

Motor Patterns and Kinematics During Backward Walking in the Pacific Giant Salamander: Evidence for Novel Motor Output

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Ashley-Ross, Miriam A. and George V. Lauder. Motor patterns and kinematics during backward walking in the Pacific Giant Salamander: evidence for novel motor output. *J. Neurophysiol.* 78: 3047–3060, 1997. Kinematic and motor patterns during forward and backward walking in the salamander *Dicamptodon tenebrosus* were compared to determine whether the differences seen in mammals also apply to a lower vertebrate with sprawling posture and to measure the flexibility of motor output by tetrapod central pattern generators. During treadmill locomotion, electromyograms (EMGs) were recorded from hindlimb muscles of *Dicamptodon* while simultaneous high-speed video records documented movement of the body, thigh, and crus and allowed EMGs to be synchronized to limb movements. In forward locomotion, the trunk was lifted above the treadmill surface. The pelvic girdle and trunk underwent smooth side-to-side oscillations throughout the stride. At the beginning of the stance phase, the femur was protracted and the knee joint extended. The knee joint initially flexed in early stance and then extended as the foot pushed off in late stance, reaching maximum extension just before foot lift-off. The femur retracted steadily throughout the stance. In the swing phase, the femur rapidly protracted, and the leg was brought forward in an “overhand crawl” motion. In backward walking, the body frequently remained in contact with the treadmill surface. The pelvic girdle, trunk, and femur remained relatively still during stance phase, and most motion occurred at the knee joint. The knee joint extended throughout most of stance, as the body moved back, away from the stationary foot. The knee flexed during swing. Four of five angles showed significantly smaller ranges in backward than in forward walking. EMGs of forward walking showed that ventral muscles were coactive, beginning activity just before foot touchdown and ceasing during the middle of stance phase. Dorsal muscles were active primarily during swing. Backward locomotion showed a different pattern; all muscles except one showed primary activity during the swing phase. This pattern of muscle synergy in backward walking never was seen in forward locomotion. Also, several muscles demonstrated lower burst rectified integrated areas (RIA) or durations during backward locomotion. Multivariate statistical analysis of EMG onset and RIA completely separated forward and backward walking along the first principal component, based on higher RIAs, longer durations of muscle activity, and greater synergy between ventral muscles during early stance in forward walking. Backward walking in *Dicamptodon* uses a novel motor pattern not seen during forward walking in salamanders or during any other locomotor activity in previously studied tetrapods. The central neuronal mechanisms mediating locomotion in this primitive tetrapod are thus capable of considerable plasticity.

INTRODUCTION

Our understanding of pattern-generating circuits in the nervous system is based largely on analyses of regularly repeating behaviors. In vertebrates, the best-characterized such behavior is forward walking. Studies in mammals have demonstrated

that the basic pattern of forward locomotion may be produced by spinal circuits (Grillner and Wallen 1985; Grillner and Zanger 1979) with input from supraspinal centers and proprioceptive and cutaneous feedback necessary to produce finely controlled stepping (e.g., Andersson and Grillner 1981; Armstrong 1988; Conway et al. 1987). Thus forward walking has been used as a model system with which to understand the generation of relatively simple behaviors. Numerous studies have detailed the patterns of limb kinematics (e.g., Ashley-Ross 1994a; Goslow et al. 1973, 1981, 1989; Goslow and Van De Graaff 1982; Hildebrand 1976, 1980; Jenkins and Weijs 1979; Johnston and Bekoff 1992) and muscle synergies (Ashley-Ross 1995; Bekoff et al. 1975, 1987b; Engberg and Lundberg 1969; Goslow et al. 1981; Hoffer et al. 1987; Jenkins and Goslow 1983; Jenkins and Weijs 1979; Nicolopoulos-Stournaras and Iles 1984; Rasmussen et al. 1978; Szekely et al. 1969; Wentink 1976). Recently, backward walking has become the subject of several studies attempting to discover how the output of a central pattern generator (CPG) may be modified. Most of these reports have focused on humans (Bates et al. 1984; Flynn et al. 1994; Miller et al. 1978; Myatt et al. 1995; Thorstensson 1986; Vilensky et al. 1986, 1987; Winter et al. 1989) and cats (Buford and Smith 1990, 1993; Buford et al. 1990; Perell et al. 1991, 1993), mammals for which posture may be characterized as “upright”, meaning that the body is positioned directly over the limbs.

Grillner (1981, 1985) suggested that backward walking could be produced by simply switching muscle synergies about the hip and knee joints. Whereas forward walking is characterized by coactivation of hip and knee extensors during stance and cocontraction of hip and knee flexors during swing, it was predicted that backward walking would be produced by coactivation of hip flexors with knee extensors during stance, and hip extensors with knee flexors during swing. Results from some human and cat studies generally have supported Grillner's hypothesis of predicted patterns of hindlimb movement (Thorstensson 1986; Winter et al. 1989), although Vilensky et al. (1987) found notable differences between forward and backward kinematics. However, the relative activity periods for several muscles (particularly hip extensors) remained the same in both forward and backward walking, suggesting a change in muscle function (e.g., from concentric to eccentric contraction) (Buford and Smith 1990; Thorstensson 1986).

Few studies (Axon et al. 1987; Eilam and Shefer 1992) have examined the kinematics or motor patterns of backward walking in animals without an upright posture. Therefore, it

is unclear whether any observed similarities in limb kinematics and motor patterns arise from a constraint on the neural output of the locomotor CPG or simply result from the gravitational demands of balancing the body over the limbs. In addition, the extent of plasticity in central neuronal mechanisms mediating locomotion is unknown for a primitive tetrapod even though such data are critical for understanding the evolution of CPGs (Cohen 1988). We investigate these questions by examining the kinematics and motor patterns of forward and backward walking in a primitive vertebrate with a sprawling posture (where the limbs extend out to the sides of the body), the Pacific Giant Salamander, *Dicamptodon tenebrosus*. Some of the results presented here have appeared previously in abstract form (Ashley-Ross 1994b).

METHODS

Animals

The Pacific Giant Salamander, *D. tenebrosus* (Good 1989), was chosen for this study as this species demonstrates good walking ability and has robust limbs, hindlimb anatomy has been described (Ashley-Ross 1992), and kinematic and electromyographic (EMG) data for forward walking are available for comparison (Ashley-Ross 1994a, 1995). Eight animals collected as larvae in Mendocino County, CA (California scientific collector permits Nos. 7058 and 7614) were used. Salamanders were housed in 40-l terraria that provided free access to water and were fed earthworms and crickets two or three times a week. All animals were maintained in the same facility on a 12L:12D photoperiod. At the time of experiments, all salamanders were a minimum of 2 mo postmetamorphic. Snout-vent lengths (SVL) of animals ranged from 12.15 to 13.80 cm at the time of the experiments.

Video recording

Forward and backward walking occurred on a variable-speed, motor driven treadmill (belt material made of rubberized nylon weave) with an effective working area of 18-in length by 8-in width (Fig. 1). Salamanders readily walked forward toward a "hiding place", a section of black PVC tubing suspended at the far end of the treadmill or were encouraged to walk by touching or gently squeezing the base of the tail. Backward walking was elicited by gently tapping the animals on the nose with a blunt dowel. Animals always were placed on the treadmill so that their left side faced the cameras. White dots were painted (using Testor's flat white model paint) over the dorsal ends of the two ilia, at the left knee, at the distal end of the left fibula, and in a series of seven to nine dots along the animal's dorsal midline (points were spaced equally, beginning at a point between the 2 scapulae and ending between the 2 ilia) to ensure reliable identification of these points. Animals were videotaped using a NAC HSV-400 High-Speed Video System (NAC Industries, Japan; sampling frequency of 200 fields per second) equipped with two cameras: one for a direct lateral view and the other, which was equipped with a zoom lens, for a dorsal view through a front-surface mirror inclined above the treadmill surface at a 45° angle. The zoom lens was adjusted so that both lateral and dorsal views of a scale bar were the same size. A 1-cm grid was affixed to the rear wall of the treadmill, below the mirror, and the treadmill belt was marked with lines 10 cm apart to enable accurate distance measurements to be taken from the videotape fields. Elapsed time (in 5-ms intervals) and a 100-Hz signal for synchronizing EMG recordings were recorded on each video field. Kinematic events of the stride (i.e., step cycle duration, beginning of stance and swing phases) were determined by examining these high-speed videos for each experiment. To

exclude any potential complicating effects of starting or stopping on kinematic and motor patterns, only sequences of strides where the animal sustained a constant speed were used for analysis.

Backward locomotion was always slower than forward locomotion (backward locomotion was likely limited by a shorter stride length; see Fig. 1), thus different speeds were measured for the two directions of walking. However, whereas forward locomotion may occur at a variety of speeds, backward walking generally occurred at one natural pace that the animal would comfortably maintain without resorting to an escape response. We therefore feel that the speeds used in this study, although not matched in terms of SVL/s, represent a valid comparison between the highest obtainable speed of backward locomotion and a modest forward speed. In addition, as described below, the kinematic and EMG differences observed between backward and forward locomotion are numerous and distinct; patterns exhibited during backward locomotion were not observed during forward locomotion at any speed (Ashley-Ross 1994c). Kinematics from a total of eight forward and seven backward strides from three animals were analyzed. For this kinematic data set, only strides in which movement of the salamander was perpendicular to the camera axis were included.

