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Patterns of hind limb motor output during walking in the salamander *Dicamptodon tenebrosus*, with comparisons to other tetrapods

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Abstract Based on similarity of motor patterns of lizards, crocodiles, birds and mammals, various authors have concluded that a number of homologous muscles across these taxa demonstrate neuromuscular conservatism. This hypothesis remains untested for more basal taxa. Therefore, a quantitative electromyographic study of the hind limb during treadmill walking (mean speed of 0.75 SVL/s) in the salamander *Dicamptodon tenebrosus* was undertaken. Muscles located ventrally on the hind limb become active just before foot placement on the substrate, and maintain activity through the first half of the stance phase. Dorsally located muscles begin activity at or just before the start of the swing phase, and fire through the first half of swing. Several muscles showed a secondary EMG burst during the stride. The second burst in most ventral muscles occurred in late stance. In all dorsal muscles with double bursts, the second burst occurred in the middle of stance. Comparison of electromyographic onset and offset values for *Dicamptodon* to those for presumed homologues in other tetrapods reveals similarity in activity patterns for all ventral and two dorsal muscles despite anatomical rearrangements, supporting the hypothesis of neuromuscular conservatism for some muscles but not others.

Key words Salamander · Locomotion · Electromyography · Tetrapod · Neuromuscular conservatism

Abbreviations *BF* biceps femoris muscle · *CDF* caudofemoralis muscle · *CPIT* caudalipuboischiotibialis muscle · *Dist* distal · *EDC* extensor digitorum communis muscle · *EMG* electromyogram · *EXF* extensor cruris et tarsi fibularis muscle ·

EXT extensor cruris tibialis muscle · *FMFB* femorofibularis muscle · *FPC* flexor primordialis communis muscle · *Gastroc* gastrocnemius muscle · *ILFB* iliofibularis muscle · *ILFM* iliofemoralis muscle · *ILTA* extensor iliotibialis pars anterior muscle · *ILTP* extensor iliotibialis pars posterior muscle · *ISC* ischiocaudalis muscle · *ISF* ischioflexorius muscle · *ISFM* ischiofemoralis muscle · *ITCR* iliotrochantericus cranialis muscle · *ITM* iliotrochantericus medius muscle · *MG* medial gastrocnemius muscle · *PFM* pubifemoralis muscle · *PIFE* puboischiofemoralis externus muscle · *PIFI* puboischiofemoralis internus muscle · *PIT* puboischiotibialis muscle · *Prox* proximal · *PTB* pubotibialis muscle · *Sol* soleus muscle · *ST* semitendinosus muscle · *SVL* snout-vent length

Introduction

The hypothesis of neuromuscular conservatism

When Jenkins and Goslow (1983) reported that the pattern of motor outflow controlling certain muscles of the tetrapod shoulder joint during locomotion had been conserved in spite of major anatomical reorganization across the evolutionary transition from reptiles to mammals, they sparked the interest of many researchers interested in the neural control of behavior. One of the outstanding questions concerning the evolution of complex systems has been whether evolutionary change in one aspect of the system (such as the morphology of the locomotor or feeding system) is necessarily accompanied by change in all the other aspects (such as motor pattern or organization of neural circuits driving the behavior). Lauder (1990, 1991, 1994)

and Striedter and Northcutt (1991) have argued that such complex morphological systems may be broken down conceptually into several levels, which may be addressed by different types of analyses. Thus, one may dissect the locomotor organs/appendages to study their morphology, film the locomotor movements to characterize the external behavior, or implant electrodes in the locomotor muscles to study the motor pattern. A variety of studies (Jenkins and Goslow 1983; Marder and Hooper 1985; Wainwright and Lauder 1986; Meyrand and Moulins 1988; Goslow et al. 1989b; Reilly and Lauder 1989, 1990; Wainwright et al. 1989; Paul 1991; Shultz 1992; see also Lauder 1990, 1991, and references therein) have shown that changes in complex systems occur at discrete levels of biological organization, with not all levels showing the same amount of change. It has been shown in several cases that peripheral morphology may change, while the motor pattern driving the anatomical structures during performance of the behavior remains relatively conservative across taxa (Wainwright and Lauder 1986; Shaffer and Lauder 1988; Paul 1991; see also Lauder 1990, 1991, 1994 and references therein).

In the vertebrate locomotion literature, studies subsequent to that of Jenkins and Goslow (1983) have identified a number of muscles of both the fore- and hind limbs of tetrapods for which the motor pattern does indeed seem to show conservation (in timing within the phases of the stride and relative to other muscles) across various taxa (e.g., Nicolopoulos-Stournaras and Iles 1984; Dial et al. 1991; Gatesy 1994), as well as some muscles whose motor patterns have diverged across taxa, possibly due to functional demands (Dial et al. 1991; Gatesy 1994). Those motor patterns that are conserved (in timing of muscle activity within the locomotor cycle or relative to other muscles) for all tetrapod groups were proposed by Jenkins and Goslow (1983) to be inherited, largely intact, from the common ancestor of all tetrapods. This hypothesis of neuromuscular conservatism, as it applies to limbed locomotion, postulates that homologous muscles in different taxa will share common periods of activity within a movement cycle (stride or wing beat), in spite of differences in the anatomical position and attachments of the homologues. Thus, when a muscle and its homologues in all tetrapod taxa are considered, relative periods of muscle activity onset, offset, and duration are expected to be similar. To date, most studies of locomotion that have addressed this question have been restricted to the more derived end of the tetrapod cladogram, focusing on lepidosaurian/mammalian (Jenkins and Goslow 1983) and crocodylian/avian comparisons (Dial et al. 1991; Gatesy 1994), or comparing cursorial and non-cursorial species within mammals (Nicolopoulos-Stournaras and Iles 1984). Only one study has measured motor patterns and addressed the issue of neuromuscular conservatism in an amphibian during walking (Peters and Goslow 1983), and that study

reported data for only two salamander hind limb muscles. However, based on their electromyographic recordings and basic kinematic data, Peters and Goslow (1983) suggested that major kinematic features of the salamander and mammalian hind limb step cycles are strikingly similar, which in turn suggested the possibility that the neural control mechanisms underlying limb movements may likewise be conserved.

