompanying paper (Fig. 1 in Ashley-Ross, 1994), but will be reiterated here. A *stride* was defined as the time from left hindfoot contact with the treadmill belt to the subsequent left hindfoot contact. *Cycle duration* of the stride was recorded in milliseconds. *Pelvic girdle angle* was defined as the angle between the line connecting the two marker points over the ilia ('pelvic girdle line') and the edge of the treadmill. *Pelvic girdle–femur angle* was measured between the pelvic girdle line and the line connecting the calculated position of the acetabulum (see Animals, above) and the knee marker dot ('femur line'). This angle was zero when the femur was in line with the pelvic girdle line and assumed positive values when the femur was protracted forwards from that line and negative values when retracted behind that line. *Femur–crus angle* was measured between the femur line and the line connecting the knee marker and the dot at the distal end of the fibula ('crus line'). A measure of the overall lateral bending of the trunk was provided by the angle between the lines connecting the anteriormost and posteriormost pairs of marker dots in the trunk region (*anterior–posterior angle*). Localized bending in the anterior, middle and posterior regions of the trunk was measured by the angle between adjacent pairs of midline markers in the appropriate trunk regions (*trunk segment angle*). All of the preceding angle variables were calculated from coordinates digitized in a dorsal view of the salamander. One additional angle variable, the *lateral crus angle*, was digitized from a lateral view of the animal and was measured as the angle between the line connecting the knee and fibula marker points and the treadmill surface. The *initial crus angle* is the value of the lateral crus angle at the beginning of the stride.

Several timing variables were also measured. *Contact interval* (footfall to lifting of the foot), phase lag between the beginning of stance and the maximum value of several variables (pelvic girdle angle, pelvic girdle–femur angle) and phase lag between the beginning of swing and the minimum value of several variables (pelvic girdle angle, pelvic girdle–femur angle, femur–crus angle) were all expressed as a percentage of the step cycle duration. Hildebrand-style footfall diagrams (Hildebrand, 1966, 1976) were generated by plotting foot contact intervals as a percentage of the step cycle.

Statistical analysis

A three-way analysis of variance (ANOVA, Zar, 1984) was used to test simultaneously for speed and metamorphic differences among the four salamanders videotaped as both larvae and metamorphosed individuals. The three-way design expressly considers any effects of the transformation from larva to metamorph, and effects of changing speed, to be the same for each individual. Metamorphic stage was treated as a fixed effect with two levels (larval or transformed), speed was treated as a fixed effect with three levels (slow walk, medium walk and trot) and individual was treated as a random effect. To increase statistical power, a separate two-way ANOVA testing for speed effects was run on all five transformed salamanders. Again, speed was treated as a fixed effect, and individual was treated as a random effect. In both ANOVAs, fixed effects were tested over the interaction term (effect × individual), while individual effects were tested over the error residual. For each ANOVA performed, the large number of simultaneous statistical comparisons makes it highly probable that some will be found significant by chance alone. To correct this problem, the sequential Bonferroni test described by Rice (1989) was used on each effect (i.e. each table column). The table-wide adjustment to P-values advocated by Rice (1989) was not made as the large number of comparisons (126 in the three-way ANOVA) combined with the small number of degrees of freedom virtually guarantees type II errors. It should also be noted that the use of values derived from the smoothed kinematic profiles (see Video analysis, above) reduces the effect of digitized 'outliers' on the results of the analysis of variance and therefore makes the ANOVA more conservative than would otherwise be the case. Therefore, column-wise correction of P-values was adopted as a compromise between these two competing effects.

To summarize multivariate effects of speed and metamorphic stage, a principal components analysis (PCA) was performed on a subset of variables chosen *a priori* to represent the overall characteristics of the stride (stride length, cycle duration, contact interval), summary angle values (pelvic girdle angle range, pelvic girdle–femur angle range, femur–crus angle range, initial crus angle, anterior–posterior trunk angle range) and phase variables (minimum pelvic girdle angle/swing initiation phase, minimum pelvic girdle–femur angle/swing initiation phase). Equality of variances between metamorphic stages and among speed categories was tested using Levene's test (Van Valen, 1978) on the PCA scores for each category.