Video analysis

Gaits were classified according to the conventions of Hildebrand (1976). At least 20 fields from each stride, equally spaced in time, were captured to optical media for later digitizing. Video digitizing and analysis procedures are described in Ashley-Ross (1994a). Briefly, a custom video-analysis program was used to digitize two-dimensional coordinates of the painted marker points. The location of the hip joint (acetabulum) was obscured by the knee and crus for large portions of the stride in the lateral view, and hence a complete three-dimensional reconstruction of limb movements was not possible. The two-dimensional coordinates were imported into a spreadsheet program, which was used to compute angular variables (defined below). The kinematic profiles 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 Pro (WaveMetrics, Lake Oswego, OR) to reduce digitizing error. Minimum and maximum values for each kinematic variable were calculated subsequently from the smoothed kinematic profiles. To aid visual comparison of forward and backward kinematics, traces from backward walking were reversed following the procedure of Winter et al. (1989).

Definition of variables

A *stride* was defined as the time (in s) from hind foot contact with the treadmill belt to the subsequent contact of the same foot. The time during the stride in which the foot is in contact with the substrate is termed the *stance phase* or *contact interval*, whereas the time that the foot is elevated and being moved into position for the start of the next stride is termed the *swing phase*. Because each stride may have differing relative proportions of stance and swing phase, all timing variables were normalized further by converting them into the corresponding values for a standardized stride consisting of 75% stance and 25% swing as advocated in Ashley-Ross (1995).

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 (see Ashley-Ross 1994a, Fig. 1 for detailed variable descriptions). *Pelvic girdle-femur angle* was measured between the pelvic girdle line and the line connecting the calculated position of the acetabulum 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

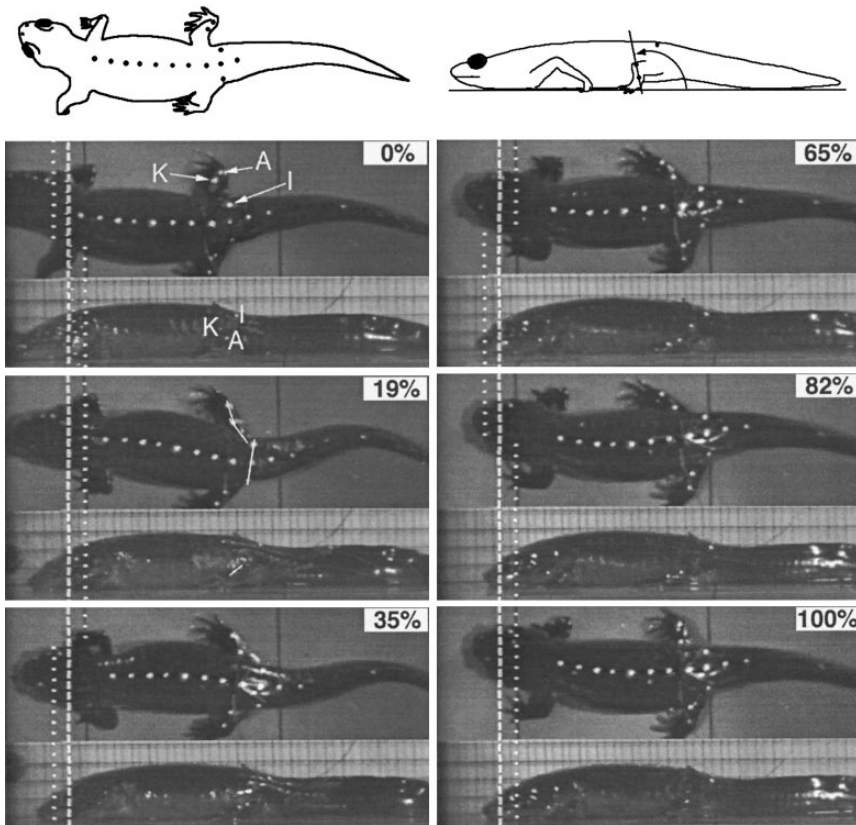


FIG. 1. Sequence of fields taken from the high-speed video showing one complete backward stride (lasting 1,840 ms; speed 0.10 snout-vent length (SVL)/s) of *Dicamptodon tenebrosus*. Sequence progresses down columns. Fields from the original video tape were cropped and composited using Adobe Photoshop (Adobe Systems, Mountain View, CA). White pattern of right and left square pulses on the left side of each field is the synchronizing pulse, which was used to match kinematic to electromyographic (EMG) events. Dorsal view of each field (top) is filmed through a 45° mirror, so the marked left leg is toward the top of each field. First field (0%) indicates the position of painted markers over the left ilium (I), left knee (K), and left ankle (A) in both dorsal and lateral views. In the second field (19%), line segments connecting the 2 ilia (dorsal view), the acetabulum and knee (dorsal view), and the knee and ankle (both views), are drawn in to illustrate the orientation of the limb segments used to calculate kinematic variables. Line drawings (top) of the figure show the position of the body in the dorsal and lateral views to aid in interpreting the video fields below, and the lateral view illustrates how the crus orientation angle was measured. Note the small percentage of time spent in the swing phase of the stride.

was inclined forward of that line (protracted), and negative values when inclined back of that line (retracted). Note that femoral protraction in the salamander is analogous to hip flexion in mammals and birds, and femoral retraction is equivalent to hip extension (Ashley-Ross 1992, 1994a). *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). Overall bending of the trunk was measured by the angle between the lines connecting the rostral-most and caudal-most pairs of marker dots in the trunk region (*anterior-posterior angle*). One additional angle variable, the *lateral crus angle* (or orientation angle), was digitized from the 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. A value of 90° indicates the crus is perpendicular to the substrate; an acute angle results when the knee joint is located caudal to the ankle, and an obtuse angle occurs when the knee is cranial to the ankle.

Electromyography

Electrical activity patterns of 13 hindlimb muscles of *Dicamptodon* were recorded from a total of 83 forward and 26 backward strides from eight salamanders. EMG recording methods are described in Ashley-Ross (1995). Briefly, insulated bipolar steel alloy electrodes (0.051-mm diam wire) were implanted into hindlimb muscles while the animal was anesthetized with a solution of tricaine methane sulfonate (MS-222; 0.4 g/l final concentration). Electrical activity was recorded simultaneously from ≤ 13 electrodes in each animal. Electrode tips were bared for ≤ 0.5 mm and intertip distances were 1–2 mm. Implants were made percutaneously. A ground electrode was implanted into connective tissue directly over the vertebral column. Animals recovered from anesthesia within 1 h, and all recordings were made within four hours postanesthesia. Immediately after each experiment, animals were

killed by anesthetic overdose and preserved in 10% formalin. Electrode position was confirmed by dissection.

EMGs were amplified 10,000 times with Grass P511J preamplifiers with a 60-Hz notch filter and a band-pass of 100–3,000 Hz before being recorded on a Teac XR5000 data recorder. The EMG records were played back at an 8-kHz sample rate for each channel (see Jayne et al. 1990) through a Keithley A/D converter into a microcomputer. The EMGs for each stride sequence were analyzed using two custom analysis programs. One program calculated EMG burst onset time, offset time, duration, and rectified integrated area (RIA). The other program used the algorithm of Beach et al. (1982) to analyze within-burst variation by calculating number of spikes, number of large spikes (spikes that exceeded a threshold magnitude set initially by visual inspection of the burst), average spike amplitude, and RIA for each 25-ms bin within an EMG burst. Because RIA captures information about both the number of spikes and their amplitude, this variable was chosen for subsequent analyses of variation in EMG bursts due to direction of locomotion. Comparison of all EMG channel recordings for each animal confirmed that each muscle's EMG profile was distinct from that of adjacent implanted muscles in onset and offset times, and EMG burst shape. Thus potential complications due to muscle cross-talk were ruled out.

The onset, offset, and burst RIA variables listed above were imported into a spreadsheet program and standardized by dividing by the step cycle duration (all kinematic timing variables were derived from analysis of videos of each experiment). Each variable therefore is expressed as a percentage of the step cycle duration.

Statistical analysis

Kinematic angular data were tested for differences between forward and backward walking by performing unpaired *t*-tests on the angle ranges. Tests of directional differences between cycle

durations and contact intervals were performed using the full data set (83 forward and 26 backward strides). Two-way analyses of variance (ANOVAs) were performed with direction of travel as a fixed effect and individual as a random effect. Significance of the direction effect was tested over the direction \times individual interaction term. The sequential Bonferroni method described by Rice (1989) was used to correct significance levels for multiple comparisons.

EMG differences between forward and backward walking were summarized by a principal component analysis (calculated from the correlation matrix) on EMG onset and burst RIA for a reduced data set of eight muscles [proximal and distal puboischiofibularis muscle (PIT), caudofemoralis muscle (CDF), puboischiofibularis externus muscle (PIFE), puboischiofibularis internus muscle (PIFI), flexor primordialis communis muscle, extensor iliotibialis pars anterior muscle (ILTA), and extensor iliotibialis pars posterior muscle (ILTP)]. A reduced data set of 28 strides was used to eliminate missing values that arose for some muscles because not all animals had the same muscles implanted. This reduced data set had the same muscles represented for all animals, with no missing cells.

RESULTS

Kinematics

A representative backward stride is shown in Fig. 1. The majority of the cycle was occupied by the stance phase, and the swing phase was confined to 23% of the total stride. The stance phase began with the foot placed on the substrate in a position lateral to the hip. The short swing phase was characterized by the foot being lifted slightly above the surface of the treadmill and slid posteriorly to a position lateral to the hip joint in preparation for the next stride (Fig. 1, 82–100%). Note that the body was not clearly lifted off of the substrate (Fig. 1, lateral view) in this representative stride. Salamanders varied in whether they lifted the body from the substrate during backward locomotion, in contrast to forward walking, when the body always was held clear of the substrate (Ashley-Ross 1994a). To ensure that any differences observed between forward and backward walking were real and not due to differences caused by the body being lifted in some backward strides versus slid along the substrate in others, all kinematic and EMG variables were tested by a nested ANOVA with body position as the main effect and individual nested within the main effect. No significant differences were seen ($P > 0.05$ for all variables), and therefore all backward strides were considered together for subsequent analyses. The lack of kinematic differences in the present case paralleled the lack of difference with body position previously seen in forward walking for metamorphosed (always raise the body) versus larval (often drag the body) *Dicamptodon* (Ashley-Ross 1994c).

In forward locomotion, all salamanders used a lateral sequence walk (Fig. 2, top), defined as a walking gait wherein the first foot to strike the substrate after a given hind foot is the fore foot on the same side of the body (Hildebrand 1976). The stance phase is initiated by heel contact and ends with toe off. In backward locomotion, the sequence of footfalls used did not conform to any gait pattern described by Hildebrand (1976) (Fig. 2, lower panel). The stance phase of backward locomotion begins with toe contact and ends with heel off.

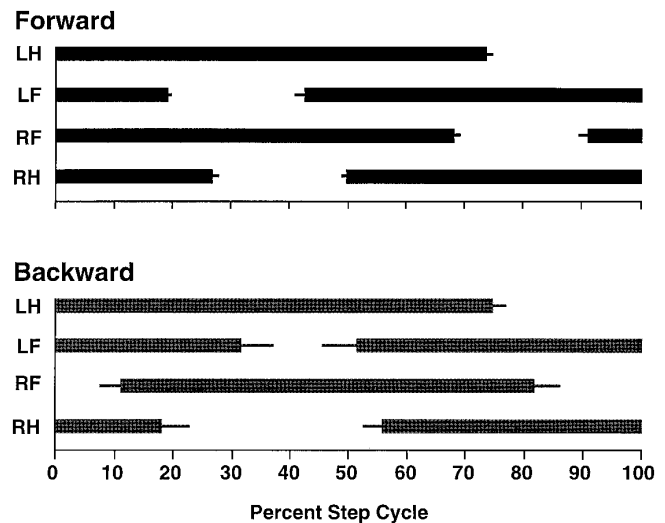


FIG. 2. Gait diagrams for *D. tenebrosus*. Thick bars represent the time when the foot is contacting the ground. Ends of the bars are calculated as the mean touchdown/lift off times for all animals. Thin bars represent 1 SE error of the mean. LH, left hind foot; LF, left fore foot; RF, right fore foot; RH, right hind foot. Top: forward walking, classified as a diagonal couplets lateral sequence walk (Hildebrand 1976). Bottom: backward locomotion. This footfall pattern does not conform to any gait in Hildebrand's classification.

Average speed was ~ 0.75 SVL/s during forward locomotion, in contrast to an average speed of 0.13 SVL/s used during backward walking. Cycle duration differed significantly between direction of travel ($P < 0.002$), averaging 1.14 and 1.76 s for forward and backward locomotion, respectively. Mean contact interval also differed significantly between locomotor directions ($P < 0.02$), with an average of 67% for forward walking and 79% for backward locomotion, respectively (see Table 2).

Figure 3 shows average kinematic profiles of the angular variables measured for forward and backward walking. Forward walking is plotted from left to right, whereas backward walking is plotted from right to left. In forward walking, the pelvic girdle (Fig. 3A) and trunk (Fig. 3E) underwent a smooth oscillation throughout the stride. At the beginning of the stance phase, the femur was angled forward relative to the pelvic girdle (Fig. 3B), and the crus was extended, making an acute angle with the treadmill surface (Fig. 3, C and D). As the stance phase progressed, the trunk straightened (toward 0°; Fig. 3E) followed by flexion toward the opposite side. The knee joint (Fig. 3C) showed flexion in early stance as the salamander body moved toward the foot, followed by extension in the second half of stance. The knee joint reached its greatest extension just before foot lift-off, which occurred as the posterior tarsal bones left the substrate. The femur was retracted relatively steadily throughout the stance phase. During the swing phase, the pelvic girdle–femur angle increased rapidly (from negative to positive values), and the lateral crus angle dropped abruptly as the entire leg was protracted by being swung straight out to the side of the animal in an “overhand crawl” motion.

Kinematic angles and EMGs recorded for the backward stride shown in Fig. 1 are diagrammed in Fig. 4. In backward walking, the pelvic girdle, trunk, and femur were held relatively still (Figs. 1, 0–82%; 3, A, B, and E; and 4), and the

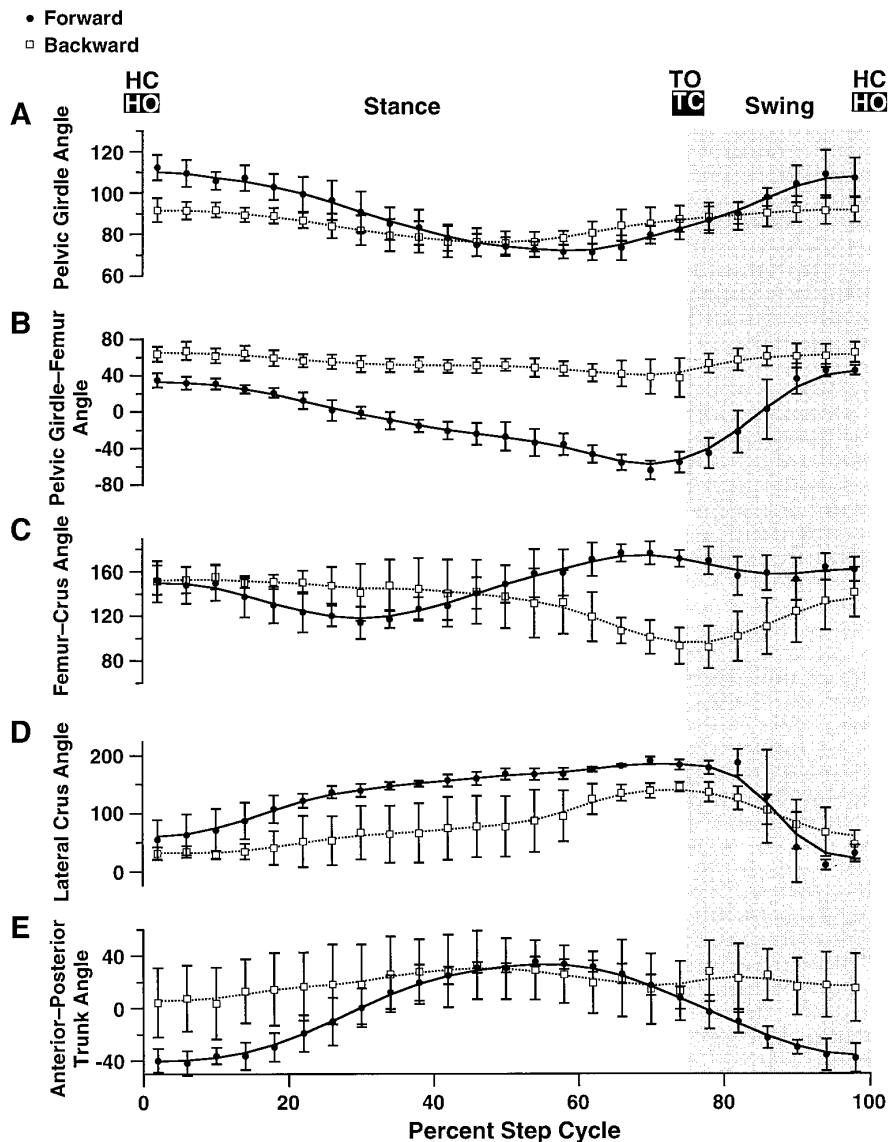


FIG. 3. Mean kinematic profiles of 5 angle variables for forward (— and ●) and backward (··· and □) walking in *D. tenebrosus* vs. percent step cycle. Gray region represents the swing phase of the stride. Note that the forward traces read left to right, whereas the backward traces read right to left. All angles are measured in degrees. Error bars are SD. In all panels, symbols are the calculated means, lines are the smoothed kinematic profiles used to calculate variable minima and maxima (see METHODS, *Video analysis*). Note that in (E), negative angle values indicate the trunk is concave to the left. HC, heel contact in forward locomotion; TO, toe-off in forward walking; TC, toe contact in backward locomotion; HO, heel-off in backward walking.