Results

In spite of a previously aquatic existence, larval *Dicamptodon tenebrosus* are able to walk on land and to use the same gaits (lateral sequence walk and trot; classified according to Hildebrand, 1976; see Fig. 2 in Ashley-Ross, 1994, for a representative walking stride) as metamorphosed *Dicamptodon*. Mean gait diagrams for larval and transformed stages are indistinguishable at walking paces (Fig. 1, L1 and T1, L2 and T2) and show only minor variation at a trot (Fig. 1, L3 and T3). The average gait diagram for the larval trot shows more overlap between contact times for diagonal limb pairs than does the metamorphosed trotting gait diagram, even though the average speed of the
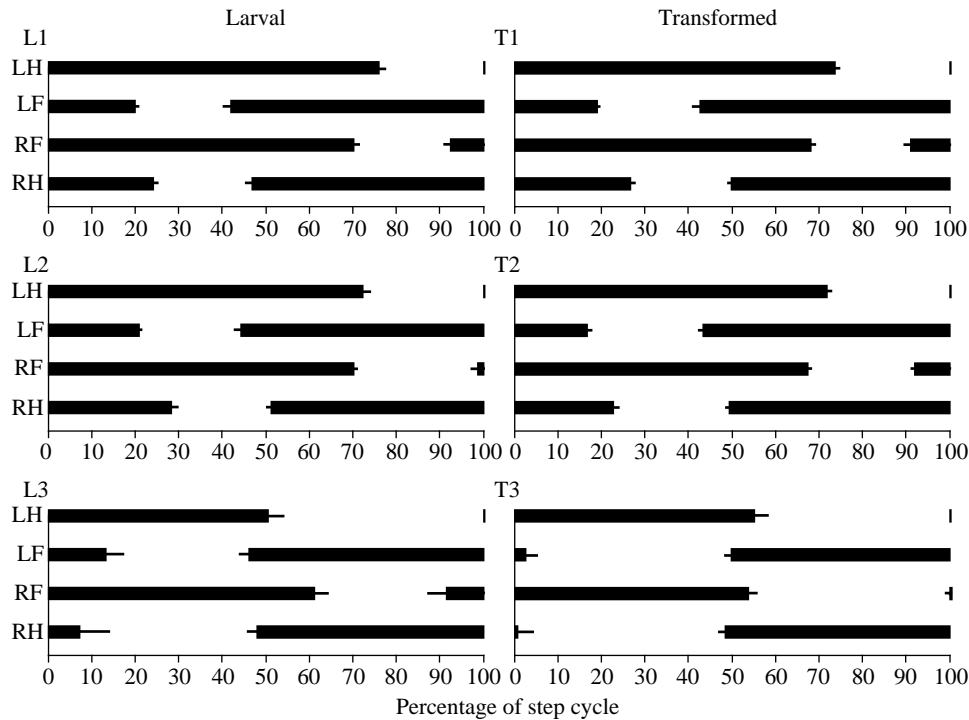
Fig. 1. Average gait diagrams for larval (L) and transformed (T) *Dicamptodon tenebrosus* locomoting at three different speed categories (slow walk=1; medium walk=2, trot=3). Mean speed ± S.E.M. for each panel are: (L1) 0.38±0.018 SVL s^{-1}, (L2) 0.72±0.031 SVL s^{-1}, (L3) 2.77±0.54 SVL s^{-1}, (T1) 0.44±0.015 SVL s^{-1}, (T2) 0.78±0.030 SVL s^{-1}, (T3) 2.23±0.21 SVL s^{-1}. Thick bars represent mean foot contact durations; thin bars are standard errors of the mean for foot-down and foot-up. For each speed category, larval values are computed from footfall data for three strides from each of four individuals (total *N*=12 for L panels); transformed values are calculated for three strides from each of five individuals (total *N*=15 for T panels). LH, left hindfoot; LF, left forefoot; RH, right hindfoot; RF, right forefoot.

larval trot sequences (2.77 SVL s^{-1}) is higher than that for the metamorphosed trot sequences (2.23 SVL s^{-1}). Though the average gait diagrams represent the typical sequence of footfalls, there is appreciable variation from stride to stride within individuals. For instance, in a lateral sequence walk, the expected order of foot movements beginning with the left hindlimb is LH down, LF up, RH up, LF down, RH down, RF up, LH up, RF down and finally LH down. 58 % of the 53 strides analyzed for transformed individuals followed the expected pattern, while only 35 % of the 43 larval strides did so. The most common variation observed was that in a diagonal pair of limbs, where the order in which footfalls occurred was reversed from the expected order. In the LF/RH diagonal limb pair, this resulted in a pattern of LF up, RH up, RH down, LF down. Strides of this pattern represented a substantial proportion of the total, 30 % in transformed individuals and 51 % in larvae. In 11 % of the transformed strides and 14 %

of the larval strides, the feet of a diagonal pair lifted off or struck the ground synchronously. No diagonal gaits were observed.

Table 1 presents mean values and standard deviations for the 21 kinematic variables measured, separated according to metamorphic stage and speed category. Comparison of larval and transformed values at each speed category reveals that means for all variables are highly similar between metamorphic stages. Many variables, particularly angular variables, also appear to remain constant with speed at either metamorphic stage. It is interesting to note, however, that there appear to be reliable differences in the amount of variation between categories. For instance, the standard deviations measured at walking speeds are larger for larvae than for metamorphs in 15 of the 21 variables. At a trot, this difference in variation is even more pronounced, with larvae showing standard deviations higher than those of metamorphs in all variables except the relative timing of the minimum femur–crus angle with the beginning of swing phase. There is less variation among the speed categories within a metamorphic stage, with most variables exhibiting similar standard deviations, although for several variables (for instance, stride length, pelvic girdle angle range and larval initial crus angle), the highest speed category has a considerably higher standard deviation than those of the two lower speed categories.

Metamorphic effects

Table 2 shows the results of a three-way ANOVA simultaneously testing for differences between metamorphic stages and among the three speed categories. Individual variation was high, with 12 of the 21 variables tested showing a significant individual effect. Several variables show differences correlated with increases in speed (discussed below). No significant effects of metamorphosis were found for any variable; note that the F-values for this effect are for the most part extremely low. One variable (anterior trunk segment angle range) showed a significant stage $\times$ individual interaction effect, and several others, though not significant, had high associated F-values. Such a pattern indicates that individuals are responding differently to metamorphosis; some salamanders increase values for these variables across metamorphosis, while others decrease them. Plots of these interaction effects are given in Fig. 2. Though the precise patterns are different for each variable, all the plots are characterized by pronounced crossing of the lines connecting variable means for larval and transformed stages. Again, this suggests that the effect of metamorphosis on these variables is inconsistent for different individuals.

Speed effects

Speed effects were also tested by a two-way ANOVA for five transformed individuals (Table 3). In this analysis, individual variation was again high, with 11 of the 21 variables showing significant individual effects. The two-way and three-way ANOVAs gave slightly different results regarding significant speed effects. However, variables that were significant in one analysis had high associated F-values in the other, so the trends observed appear reliable. Variables demonstrating significant effects of speed in either analysis reflect either timing variables of the stride (cycle duration, contact interval) or timing of hindlimb movements within the stride (minimum pelvic girdle–femur

Table 1. *Means (standard deviation) for kinematic variables measured for three-way ANOVA presented separately for each stage and speed*

Variable	Larval			Transformed		
	<0.5 SVL s⁻¹ (N=15)	0.5–1.0 SVL s⁻¹ (N=16)	>1.0 SVL s⁻¹ (N=14)	<0.5 SVL s⁻¹ (N=13)	0.5–1.0 SVL s⁻¹ (N=15)	>1.0 SVL s⁻¹ (N=13)
Stride length	64.7 (6.7)	71.3 (9.1)	91.8 (31.4)	65.6 (4.8)	74.0 (5.3)	96.8 (15.8)
Cycle duration	1751 (312)	1026 (268)	4256 (152)	1504 (207)	971 (125)	477 (134)
Contact interval	76.0 (6.7)	72.0 (7.2)	51.6 (11.4)	72.3 (3.2)	70.7 (4.9)	58.3 (5.9)
Pelvic girdle angle range	46.5 (21.8)	47.4 (18.6)	70.5 (25.2)	47.6 (11.1)	40.1 (6.5)	58.5 (15.6)
Minimum pelvic girdle–femur angle	−70.0 (9.3)	−67.5 (13.6)	−66.2 (20.4)	−58.3 (11.7)	−58.9 (19.5)	−55.5 (10.8)
Maximum pelvic girdle–femur angle	55.9 (15.0)	50.9 (14.8)	54.9 (20.3)	49.3 (7.0)	49.0 (8.0)	57.9 (10.3)
Pelvic girdle–femur angle range	126.0 (17.6)	118.5 (14.6)	121.2 (29.0)	107.6 (11.6)	107.9 (14.2)	113.4 (11.0)
Minimum femur–crus angle	114.1 (17.9)	124.8 (17.9)	116.8 (13.8)	106.8 (12.6)	112.3 (15.7)	109.0 (12.7)
Maximum femur–crus angle	178.5 (10.9)	178.8 (11.6)	174.9 (13.5)	170.4 (11.6)	175.8 (20.0)	164.9 (11.0)
Femur–crus angle range	64.4 (15.0)	54.0 (14.2)	61.2 (17.1)	63.6 (7.1)	63.4 (22.6)	56.0 (12.0)
Maximum lateral crus angle	196.0 (6.1)	190.1 (5.2)	185.5 (8.8)	190.9 (6.6)	186.0 (7.7)	190.1 (5.6)
Initial crus angle	28.0 (9.0)	36.3 (10.0)	52.8 (43.5)	39.3 (20.7)	40.3 (16.4)	47.7 (20.4)
Anterior trunk segment angle range	14.4 (5.2)	13.4 (5.7)	17.6 (8.4)	15.6 (4.3)	13.1 (3.2)	14.8 (4.3)
Middle trunk segment angle range	24.8 (8.2)	20.5 (7.4)	24.8 (9.0)	19.6 (3.4)	18.7 (2.9)	20.7 (2.3)
Posterior trunk segment angle range	17.8 (5.9)	17.2 (6.5)	25.4 (8.7)	19.7 (5.0)	18.1 (4.0)	23.4 (4.2)
Anterior–posterior trunk angle range	71.2 (33.3)	70.8 (30.7)	105.4 (40.6)	78.3 (11.3)	69.4 (12.1)	82.4 (9.4)
Minimum pelvic girdle angle/swing phase	−19.6 (15.1)	−16.8 (10.1)	−9.5 (9.4)	−18.7 (5.6)	−22.2 (5.8)	−14.5 (6.7)
Maximum pelvic girdle angle/stance phase	−0.2 (4.0)	1.5 (4.0)	−10.1 (9.2)	1.1 (2.8)	0.9 (3.6)	−5.7 (7.6)
Minimum pelvic girdle–femur angle/ swing phase	−3.7 (6.6)	−0.9 (9.2)	7.0 (5.8)	−5.5 (6.3)	−0.6 (4.9)	5.9 (5.4)
Maximum pelvic girdle–femur angle/ stance phase	−4.2 (4.4)	−1.3 (3.1)	−8.1 (7.4)	−5.1 (3.4)	−3.7 (3.6)	−7.2 (5.1)
Minimum femur–crus angle/swing phase	−45.1 (17.8)	−39.0 (24.9)	−5.7 (24.9)	−46.8 (7.1)	−41.6 (7.9)	−18.4 (29.5)

Stride length is given as a percentage of SVL, cycle duration is in ms, contact interval is in percentage of step cycle, all angle variables are in degrees and all phase variables are in percentage of step cycle (a negative phase value indicates the minimum/maximum angle occurred before the onset of swing/stance phase, a positive value indicates it occurred after). Sample sizes given under each category.

Table 2. *Three-way analysis of variance for 21 kinematic variables measured from four* Dicamptodon tenebrosus *at two metamorphic stages (larval and transformed) and three speed categories ($<0.5\,SVL\,s^{-1}$, $0.5–1.0\,SVL\,s^{-1}$ and $>1.0\,SVL\,s^{-1}$)*