major motion occurred at the knee joint (Fig. 3, C and D). The knee joint extended throughout the stance phase, with most of the extension occurring during the first third of stance (Figs. 3C and 4). During the middle portion of the stance phase, there is relatively little change in femur-crur angle. Flexion of the knee begins late in stance, with most of the angular change occurring during swing (Figs. 3C and 4). The total amount of knee flexion and extension was similar for forward and backward walking. Values for pelvic girdle-femur angle never became negative in backward locomotion, indicating that the femur was never retracted past a point perpendicular to the body. The crus started from an initial angle with the substrate of $\sim 90^\circ$ or greater at the beginning of stance and progressed to more acute angles as stance continued and the body was pushed back, away from the stationary foot (Figs. 3D, 1, and 4). As the animal moved backward, progressively more of the plantar surface of the foot came into contact with the substrate. Four of the five angle variables showed significantly smaller ranges in backward walking than in forward walking (pelvic girdle

angle, pelvic girdle-femur angle, lateral crus angle, and anterior-posterior trunk angle; all with $P < 0.001$). Overall, limb kinematics were more variable in backward locomotion than in forward walking (compare error bar sizes in Fig. 3).

Electromyography

Recordings were made from 11 distinct muscles of the salamander hindlimb; these muscles and their respective functions are presented in Table 1. During forward walking, the ventrally located muscles plus the flexor primordialis communis (FPC; the major muscle of the salamander "calf") began activity in the late swing phase, just before the foot contacted the substrate, and demonstrated large periods of overlap in activity (Fig. 5; Table 1). The order of activation appeared to progress in an anterior to posterior fashion, beginning with pubotibialis muscle (PTB), followed by PIT, ISF, caudalipuboischiotibialis muscle (CPIT), and finally CDF (Fig. 5). The distal portions of the PIT and ISF had a mean onset prior to the proximal

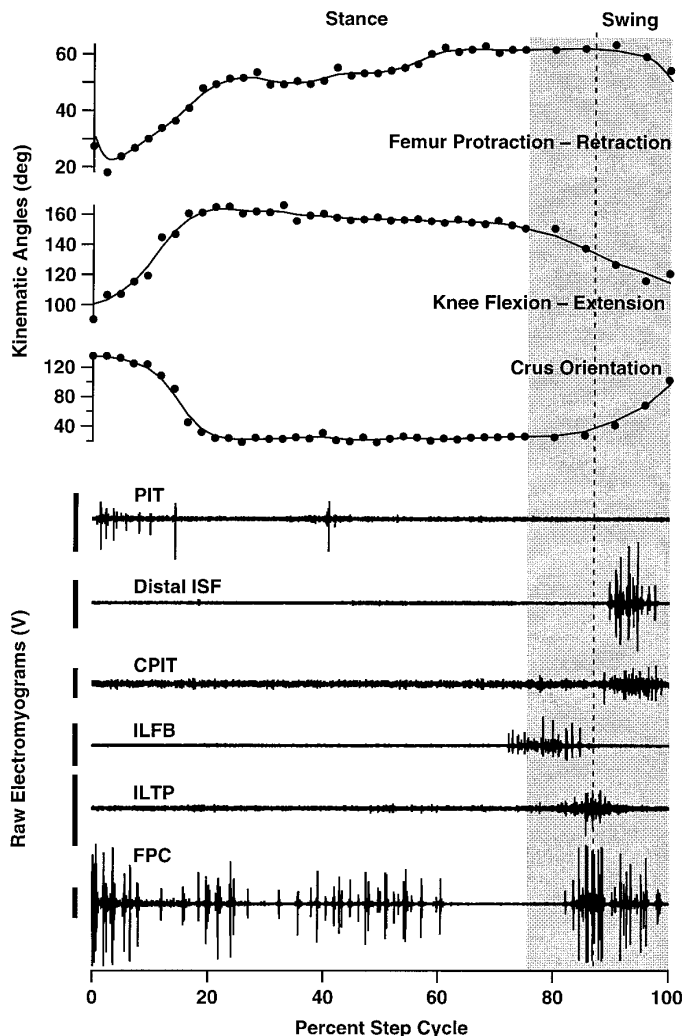


FIG. 4. Limb kinematic angles and EMGs from the same backward stride shown in Fig. 1 vs. percent step cycle. Gray region indicates the time when the limb is being elevated. During the *left portion* of the shaded area, the femur is elevated and the foot is being lifted off the treadmill, while the region to the *right* of the vertical dashed line represents the time when the limb is elevated completely in the swing phase. *Top*: changes in angles associated with limb segments of the left hindlimb: pelvic girdle–femur angle (*top*), femur–crus angle (*middle*), and lateral crus angle (*bottom*). In the angle plots, symbols are the raw data calculated from video analysis, whereas lines are smoothed kinematic profiles. *Bottom*: raw EMGs for 6 hindlimb muscles. EMGs for the puboischiotibialis (PIT), distal ischioflexorius (ISF), caudalipuboischiotibialis (CPIT), and extensor iliotibialis pars posterior (ILTP) were taken from the right hindlimb, and these traces have been shifted in time so as to line up with the stride of the left hindlimb for easy comparison between kinematic and EMG events. EMGs from the iliofibularis (ILFB) and flexor primordialialis communis (FPC) are from the left hindlimb. Scale bars to the left of each plot represent 0.25 mV for all muscles.

sections. PIFE, one of the deepest muscles of the ventral pelvic girdle, became active in concert with the other ventral muscles and persisted in activity throughout most of the stance phase. Several ventral muscles showed “variable secondary bursts” in addition to the consistent bursts of electrical activity described above (the muscle’s “primary burst”, present in 100% of forward strides). These secondary bursts were not consistently present in stride sequences, but when present, were distinct from the primary burst. For individual

muscles, secondary activity periods were present from 16 to 90% of the time (mean for all muscles of 43%). Secondary bursts in the PIT and the FPC occurred during the stance phase, whereas those in the PIFE occurred during the early swing phase (shaded bars in Fig. 5). Dorsal hindlimb muscles were all recruited during the swing phase and had considerable overlap in their periods of activity (Fig. 5, Table 1). The order of activity also showed a progression, this time in a posterior to anterior direction; iliofibularis muscle (ILFB) became active just before the start of swing phase, followed by ILTP and then ILTA (Fig. 5). The deeply located PIFI activated in tandem with the ILFB and continued to be active through the first half of the swing phase. All dorsal muscles except the ILTA showed a variable secondary burst of activity (shaded bars in Fig. 5). When present, the secondary burst occurred during the middle of the stance phase.

Different patterns of EMG activity were seen in backward locomotion. Overall, motor patterns of backward walking were more variable than those of forward walking (Figs. 4 and 5). Bursts of activity were not always present for each muscle in each stride; primary bursts were present from 50 to 100% of the time (mean of 86% for all muscles), whereas variable secondary bursts occurred in 6–82% of strides (average for all muscles was 41%). The primary period of activity of all muscles except the PIFE (and to a lesser extent, the PTB) shifted into the swing phase during backward walking (Fig. 5). The long overlapping bursts of ventral muscles during the stance phase of forward walking (which would help support the body) largely disappeared in backward locomotion (Figs. 1 and 4), even when the body was raised off the substrate. The FPC (Fig. 4) and PTB are the only muscles that consistently showed long periods of activity during backward locomotion. As in forward walking, dorsal muscles displayed their primary activity during the swing phase, with secondary bursts (in PIFI and ILTA) during stance (hatched bars in Fig. 5). Surprisingly, there was little consistent activity of the expected hip flexor (PIFI) and knee extensor muscles (ILTA, ILTP; see also Fig. 4) in the stance phase, given that one would predict that the “power stroke” of backward locomotion should be femoral protraction and knee extension during stance. However, the PTB originates rostral to the hip joint on the pelvic girdle (Ashley-Ross 1992) and thus is in a position to protract the femur (Table 1). The consistent recruitment of the PTB during mid to late stance thus may provide the major contribution to hip flexion at this time. Finally, the pattern of synergy of EMG activity was very different in backward locomotion, with both dorsal and ventral muscles being primarily coactive during the swing phase. These muscles were never all simultaneously active in forward locomotion (Fig. 5).