Variable	Metamorphic stage (1, 3)	Speed (2, 6)	Individual (3, 62)	Stage × individual (3, 6)	Speed × individual (6, 6)	Stage × speed (2, 6)
Stride length	0.083	9.646	4.228	2.449	1.985	0.042
Cycle duration	1.005	65.876**	11.940**	2.564	2.276	2.786
Contact interval	0.048	29.669*	4.152	1.892	0.976	2.235
Pelvic girdle angle range	0.470	2.342	5.859*	7.469	3.846	1.488
Minimum pelvic girdle–femur angle	1.512	0.237	15.937**	10.483	4.192	0.347
Maximum pelvic girdle–femur angle	0.316	0.429	8.922**	1.274	0.871	0.587
Pelvic girdle–femur angle range	6.752	0.031	3.572*	0.715	1.680	0.516
Minimum femur–crus angle	1.282	1.371	2.482	1.658	0.463	0.023
Maximum femur–crus angle	0.606	0.927	7.619**	7.369	2.375	0.912
Femur–crus angle range	0.315	0.548	1.547	0.276	0.852	0.560
Maximum lateral crus angle	0.137	2.747	1.950	6.003	2.234	5.330
Initial crus angle	0.102	1.550	6.115*	1.642	1.695	0.897
Anterior trunk segment angle range	0.024	0.548	38.496**	24.570*	5.601	2.519
Middle trunk segment angle range	0.851	0.468	16.566**	5.543	1.351	0.110
Posterior trunk segment angle range	0.0005	3.532	4.362	9.713	3.020	1.072
Anterior–posterior trunk angle range	0.233	1.787	39.540**	3.876	1.790	1.552
Minimum pelvic girdle angle/swing phase	0.796	4.210	0.286	2.581	1.128	0.682
Maximum pelvic girdle angle/stance phase	2.189	4.651	6.663*	2.553	7.644	3.032
Minimum pelvic girdle–femur angle/ swing phase	0.079	19.818*	2.426	2.404	0.419	0.063
Maximum pelvic girdle–femur angle/ stance phase	0.576	2.565	4.253	1.237	3.358	0.699
Minimum femur–crus angle/swing phase	0.845	10.888	12.374**	2.718	1.648	0.589

Table entries are *F*-values.

Degrees of freedom are given below each effect.

*$P \leq 0.05$ (sequential Bonferroni corrected); **$P \leq 0.05$ (sequential Bonferroni corrected).

Table 3. *Two-way analysis of variance for 21 kinematic variables measured from five transformed* Dicamptodon tenebrosus *at three speed categories ($<0.5\,SVL\,s^{-1}$, 0.5–$1.0\,SVL\,s^{-1}$ and $>1.0\,SVL\,s^{-1}$)*

Variable	Speed (2, 8)	Individual (4, 38)	Speed × individual (8, 38)
Stride length	12.327	2.426	4.148*
Cycle duration	82.881**	5.047*	4.453*
Contact interval	7.476	1.477	6.675*
Pelvic girdle angle range	3.456	5.141*	8.457*
Minimum pelvic girdle–femur angle	0.269	20.339**	3.137
Maximum pelvic girdle–femur angle	2.138	11.070**	5.215**
Pelvic girdle–femur angle range	0.374	12.409**	3.700*
Minimum femur–crus angle	0.196	9.902**	3.536*
Maximum femur–crus angle	2.009	11.641**	2.280
Femur–crus angle range	1.375	1.819	1.353
Maximum lateral crus angle	2.621	2.555	1.725
Initial crus angle	1.633	3.648	1.010
Anterior trunk segment angle range	0.875	22.357**	8.140**
Middle trunk segment angle range	2.082	40.501**	11.778**
Posterior trunk segment angle range	3.931	18.653**	8.353**
Anterior–posterior trunk angle range	3.061	8.585**	8.092**
Minimum pelvic girdle angle/swing phase	4.976	1.938	1.703
Maximum pelvic girdle angle/stance phase	6.434	2.755	1.886
Minimum pelvic girdle–femur angle/ swing phase	6.972	1.440	2.196
Maximum pelvic girdle–femur angle/ stance phase	4.864	3.429	1.083
Minimum femur–crus angle/swing phase	6.919	2.721	2.224

Table entries are *F*-values.
Degrees of freedom are given below each effect.
*$P \leqslant 0.05$ (sequential Bonferroni corrected); **$P \leqslant 0.05$ (sequential Bonferroni corrected).

angle/swing phase). Several variables, while not statistically significant in either ANOVA, nevertheless had high *F*-values and may be indicative of trends. Examination of mean values for all of these variables in Table 1 reveals two basic patterns of change. The first pattern is that, as speed increases, the variable changes in a roughly linear fashion; variables following this pattern are cycle duration (decreases with speed), stride length (increases) and minimum pelvic girdle–femur angle/swing phase (with increasing speed, occurs progressively later in the step cycle relative to the start of swing phase). The second general pattern is one in which the values are similar for both the slow and moderate walks, but are increased or decreased for the trot. Variables conforming to this pattern are contact interval (decreases in going from a walk to a trot), maximum pelvic girdle angle/stance phase (occurs earlier in the step cycle relative to the start of stance phase in the trot) and the minima of both pelvic girdle angle and femur–crus angle relative to the beginning of swing phase (both occur relatively earlier in the trot).
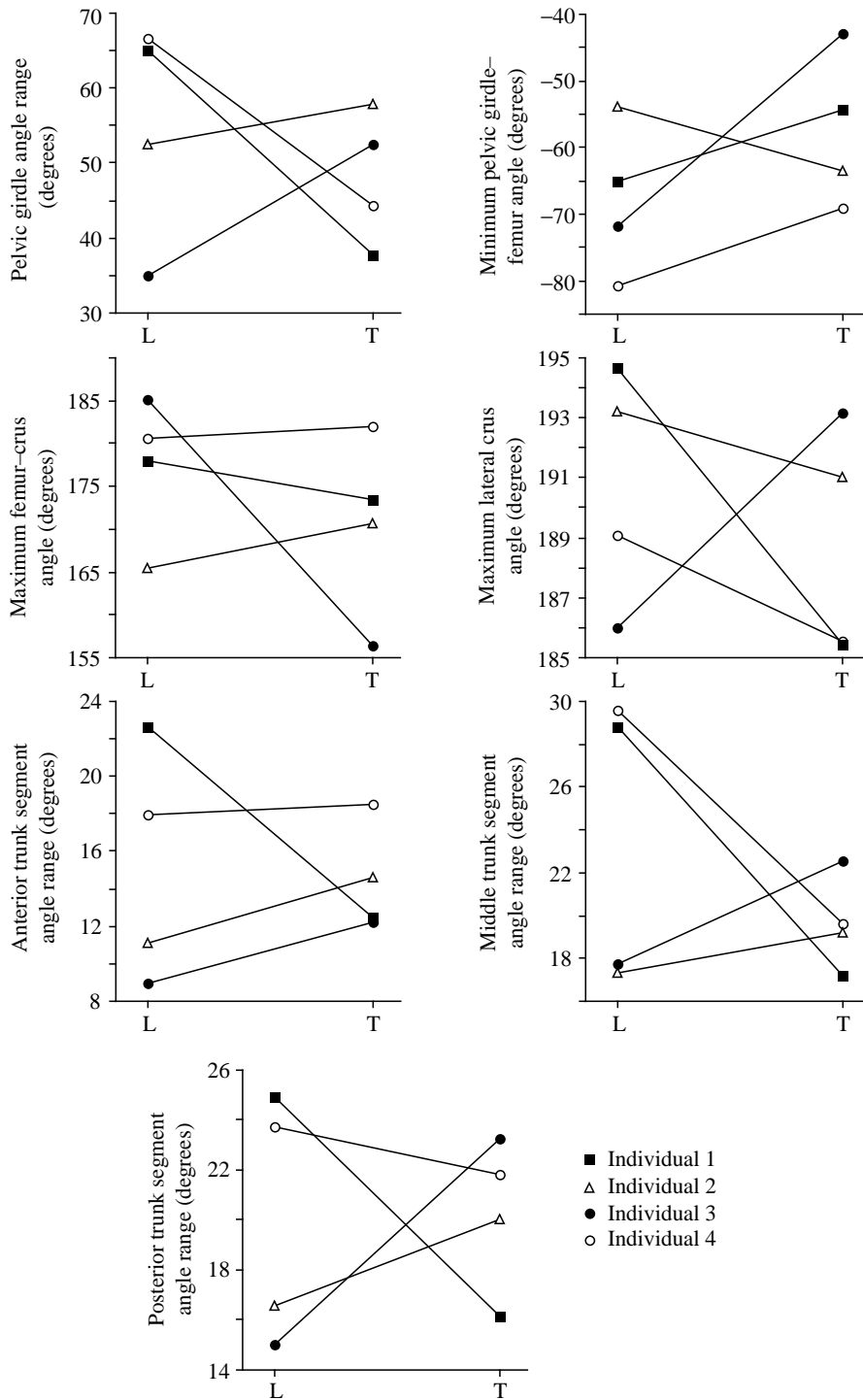
Fig. 2

Fig. 2. Interaction plots for seven variables that had high associated stage × individual *F*-values in the three-way ANOVA. The interaction term for anterior trunk segment angle range was statistically significant. Mean values at the two metamorphic stages for each individual are connected by lines. L, larval stage; T, transformed salamanders.

The majority of variables tested in the two-way ANOVA (11 of 21) also showed significant speed × individual effects, indicating that these values for all individuals do not change in the same direction as they increase speed. Interaction plots for these significant variables are presented in Fig. 3. Variables such as stride length, cycle duration, contact interval and pelvic girdle angle range, and the variables describing trunk bending (with the exception of the anterior trunk segment angle), show similar patterns for each individual, with the primary difference being one of amplitude, not direction of change. Variables describing the amplitude and phasing of limb movements, in contrast, show more individual differences in response to speed changes. It is difficult to detect any clear pattern; however, a few generalizations may be made. In four of the five salamanders, pelvic girdle angle range is least in the 0.5–1.0 SVL s^{-1} speed category; the greatest amplitude occurs at the highest speed for three salamanders, and at the lowest speed for the other two. This pattern is exactly the same (for the corresponding individuals) for the anterior–posterior trunk angle. Apparently, lateral flexion of the trunk and pelvic girdle rotation are positively coupled. It also appears that individuals that show large changes in the amplitude of trunk and pelvic girdle oscillations (salamander 5, Fig. 3) show relatively small changes in the amplitude of limb movements, and *vice versa* (salamanders 2 and 4). However, it must be emphasized that there is no clear pattern of a trade-off between the amplitudes of axial movements and limb movements with changes in speed.