Spike analysis

Figure 6 illustrates the activity envelopes (RIA per 25 ms bin) of the primary bursts of eight representative muscles during forward and backward locomotion. All panels of Fig. 6 are plotted on the same x and y scales so comparisons may be made among muscles. Most muscles showed a pattern of activity in which the RIA either increased rapidly to a maximum early in the burst and then slowly declined (Fig. 6;

TABLE 1. *Summary of salamander muscle names and functions*

Salamander			Mammalian	
Muscle	Abbreviation	Function	Homologue	Function
Puboischiotibialis	PIT	Adduct and retract femur, flex knee	Gracilis	Adduct femur, flex knee
Pubotibialis	PTB	Adduct and protract femur, flex knee	Adductor magnus	Adduct and protract femur
Ischioflexorius	ISF	Flex knee and toes, retract femur	Semimembranosus, semitendinosus, biceps femoris	Flex knee, retract femur
Puboischiofemoralis externus	PIFE	Adduct femur	Obturator externus, quadratus femoris	Rotate femur
Caudalipuboischiotibialis	CPIT	Retract femur, flex tail	Piriformis	Rotate femur
Caudofemoralis	CDF	Retract femur, flex tail	Medial gastrocnemius, soleus	Plantarflex ankle, flex knee
Flexor primordialis communis	FPC	Plantarflex foot, flex digits and tarsus, flex knee		Plantarflex ankle
Puboischiofemoralis internus	PIFI	Protract femur	Iliopsoas	Protract and rotate femur
Extensor iliotibialis, pars anterior	ILTA	Extend knee, elevate femur	Sartorius	Elevate femur, flex/extend knee (depending on compartment recruited)
Extensor iliotibialis, pars posterior	ILTP	Extend knee, elevate femur	Rectus femoris	Elevate femur, extend knee
Iliofibularis	ILFB	Flex knee, elevate femur	Gluteus maximus	Retract femur

Mammalian homologs, where known, are given to aid comparison with human and cat studies. See text for discussion.

e.g., proximal and distal PIT, PIFI during forward walking) or maintained a relatively stable level (Fig. 6; e.g., PIFI, ILTA in backward locomotion). There was no consistent pattern to the envelope of activity that could be correlated to either the direction of locomotion or period of activity

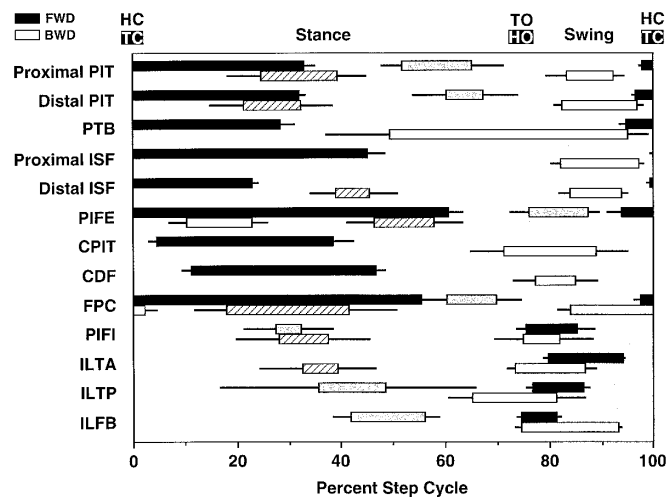


FIG. 5. Summary bar diagram of EMG activity in hindlimb muscles vs. percent step cycle, normalized to 75% stance/25% swing. Gray region indicates the swing phase of the stride. Ends of the thick bars represent the mean onset and offset of muscle activity, and the thin bars show 1 SE. Primary EMG bursts (present in most strides) are represented by black bars for forward walking, and white bars for backward walking. Secondary, variable EMG bursts are represented by shaded bars for forward walking and hatched bars for backward walking. Note that in backward locomotion, EMG bursts were more variable than in forward walking and in some strides EMG activity in certain muscles was absent (e.g., the lack of PIT activity in Fig. 4). In this figure, the direction of the backward trace has not been reversed (i.e., both forward and backward traces read left to right). HC, heel contact in forward locomotion; TO, toe-off in forward walking; TC, toe contact in backward locomotion; HO, heel-off in backward walking.

for all muscles (indicated by the *insets* in Fig. 6). However, one generalization that may be made is that muscles that are in a position (and activated at an appropriate time) to effect a reversal in direction of the limb tend to show an early peak in activity (e.g., proximal and distal PIT during forward stance, PIFI and ILTP during forward swing, FPC during backward swing). This initial intense activation presumably is responsible for overcoming the inertia of the limb and starting movement in the new direction and is followed by a lower level of activation as the limb continues to move. In contrast, muscles that act to continue or retard the movement of the limb in a given direction (e.g., CDF during forward locomotion, PIFI during backward swing) showed a low steady level of activity. Note that some muscles showed approximately the same absolute duration of activity in both forward and backward walking (proximal and distal PIT, ILTA, ILTP), whereas for other muscles, the duration of the EMG burst was distinctly different according to the direction of travel (CDF, PIFE, FPC, PIFI). Finally, the amplitude of the RIA varied between forward and backward walking for some muscles (e.g., ILTA, ILTP, distal PIT, PIFI; see also Table 2). In all but one case (ILTP), the per-bin RIA was higher in forward than in backward walking, indicating that these hindlimb muscles were recruited more intensely during forward walking. One interesting difference between forward and backward locomotion is seen by comparing the activity envelopes for the ILTA and ILTP. In forward walking, ILTA had a larger RIA envelope than ILTP, whereas exactly the opposite was true during backward progression. Also, the amplitude of the RIA envelope was similar between ILTA in forward walking and ILTP in backward walking. This suggests that of this pair of muscles, the one that is most "anterior" (when considered in respect to the direction of locomotion) plays the major role in swinging the limb into position for the beginning of the next stride.

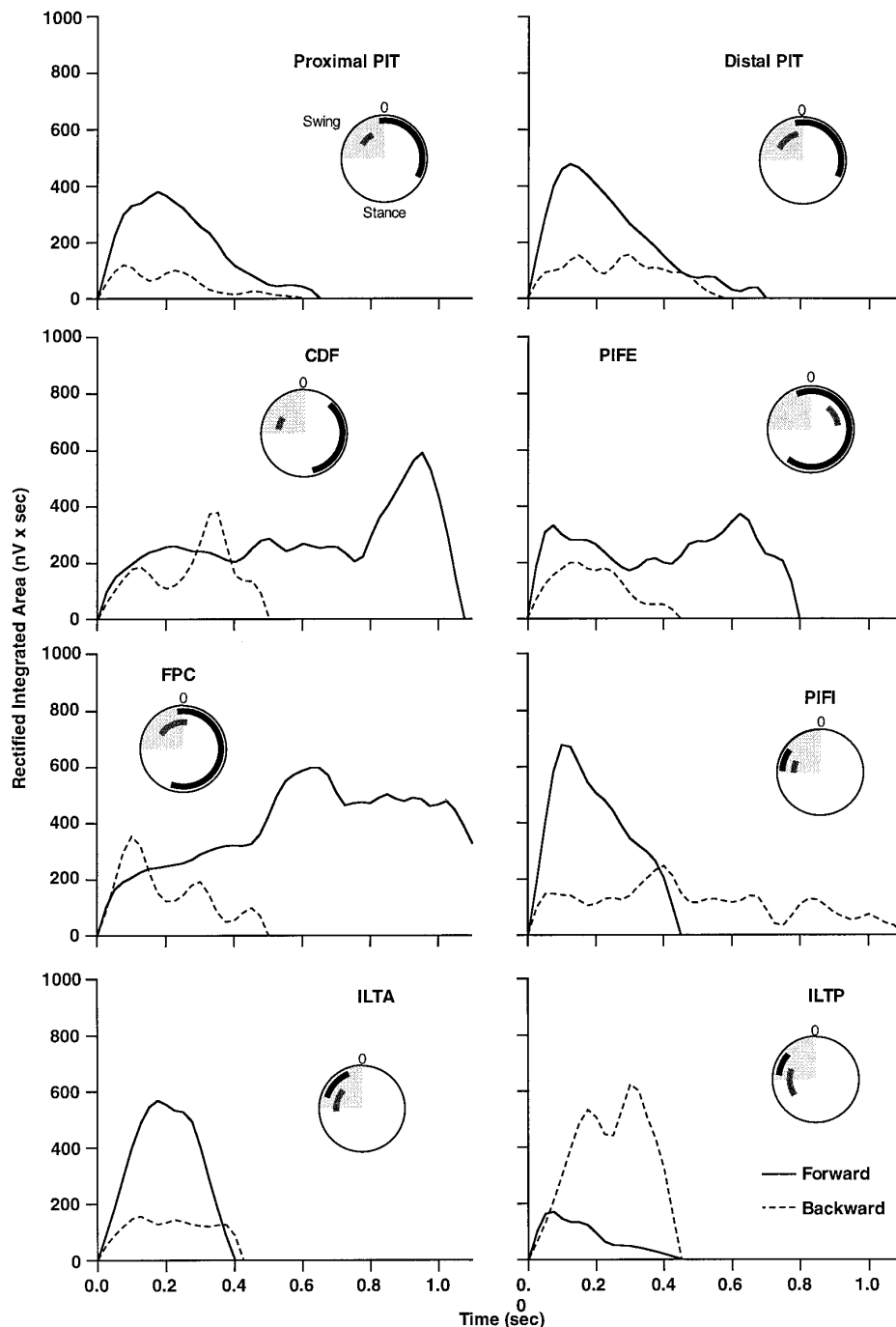


FIG. 6. Smoothed outlines for mean rectified integrated area (RIA) per 25-ms bin for primary bursts of 8 hindlimb muscles during forward (—) and backward (---) walking. Bins are based on absolute time, not percent cycle time. All panels are plotted on the same x and y scales, and bursts are aligned to start at time 0 s. *Insets*: circular bar diagrams illustrating the mean period of primary burst activity of the muscle during the stride. Clear portion of the circle indicates the stance phase, and the shaded quarter of the circle indicates the swing phase. Note that burst durations in the inset bar diagrams are scaled as a proportion of cycle time during forward and backward locomotion as appropriate; hence, relative burst durations may be different from the plots of burst RIA. Forward walking is represented by ■, whereas backward walking is represented by ▨. Note the difference in duration of bursts for several muscles (PIFE, CDF, FPC, PIFI) between forward and backward walking. Muscles illustrated in this figure are the same ones used in the principal component analysis shown in Fig. 7.