Multivariate analysis

Table 4 shows loadings on principal components 1–4 for ten variables selected to

Table 4. *Loadings of selected variables on principal components 1–4*

| Variable	PC 1	PC 2	PC 3	PC 4
Stride length	0.746	0.082	−0.102	−0.392
Cycle duration	−0.652	0.275	0.442	0.209
Contact interval	−0.896	−0.055	0.117	−0.206
Pelvic girdle angle range	0.802	0.218	0.469	0.040
Pelvic girdle–femur angle range	−0.137	0.811	−0.353	−0.114
Femur–crus angle range	−0.258	0.577	−0.224	0.496
Initial crus angle	0.448	−0.624	0.117	0.295
Anterior–posterior trunk angle range	0.677	0.468	0.451	0.201
Minimum pelvic girdle angle/swing initiation phase	0.451	−0.236	−0.526	0.405
Minimum pelvic girdle–femur angle/ swing initiation phase	0.732	0.305	−0.183	−0.174
Percentage of total variance explained	39.110	18.812	11.411	8.223
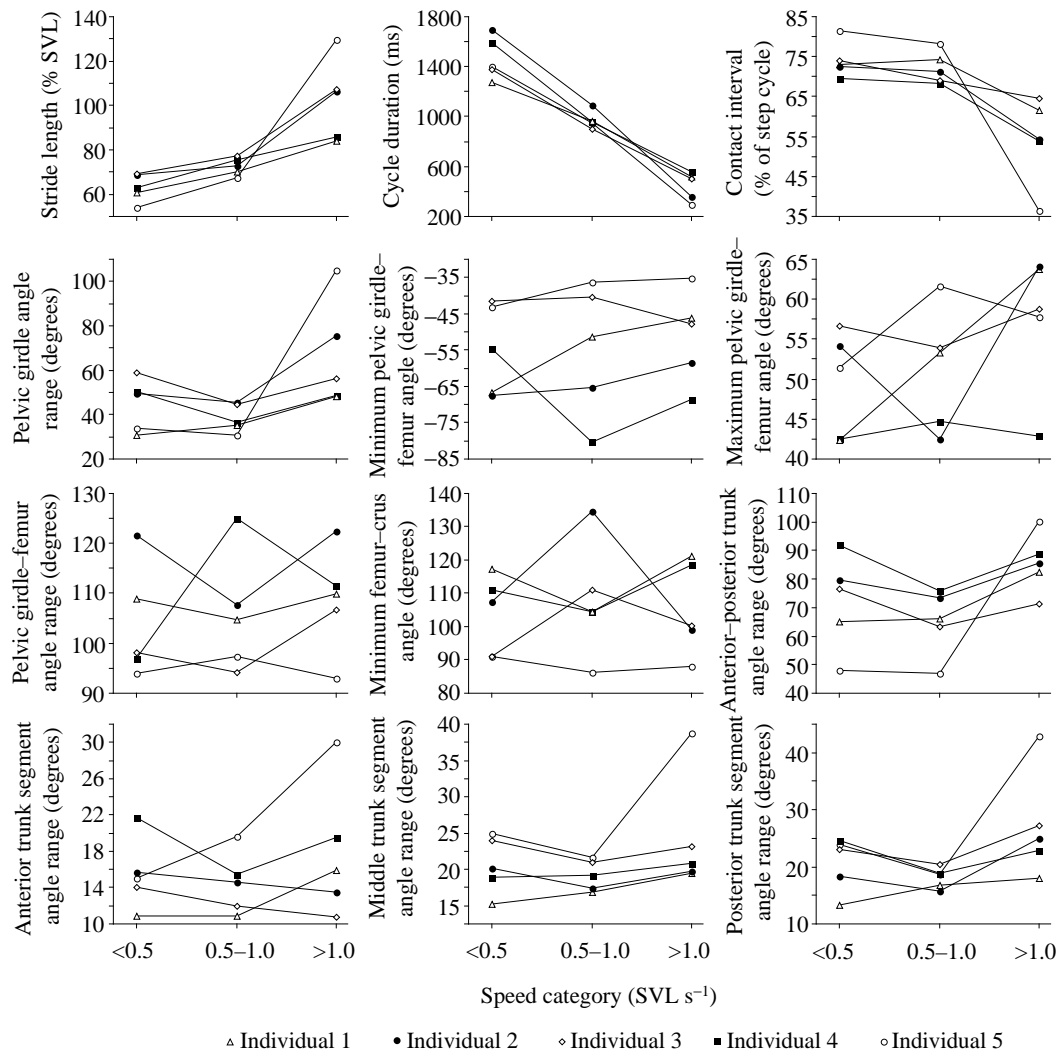
Fig. 3. Interaction plots for 11 variables from the two-way ANOVA that had significant speed × individual terms and one variable (minimum pelvic girdle–femur angle) that had an F-value of the same general magnitude. Mean values at the three speed categories for each individual are connected by lines.

represent a mixture of overall stride descriptors (stride length, cycle duration, contact interval), limb angle variables and limb movement timing variables. Principal component (PC) 1 correlates with speed (see also Fig. 4), with high PC 1 scores having longer stride lengths and shorter cycle durations and contact intervals, reflecting higher-speed locomotion. High PC 1 scores also correlate with larger pelvic girdle oscillations, larger trunk oscillations and with maximal femoral retraction (minimum pelvic girdle–femur
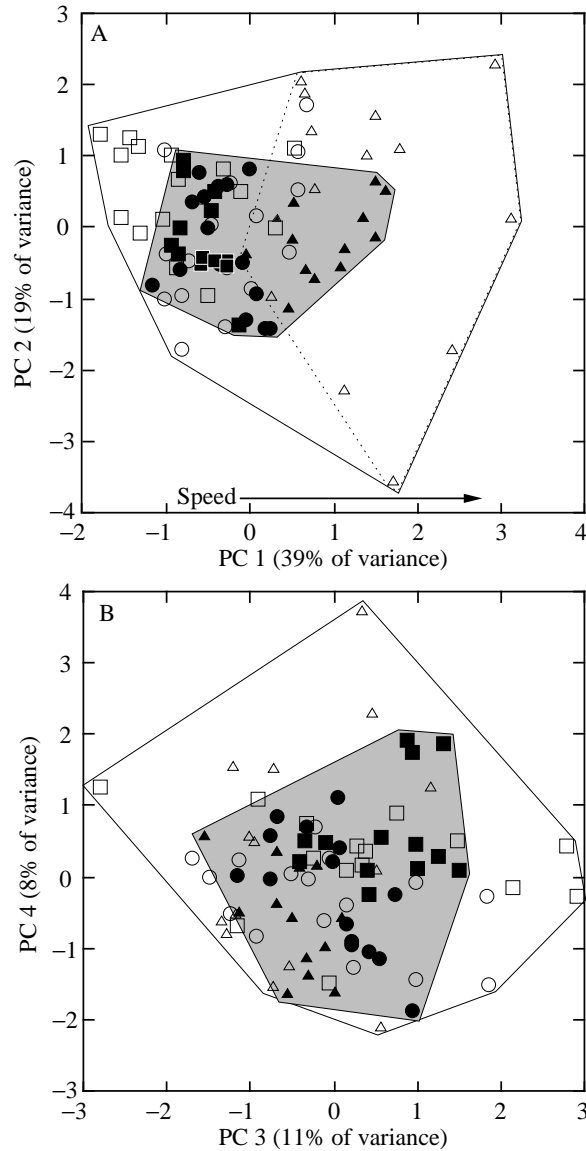
Fig. 4. Plots of principal component 1 *versus* principal component 2 (A) and principal component 3 *versus* principal component 4 (B) for ten representative kinematic variables. Larvae are represented by open symbols, transformed individuals by filled symbols. Lowest speed category ($\leqslant 0.5\,\mathrm{SVL\,s^{-1}}$) is represented by squares, middle speed category ($0.5\text{--}1.0\,\mathrm{SVL\,s^{-1}}$) by circles and highest speed category ($\geqslant 1.0\,\mathrm{SVL\,s^{-1}}$) by triangles. Open polygons enclose values for larvae, shaded polygons enclose values for transformed salamanders. Dotted line in A forms the left-hand border of a polygon enclosing all symbols for the highest speed category. Principal component 1 is roughly equivalent to a speed axis, as the three speed categories form a progression from low to high as PC 1 values increase.