Multivariate analysis

Multivariate effects of direction of travel were examined by a principal component analysis on the onset and burst RIA of the muscles shown in Fig. 6. Burst RIAs (equivalent to the area under the curves shown in Fig. 6) for all muscles are listed in Table 2. Scatter plots on principal components 1–4 of individual strides from the reduced data set used for multivariate analysis are shown in Fig. 7. The strides are keyed by direction of walking. Principal component (PC) 1 completely separated forward from backward strides, with no overlap between the groups (Fig. 7, *top*). Principal components 2–4 failed to separate forward from backward walk-

ing. Loadings on the first four principal components for the individual EMG variables are listed in Table 3. PC 1 explains ~39% of the variance in the data set, and the first four PCs collectively account for >75% of the variance. High loadings on PC 1 reflect higher RIAs in forward walking than in backward locomotion (e.g., CDF; Tables 2 and 3), whereas negative loadings reflect higher RIAs in backward walking (e.g., ILTP; Tables 2 and 3). High PC 1 loadings also reflect EMG onsets during forward walking that occur at the end of the swing phase, with the onset for backward locomotion occurring at a distinctly different time during the cycle (e.g., proximal and distal PIT; Table 3, Fig. 5).

TABLE 2. Rectified integrated area (RIA) of electromyogram bursts of hindlimb muscles and kinematic cycle parameters during forward and backward walking in *Dicamptodon tenebrosus*

Muscle	Forward	Backward
Proximal PIT 1°	10.7 (7.8)	4.1 (5.1)
Proximal PIT 2°	2.9 (2.2)	2.7 (2.3)
Distal PIT 1°	13.4 (7.5)	4.5 (3.6)
Distal PIT 2°	2.4 (1.9)	6.4 (8.1)
PTB	13.6 (5.6)	25.1 (21.1)
Proximal ISF	12.2 (7.0)	5.3 (2.4)
Distal ISF 1°	7.8 (5.0)	1.8 (0.7)
Distal ISF 2°	N/A	2.9 (2.8)
PIFE 1°	13.3 (9.7)	6.0 (5.5)
PIFE 2°	1.7 (1.1)	5.3 (5.4)
CPIT	8.3 (5.7)	6.5 (3.9)
CDF	11.3 (12.6)	2.5 (2.1)
FPC 1°	23.5 (19.7)	13.1 (15.1)
FPC 2°	9.1 (10.5)	16.1 (10.1)
PIFI 1°	11.2 (5.5)	9.1 (11.2)
PIFI 2°	1.6 (0.9)	3.5 (2.9)
ILTA 1°	10.8 (3.7)	4.3 (2.8)
ILTA 2°	N/A	2.5 (1.7)
ILTP 1°	3.5 (2.1)	10.5 (8.3)
ILTP 2°	3.0 (2.3)	N/A
ILFB 1°	2.1 (1.3)	11.4 (6.7)
ILFB 2°	3.4 (2.4)	N/A
Cycle duration, s	1.14 (0.19)	1.76 (0.49)
Contact interval, %	66.64 (12.17)	78.61 (9.16)

Values are given as means \pm SD. RIA has units of mV \cdot percent step cycle. See Table 1 for abbreviations.

A negative loading (CDF; Table 3) correlates with EMG onset in forward locomotion at mid-stance (Fig. 5). No consistent patterns can be seen in examination of the loadings on PCs 2–4 with respect to the direction of travel.

DISCUSSION

Kinematics and muscle synergies during forward and backward walking in *Dicamptodon*

Interlimb coordination during forward walking in salamanders conformed to the pattern of a diagonal–couplets lateral sequence walk (Fig. 3, *top*) (Hildebrand 1976). Intralimb coordination demonstrated the following sequence of kinematic and EMG events. During the late swing phase (before the foot contacts the substrate), the ventral hindlimb muscles plus the FPC began activity (Fig. 5). Thus when the foot contacted the treadmill surface, those muscles are ready to support immediately the weight of the body (ventral group, especially PIFE) and plantarflex the foot (FPC). The foot initially was placed forward of the knee joint; the knee was extended, and the hip was flexed (Fig. 3). As the ventral muscles [PTB, PIT, and ischioflexorius muscle (ISF)] continued their activity, the femur retracted and the knee flexed in early stance as the salamander body moved toward the foot. In later stance the femur continued to retract, probably as a result of activity in the CPIT and CDF, and the knee extended as the animal pushed off of the foot. Knee extension was associated with activity of the ILTP and also with the movement of the contralateral limb, which may pull the salamander's body toward the opposite side. The knee joint

was extended most just before the start of the swing phase. The trunk and pelvic girdle first straightened, then flexed toward the opposite side (Fig. 3) in a smooth motion during stance. In the swing phase, the entire leg was swung straight out to the side of the salamander as it was protracted in an overhand crawl motion. Superficial dorsal muscles all were recruited during this phase, beginning in late stance with the ILFB and progressing in a posterior to anterior direction, with ILTP and then ILTA activating, and had considerable overlap in their periods of activity (Fig. 5). We suggest that this order of activation occurs to first lift the limb from its caudally directed position and then swing it progressively forward. The deep PIFI, which was activated along with the ILFB and persisted in activity through the first half of swing, may function to flex the hip during the swing phase.

In backward locomotion, interlimb coordination did not follow any named gait (Hildebrand 1976) (Fig. 3, *bottom*). The body was not always lifted from the treadmill surface during backward walking, whereas it was consistently elevated in forward walking. Intralimb coordination followed a pattern quite different from that of forward locomotion.

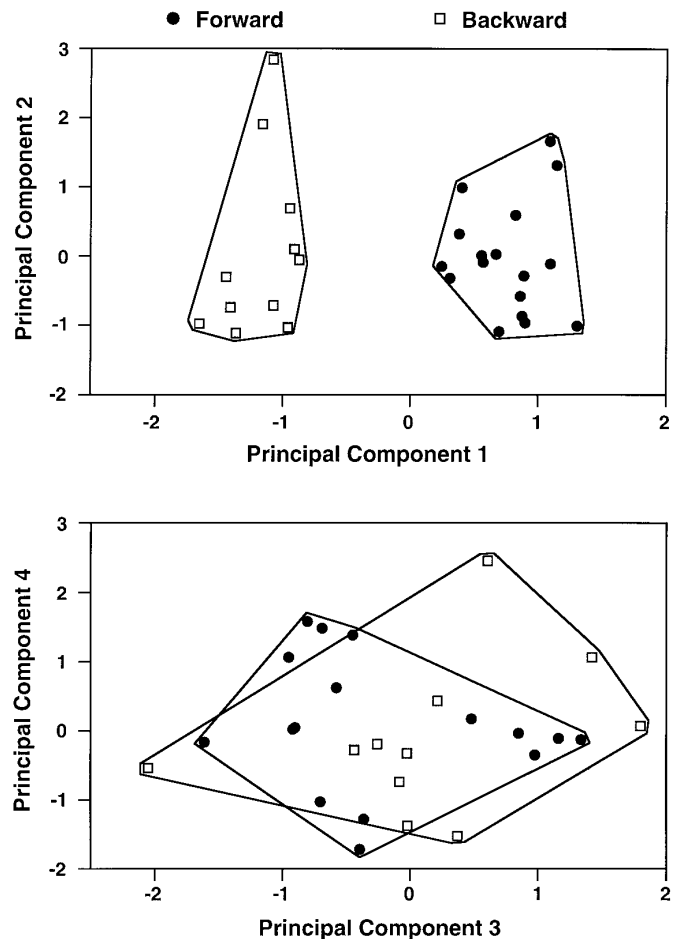


FIG. 7. Plots of principal component 1 vs. principal component 2 (*top*) and principal component 3 vs. principal component 4 (*bottom*) for 16 EMG variables. Forward strides are represented by \bullet , backward strides by \square . Each symbol represents 1 stride in the reduced data set used for multivariate analysis (see text for details). Note that principal component 1 completely separates forward and backward strides (no overlap of polygons). However, direction of stride fails to separate on all other principal components.

TABLE 3. *Loadings of electromyographic variables on the first four principal components*

Variable	PC1	PC2	PC3	PC4
Distal PIT				
Onset	0.832	0.116	-0.045	-0.204
RIA	0.546	0.067	0.268	0.689
Proximal PIT				
Onset	0.921	-0.137	0.084	0.181
RIA	0.498	-0.410	-0.379	-0.346
CDF				
Onset	-0.879	0.129	-0.073	-0.060
RIA	0.790	-0.207	-0.345	-0.279
PIFE				
Onset	0.808	0.140	0.302	0.361
RIA	-0.039	0.208	0.775	-0.307
PIFI				
Onset	0.076	-0.391	0.448	-0.474
RIA	0.774	-0.125	-0.353	-0.063
FPC				
Onset	0.304	-0.143	-0.255	0.132
RIA	0.277	0.811	0.189	-0.302
ILTA				
Onset	0.448	0.711	-0.272	-0.224
RIA	0.615	0.003	0.641	-0.110
ILTP				
Onset	0.471	-0.693	0.281	-0.081
RIA	-0.695	-0.464	0.206	0.005
Percent of variance explained	38.7	14.8	13.0	8.6

RIA was calculated for the entire burst, in units of mV · (percent step cycle), and EMG onset was in units of percent step cycle.

Backward strides started with the foot positioned lateral to the hip joint (Fig. 1). The sole muscle that began a burst during late swing and continued activity into the stance phase was the FPC, which could act to press the foot against the treadmill surface at this time. In the stance phase of a backward stride, the pelvic girdle, trunk, and femur were held nearly motionless, and most movement occurred at the knee joint (Fig. 3). All of the kinematic angles measured except for the femur–crus (knee joint) angle had significantly smaller ranges in backward walking. The knee extended during most of the stance phase, and we hypothesize that the forces producing knee extension act to push the body backward (Figs. 1 and 3). However, there was little sustained activity in the knee extensor muscles (ILTA, ILTP) during the stance phase. Only the secondary burst of the ILTA occurred during midstance; this could contribute to knee extension. The prominent ventral muscle activity during forward stance was absent from backward stance with only the PIFE and PTB regularly recruited during backward stance, possibly to support the weight of the body and (for PTB) to flex the hip. A number of muscles (PIT, the distal ISF, FPC, PIFI, as well as ILTA) showed variable secondary bursts during the stance phase. In the swing phase, the foot was lifted just above the substrate, and the knee joint flexed as the foot was slid posteriorly in preparation for the next stride (Fig. 1). As in forward walking, dorsal muscles were active primarily during the swing phase, presumably to lift the limb. However, unlike forward locomotion, the ventral muscles (with the exceptions of PIFE and PTB) also showed their primary burst of activity during swing (Fig. 5), presumably to retract the limb in preparation for the next stride. This resulted in a pattern of EMG synergy very different

from that seen in forward locomotion. Both ventral and dorsal muscles were recruited simultaneously during part of the backward stride, whereas these two groups of muscles were never coactive during a forward stride. Hence, backward walking in *Dicamptodon* involved an entirely different motor pattern than forward walking.

Modulation of limb kinematics during forward and backward walking: comparisons with other organisms

The findings presented here for *Dicamptodon* demonstrate some interesting similarities and differences when compared with the kinematics of forward versus backward locomotion in a number of other species. One of the most reliable similarities is that, in all groups examined, joint angle patterns in backward locomotion are more variable than in forward walking (salamanders: this study; cats: Buford et al. 1990; decapod Crustacea: Clarac 1982; Clarac and Chasserat 1983; stick insects: Graham and Epstein 1985; humans: Vilensky et al. 1987). In this study, individual salamanders show statistically significant differences in measured variables (although this variability is much less than that due to direction of locomotion), and individual strides are also more variable during backward walking. Additionally, the mammalian species studied also show reductions in joint angle ranges similar to those seen in *Dicamptodon* (Buford et al. 1990; Vilensky et al. 1987), although in human backward running, knee excursions increase by $\sim 10^\circ$ over values seen during forward running (Bates et al. 1984). Further, in cats, humans, and stick insects, the hip and knee joints make similar contributions to horizontal displacement as they do in the salamander. In forward walking, hip extension plays a major role in moving the animal rostrally, whereas knee extension is the primary contributor to backward movement (Buford et al. 1990; Graham and Epstein 1985; Vilensky et al. 1987; Winter et al. 1989). The position of the limb at its rostral-most extent (maximum hip flexion) is similar for backward and forward walking in salamanders (this study), cats (Buford et al. 1990), and humans (Vilensky et al. 1987), whereas the caudal-most position of the limb in backward locomotion is always less extended (hip more flexed) than in forward walking in these species. Relative proportions of the swing and stance phases do not change drastically between forward and backward walking in salamanders (Table 2), humans (Vilensky et al. 1987; Winter et al. 1989), cats (Buford et al. 1990), and decapods (Clarac and Chasserat 1983). Interlimb coordination patterns also show some similarities across species. In forward walking, the contralateral limbs of a pair typically move in strict alternation (Buford et al. 1990; Clarac and Chasserat 1983; Jamon and Clarac 1995), whereas in backward locomotion, this phasing tends to be more variable, particularly in the forelimbs (Fig. 2) (Buford et al. 1990; Clarac and Chasserat 1983).

Several differences are also apparent when forward versus backward walking is compared among species. First, when similar speeds are examined, the cycle duration for backward walking is shorter for cats (Buford et al. 1990), humans (Vilensky et al. 1987), and naked mole rats (Eilam et al. 1995), but longer for decapod crustaceans (Clarac 1982; Clarac and Chasserat 1983). Cycle durations could not be compared in the present study because forward walking was

always faster than backward walking. Second, hindlimb positions at foot touchdown in backward locomotion may be roughly in line with the hip (salamanders; cats: Buford et al. 1990), moderately behind the hip (less than the caudal excursion of the limb in forward locomotion) (humans: Vilensky et al. 1986; Winter et al. 1989), or displaced far caudally in relation to the pattern in forward walking (stick insects: Graham and Epstein 1985). Third, the pattern of interlimb coordination during backwards locomotion does not correspond to any regular gait in *Dicamptodon* (Fig. 2), whereas in the cat (Buford et al. 1990) and naked mole rat (Eilam and Shefer 1992), the footfall pattern switches from a lateral sequence to a diagonal sequence gait (although, as Eilam and Shefer point out, when viewed with respect to the direction of locomotion, the pattern is invariant at a lateral sequence walk). Finally, when backward and forward joint angle patterns are plotted so that the backward traces are reversed and shifted (Winter et al. 1989), for humans (Vilensky et al. 1987; Winter et al. 1989), cats (Buford et al. 1990), and decapod crustaceans (Clarac 1982) (in the latter 2 cases, we are drawing inferences from the authors' published unreversed traces), striking similarity is seen in the timing of joint movements. To some extent this is also true of *Dicamptodon* (Fig. 3, pelvic girdle, pelvic girdle–femur, and lateral crus angles); however, the pattern of knee joint movement is very different between forward and backward locomotion (Fig. 3, femur–crus angle). This movement pattern of the knee during backward locomotion in *Dicamptodon* thus cannot be considered as a simple reversal of the forward pattern and contrasts to the considerable similarities in knee movements noted for backward and forward movements in humans (see Vilensky et al. 1987, their Fig. 2).

Overall, the kinematic patterns seen in the vertebrate species examined thus far do not support Grillner's (1981) hypothesis that a simple reversal in hip–knee coupling would suffice to turn forward walking into backward progression in tetrapods (compare Figs. 1 and 5 in Vilensky et al. 1987 for an example of actual human kinematic patterns to those predicted by Grillner's hypothesis). The similarity of forward to reversed and shifted backward kinematic traces implies that joint relationships are largely unchanged in the two forms of locomotion. Indeed, the group that seems to best fit Grillner's hypothesis for modulating forward into backward walking is the decapod Crustacea. These animals walk as readily backward as forward (Clarac 1982), and the switch between the two directions of locomotion seems to be accomplished principally by a change in the coupling between limb protraction/retraction and limb elevation/depression (Ayers and Davis 1977). In forward walking, retraction accompanies limb depression and weak limb flexion, while protraction is associated with limb elevation. In contrast, during backward progression limb protraction is coupled to depression and weak flexion of the limb, while retraction accompanies elevation (Ayers and Davis 1977).

Modulation of limb EMG patterns during forward and backward walking: comparisons to other organisms

When motor patterns of muscles are compared across species in vertebrates, the common practice is to compare activi-

ties of evolutionarily homologous muscles so that any changes in activity patterns of the same muscles can be assessed (Ashley-Ross 1995; Gatesy 1990; Goslow et al. 1989; Lauder and Reilly 1996; Smith 1994). Homologous muscles may share common origins and insertions, but it is frequently true, especially in comparisons among phylogenetically diverse taxa such as salamanders and mammals, that muscles deemed homologous on developmental or phylogenetic grounds may not share common origins or insertions (Lauder 1994). Hence, the mechanical effect on the limb of two evolutionarily homologous muscles may be very different even if the timing of muscle activity is similar in the two species. Although analyses of limb mechanics within any one species need not consider the evolutionary homology of muscles, comparisons across species benefit from comparison based on homology. In this light, we have framed our among-vertebrate species discussion below on muscles determined to be homologous based on current developmental, anatomic, and comparative phylogenetic data (Table 1) (Ashley-Ross 1992, 1995; Jones 1979; Kerr 1955; Romer and Parsons 1986; Walker and Homberger 1992).