Table 5. *Levene's test on principal component score deviations to test for equality of variances between metamorphic stages and speed categories*

Variable	Metamorphic stage (d.f.=1)	Speed category (d.f.=2)
Principal component 1	8.267*	10.588***
Principal component 2	10.525*	2.486
Principal component 3	7.434*	1.850
Principal component 4	0.203	3.770

Degrees of freedom are listed below each effect.
Table entries are *F*-values.
*$P \leqslant 0.05$ (sequential Bonferroni corrected); ***$P \leqslant 0.05$ (sequential Bonferroni corrected).

angle) being reached after the foot has left the ground to start the swing phase. Principal component 2 correlates with a larger pelvic girdle–femur angle range (greater extent of limb protraction and retraction), a large femur–crus range (large knee joint excursions) and a small initial crus angle. Variables tend to exhibit lower loadings on principal components 3 and 4, with no interpretable pattern.

Fig. 4 shows scatterplots of individual strides keyed according to metamorphic stage and speed on the first four principal components. The three speed categories tend to separate on PC 1, with the highest category (trot; separated off by the dotted line) being more distinct from the slow and medium walks than the latter two categories are from each other. Note that the region occupied by strides in principal component space expands as one moves to increasing values of PC 1, indicating that kinematic variability increases with speed. Note also that the polygons enclosing transformed strides are considerably smaller than those enclosing larval strides. This effect is more exaggerated in Fig. 4A (PC 1 *versus* PC 2) than in Fig. 4B (PC 3 *versus* PC 4). This difference in the extent of scatter in principal component space indicates that larvae show more overall variance in their kinematics than do postmetamorphic *Dicamptodon*.

Levene's test (Van Valen, 1978) was used on the principal component scores to determine whether the differences in variances suggested by the principal component plots in Fig. 4 were significant between metamorphic stages and speeds. These results are presented in Table 5 and confirm that there are significant differences in variance between larval and transformed groups on the first three principal components (which together account for 69 % of the total variance) and among the three speed categories on PC 1 (accounting for 39 % of the total variance).

Discussion

Metamorphic effects

The transition from an aquatic to a terrestrial existence in salamander metamorphosis involves, among other major effects, a profound change in the physical environment in which locomotion must be accomplished (Noble, 1931; Duellman and Trueb, 1986). One might therefore reasonably expect to find differences in some facets of the terrestrial

locomotor pattern for larval and metamorphosed animals, either because of disparity in efficient movement patterns in the two physical media or simply because of the need for muscular effort to oppose gravity in air. However, the overwhelming conclusion from the results presented in this study is that there are no consistent metamorphic differences in limb kinematics during terrestrial locomotion in *Dicamptodon*. None of the 21 variables tested showed a significant metamorphic effect in the three-way analysis of variance. These findings are consistent with those on swimming endurance (Shaffer *et al.* 1991) and aquatic feeding kinematics and motor patterns (Lauder and Shaffer, 1988; Shaffer and Lauder, 1988) across metamorphosis. Both of these experimental systems compare behaviours common to larvae and transformed salamanders. However, the present case is somewhat different in that the larvae are solely aquatic before metamorphosis. Therefore, two hypotheses are suggested by these observations. One possibility is that the motor patterns (and their resultant kinematic pattern) that will be required by the transformed animal are present in the larval nervous system and are not used until the animal attempts terrestrial locomotion. The alternative is that the same movement patterns are equally useful both on land and in the water. These two hypotheses should be tested by filming salamanders using their limbs to walk under water and comparing those kinematics with the corresponding values for terrestrial locomotion.

The attempt to determine metamorphic differences is confounded by the large amount of individual variation among salamanders. Most of the variables measured show significant individual effects (Table 2), and several variables show pronounced individual differences across metamorphosis (Fig. 2). This indicates that individual salamanders may change facets of their kinematic patterns across metamorphosis, but the direction of these changes is different among individual salamanders. These observations further strengthen the conclusion that no particular changes in specific variables are *required* as a consequence of metamorphosis.