When motor patterns driving forward versus backward locomotion are examined in various species, one finds fewer similarities between salamanders and other groups than for kinematics. First, as seen in *Dicamptodon* (Fig. 5), stick insect motor patterns show more overlap in antagonist muscle activity (Graham and Epstein 1985) during backward progression, and these two species as well as the decapod Crustacea (Clarac and Chasserat 1983) exhibit more EMG variability in backward than forward walking (e.g., Fig. 5: CDF error bars). Second, burst amplitudes and envelopes of activity for individual muscles are distinctly different during forward and backward locomotion in many groups (salamanders, this study; cats, Buford and Smith 1990; decapod crustaceans, Clarac and Chasserat 1983; stick insects, Graham and Epstein 1985).

Differences between *Dicamptodon* and other species were pronounced when EMG patterns were examined for forward and backward walking. First, in *Dicamptodon*, all muscles examined were coactive during the swing phase of backward locomotion, whereas in forward walking, there was never a phase of the step cycle when all of the muscles were active simultaneously (Fig. 5). In other vertebrates examined, however, the same general pattern of muscle synergies seen in forward walking also is seen in backward walking, with flexor and extensor muscles being activated reciprocally (chicks, Bekoff et al. 1987a; cats, Buford and Smith 1990; Pratt et al. 1996; Trank and Smith 1996). Individual homologous muscles in the cat (Table 1) also exhibit different periods of activity during backward walking (Buford and Smith 1990) than those seen in the salamander (refer to Fig. 5 and Table 1). For instance, the semitendinosus (ST; one mammalian homologue of the ISF) begins activity before the start of the swing phase and persists until the beginning of stance, whereas the anterior biceps femoris (ABF; a second homologue of ISF) becomes active just before paw contact and sustains its activity through mid to late stance. In contrast, the ISF of *Dicamptodon* has its principal phase of activity confined to mid to late swing (Fig. 5). Likewise, the salamander FPC is recruited principally during the mid to late swing phase, turning off in early stance (Fig. 5),

whereas the homologue in the cat (lateral gastrocnemius, LG) begins activity in late swing and persists in activity throughout mid stance (Buford and Smith 1990, their Fig. 4). Interestingly, the LG also shows envelopes of activity during forward and backward walking that are opposite of the patterns seen in *Dicamptodon*; the cat's LG in forward progression peaked early in the burst and subsequently declined, whereas in backward locomotion the RIA increased steadily throughout the burst (Buford and Smith 1990, their Fig. 2). In contrast, *Dicamptodon*'s FPC shows a pattern of increasing RIA later in the burst during forward walking as the foot begins to roll up on the toes, whereas in backward movement, the RIA shows an early peak followed by a marked decline (Fig. 6).

The results from examination of motor patterns during backward walking in vertebrates bolster the conclusions reached from kinematic analyses and fail to support Grillner's (1981) hypothesis. It was predicted that a mixed synergy would be seen, with hip flexors cocontracting with knee and ankle extensors during backward stance and hip extensors cocontracting with knee and ankle flexors during swing. Contrary to this expectation, both in cats (Buford and Smith 1990; Trank and Smith 1996) and in chicks (Bekoff et al. 1987a), the same basic pattern of muscle coactivation in forward locomotion also is seen in backward progression. Hip extensors remain coactive with knee and ankle extensors during stance, and hip, knee, and ankle flexors cocontract during swing in both directions of walking. Buford and Smith (1990) suggest that this basic synergy is modified in the details of the activation pattern and intensity of recruitment to produce either forward or backward locomotion (also see Zernicke and Smith 1996). Again, arthropods seem to match more closely the predictions of Grillner's (1981) hypothesis for modification of the forward motor pattern into backward locomotion. In forward walking in lobsters, the levator muscle of the limb cocontracts with the promotor muscle to produce the swing phase, while the depressor muscle activates in concert with the remotor muscle to produce propulsion during stance (Ayers and Davis 1977; Clarac 1984; Clarac and Chasserat 1983). In backward walking, activity in the depressor muscle is coupled to recruitment of the promotor, while the levator muscle coactivates with the remotor. Thus the switch between forward and backward locomotion seemingly is mediated by a simple switch in phase of activation at a single joint. An interesting exception, however, is seen in stick insects (Graham and Epstein 1985). In these organisms, the activity pattern in forward locomotion is what one would expect, with levator muscles coactive with promotors and depressor muscles coactive with remotors in discrete bursts. In backward walking, the levators show their strongest activity in concert with the strongest bursts from the remotor muscles during the swing phase, and the depressors likewise exhibit their most intense bursts while coactivated with the promotors during stance. However, all of these muscles often show continuous low-level activity during backward locomotion (which is not seen in forward walking). Graham and Epstein (1985) suggest that this low-level activity at inappropriate times during backward progression results from small, slow motor units being strongly coupled to their normal synergists in forward walking; only fast, large motor units are capable of being

modulated to produce patterns appropriate for backward locomotion, and these are strong enough to override the output of the small units, thereby enabling the insect to walk backward.

Conclusions: modulation of motor output during locomotion

One of the key approaches to studying the design of central neuronal networks that govern vertebrate locomotion has been the analysis of motor output during both in vivo and fictive locomotion (e.g., Cohen et al. 1988; Grillner 1981, 1985). In addition, the study of different patterns of motor output that result from varied behaviors (presumably generated by these same CPGs) has provided considerable insight into how plastic central output may be used to execute different behaviors. For example, Gelfand et al. (1988, p. 171) compared motor output during two different behaviors that involve the limb musculoskeletal system, stepping and scratching. They concluded that "... we think that essentially the same nervous mechanisms are used to generate the efferent patterns in these two movements." Such conclusions about CPGs are based on similarities in motor output characteristics such as flexor and extensor muscle phase relationships between the behaviors studied. General support for the basic hypothesis that a single CPG might be modulated to drive different vertebrate locomotor behaviors is available in studies that demonstrate the conversion of forward into backward locomotion. For example, Axon et al. (1987) showed in rats that modulation of a single receptor class (dopamine D₂) converted forward into backward locomotion.

In humans, Winter et al. (1989) arrived at a similar conclusion for forward and backward walking. They indicated that "... similar muscle activation patterns could be used to produce both modes of locomotion, but the temporal cycling of muscle contraction would be reversed." Also, Smith's laboratory (Buford and Smith 1990; Buford et al. 1990; Perell et al. 1993; Trank and Smith 1996) and Bekoff et al. (1987a) have concluded from studies on cats and chicks, respectively, that similar neuronal mechanisms might control both backward and forward locomotion.

However, on the basis of the motor pattern and kinematic data presented here, the conclusions described above for humans, cats, rats, and chicks cannot be extended to salamanders. The salamander knee joint follows different movement patterns during forward and backward locomotion (Fig. 3), and there are drastic differences in the motor patterns between the two directions of locomotion (Figs. 5–7). Most significantly, the primary burst of muscle activity in backward locomotion occurs during the swing phase for most muscles. Femoral retractor muscles such as the CDF and ISF change activity periods completely between locomotor modes, whereas the hip flexor (PIFI) retains its primary burst of activity during the swing phase in both directions of walking. The relative lack of activity in the knee extensors suggests that the hindlimbs are not contributing much to locomotor power and that perhaps the forelimbs take this role (functionally becoming the hindlimbs) as suggested by Eilam and Shefer (1992) for the naked mole rat and Perell et al. (1993) for the cat.

From these data it is clear that in salamanders, backward walking does not represent a simple reversal of forward locomotion in which muscle activity shifts in phase relationship to drive the hindlimb in a reverse step cycle from forward locomotion. Rather, the extensive differences in motor output between the two directions of movement suggest that the CPG controlling forward walking by the hindlimb is either modulated extensively to produce backward locomotion or that a second pattern generator is recruited to drive hindlimb musculature during backward locomotion. It is clear that the hindlimb pattern generator that drives forward locomotion in salamanders does not constrain either the motor output or limb movements of backward walking. This suggests that the similarities seen between forward and backward walking in upright-postured animals may result, in part, from the need to balance the weight of the body over the limbs.

Finally, these data suggest that we have much to learn about the control of locomotion in primitive tetrapods and that patterns found in mammals and chicks are not generalizable to other taxa with different limb postures. In the future, data on ground reaction forces and joint torques from primitive tetrapods would be a valuable adjunct to the current comparative EMG data, as it is possible that joint torques experienced by homologous joints across vertebrates are similar despite different kinematic and motor patterns in the limb. Data from a variety of tetrapod taxa will be needed to provide a more complete picture of the evolution of central control of locomotor behavior.

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