The only consistent difference between larvae and metamorphs to emerge from this study is that the overall (multivariate) variance of larvae is significantly greater than that of the transformed group (Fig. 4; Table 5). This may result from the relative locomotor coordination of the two groups. Hildebrand (1966) observed that the variance in locomotor variables was greater for salamanders than for accomplished cursors. Larval salamanders, being inexperienced with the terrestrial environment, might be expected to show even higher variance. An additional possibility might be that larvae are neuromuscularly immature and have not settled on the more coordinated movement pattern characteristic of adults. Young mammals (cats, Peters, 1983; humans, Sutherland *et al.* 1980) have been shown to exhibit high variability in their gait variables before finally achieving the adult pattern. However, Shaffer *et al.* (1991) found that larvae are more variable than transformed salamanders in swimming burst speed. Since swimming is a behaviour common to both metamorphic stages, larger variance in larvae may reflect greater behavioural plasticity in this stage.

Speed effects

Speed increases in salamanders (from slow walk to trot) are apparently accomplished by the same suite of changes shown in a diverse array of other limbed animals. As has

been well documented for mammals (Hildebrand, 1966; Goslow *et al.* 1973; Biewener, 1983; Halbertsma, 1983; Vilensky and Gehlsen, 1984; Vilensky and Gankiewicz, 1990; Vilensky *et al.* 1990), chicks (Jacobson and Hollyday, 1982), lizards (Daan and Belterman, 1968), cockroaches (Full and Tu, 1990, 1991) and ghost crabs (Full and Weinstein, 1992), an increase in speed is accompanied by an increase in stride length and a decrease in both cycle duration and contact interval. Though not statistically significant, examination of mean values in Table 1 reveals a strong trend towards increasing the overall bending of the midline (anterior–posterior trunk angle range) in moving from the walking paces to a trot. This is consistent with the results of Edwards (1977), who found an increase in the contribution of girdle rotation (in which he included axial flexion) in salamanders at a trotting pace. However, it may not be a pattern which is applicable to all sprawling tetrapods, as Daan and Belterman (1968) reported that lizards tended to show either no increase or a decrease in lateral bending with increasing speed. Further quantitative data are needed to resolve this question, as Daan and Belterman (1968) were careful to point out that they were able to obtain a large sample size for only one lizard species.

Additionally, salamanders show changes in the timing of several variables in relation to the beginning of swing/stance phase in going from a walk to a trot (Tables 1, 2, 3). At a walking pace, the pelvic girdle is maximally advanced on the side of the leg being swung forward virtually simultaneously with the beginning of the stance phase, minimum pelvic girdle–femur angle (maximal femoral retraction) is simultaneous with the start of swing phase, and minimum femur–crus angle (maximal knee flexion) occurs almost 50 % of the stride before the beginning of swing phase (indicating that the knee joint is extending for the considerable period of the stride between those two points). In trotting, the pelvic girdle has already begun retraction (on the side of the leg in question) before the start of stance phase, minimum pelvic girdle–femur angle is achieved during the swing phase, and the minimum femur–crus angle is almost in phase with the beginning of swing phase (indicating that there is less knee extension pushing the body forward at the trot). Consistent changes in the timing of maximum and minimum angles during the step cycle have been noted for vervet monkeys (Vilensky and Gankiewicz, 1990). These authors noted that the time (in percentage of the stride) of maximal femoral protraction decreased with speed in relation to the beginning of stance (indicating that the limb begins retraction before the start of the stance phase; the limb is retracted earlier with increasing speed). They also noted that the relative time of the minimum ankle joint angle during the stride decreased with increasing speed; however, because ankle joint angle was not measured for the salamanders in this study, this result is not directly comparable. Vilensky and Gankiewicz (1990) also noted considerable individual variation, with some vervets showing consistent decreases in only some angle maxima/minima timings across speeds, while other vervets showed consistent changes in other angles. Such observations of high individual variation are consistent with those reported here, where 11 of 21 variables showed significant speed $\times$ individual effects in the two-way ANOVA. These results indicate that individuals can use different methods (changing different variables) to accomplish the same result (speed increase).

No consistent changes with increasing speed were noted for any of the angular minima,

maxima or excursions in *Dicamptodon*. Again, many of these variables showed significant speed × individual effects, indicating that, although individuals may alter these values to effect an increase in speed, none of these variables is *required* to change with speed. Similar individual variability was reported by Vilensky and Gankiewicz (1990) for vervet monkeys. Overall, the mammalian literature reports either no change (Goslow *et al.* 1973; Grillner, 1975) or a slight increase (Goslow *et al.* 1973, 1981; Halbertsma, 1983) in different angular excursions of the limb with increasing speed.

Two major conclusions may be drawn from these results for salamander locomotion. First, larvae possess the same hindlimb locomotor pattern for terrestrial locomotion as metamorphs, though they show a higher variance in their movements. This greater degree of variation in larvae is consistent with findings on locomotor performance in salamanders (Shaffer *et al.* 1991) and also with data for immature mammals (Sutherland *et al.* 1980; Peters, 1983). The higher variance in larval salamanders may be due to neuromuscular immaturity or may represent greater neuromuscular plasticity in the larval stage. Second, salamanders use the same mechanisms as other limbed animals to change speed, by increasing stride length and decreasing cycle duration and contact interval. Like mammals, salamanders also show changes in the timing of the limb movements rather than a change in the excursions of various limb segments. Since this same strategy for increasing speed is present in these two widely separated taxonomic groups, it is likely that it was adopted very early in tetrapod evolution. If primitive amphibians possessed a high degree of locomotor plasticity coupled with the ability to vary the timing of limb movements to increase speed, the combination of these two traits may have aided the invasion of the terrestrial environment.

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