Comparisons among tetrapod taxa

In a previous kinematic study (Ashley-Ross 1994a), I identified several features of the hind limb step cycle shared by all tetrapod taxa examined, and proposed that these features were plesiomorphic (inherited unchanged from the common ancestor) for tetrapods. These characteristics were: (1) pelvic girdle rotation simultaneous with lateral bending (standing wave) of the vertebral column; (2) retraction of the femur commencing at the start of and continuing throughout the stance phase; (3) knee flexion in early stance pulling the body toward the supporting foot; and (4) extension of the knee joint in late stance, after the hip has been advanced past the ankle, to thrust the body forward. The latter three movements, which directly involve the hind limb, are controlled by muscles examined in this study. Because the hind limbs of all tetrapod taxa produce these characteristic kinematic patterns while walking, conservation of motor function is especially likely to be present in the muscles producing them. I hypothesize that examination of motor patterns for the homologous muscles controlling these plesiomorphic movements in all major tetrapod groups will reveal their onset and offset times to be similar, in spite of anatomical differences in the hind limbs of the various taxa. In other words, the muscles responsible for these plesiomorphic movement patterns will show neuromuscular conservatism (though conservation of motor patterns is not necessarily limited to these muscles).

Therefore, the objectives of this study are to describe the electromyographic patterns produced by hind limb muscles during walking in *Dicamptodon tenebrosus*, which may then be compared to the corresponding data for other tetrapod taxa. In order to eliminate the potentially confounding effects of speed on locomotor patterns (e.g., see Goslow et al. 1981; Gatesy 1990; Ashley-Ross 1994b), all data presented in this paper are taken from animals moving at a walking pace. Though salamanders are known to be a side branch off the evolutionary line leading to the amniotes (Carroll 1988; Milner 1988; Panchen and Smithson 1988), they are the best extant postural model for primitive tetrapods, based on structural features, data from fossil trackways, and locomotor kinematics (Romer and Byrne 1931; Schaeffer 1941; Carroll 1988; Gans and De Gueldre 1992; see Ashley-Ross 1994a, for further discussion).

Electromyographic features common to all taxa may be concluded to be inherited from the common ancestor of all tetrapods. I also propose some standardized ways in which electromyographic data may be presented in order to facilitate comparisons among different groups. Because of the extremely limited literature on aspects of the neuromuscular control of salamander locomotion such as muscle physiology and recruitment characteristics, and information from "reduced" preparations, I have excluded *in vitro* data from other tetrapod groups due to its limited utility for illuminating trends among the taxa considered here. Some of this material has appeared previously in abstract form (Ashley-Ross 1993).

Materials and methods

Animals

Dicamptodon tenebrosus was chosen as the subject for this study as this species demonstrates good walking ability, has robust limbs, and kinematic data are available (Ashley-Ross 1994a). Eight individuals collected as larvae in Mendocino County, CA (California scientific collector permits #7058 and #7614) were used for this study. At the time of experiments, all individuals were a minimum of two months post-metamorphic. Snout-vent lengths (SVL) of animals ranged from 12.15 to 13.80 cm at the time of the experiments.

Video recording

A variable-speed, motor driven treadmill (belt material made of rubberized nylon weave) with an effective working area of 18" length by 8" width was used for locomotion experiments. Salamanders readily walked 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. Animals were videotaped using a NAC HSV-400 High-Speed Video System (NAC Industries, Japan; sampling frequency of 200 field/s). Elapsed time (in 5 ms intervals) and a 100 Hz signal for synchronizing electromyographic 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 examination of these high-speed videos for each experiment. All animals used a lateral sequence walk, 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). Average speed was approximately 0.75 SVL/s (stepping frequency of 1.03 strides/s), a value in the middle of the range of speeds for this gait (Ashley-Ross 1994a). 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.

Electromyography

Electrical activity patterns of 13 hind limb muscles of *Dicamptodon* were recorded from a total of 83 strides from 8 individuals. Insulated bipolar steel alloy (wire diameter of 0.051 mm) electrodes were implanted into hind limb muscles while the animal was under general anaesthesia induced by placing it in a solution of tricaine methane sulfonate (MS-222; 0.4 g/l final concentration). Up to 13 electrodes were simultaneously recorded from each animal. Electrode tips were bared for ≤ 0.5 mm and the insulated portions of the

wires proximal to the tips were glued together with cyanoacrylate adhesive. Intertip distances were 1–2 mm. Implants were made percutaneously under visual guidance. All electrode wire pairs were gathered into a bundle and sutured to the skin of the animal's dorsal midline at the level of the sacrum. The wires were then glued together with model airplane glue to make a flexible cable. A ground electrode was implanted into connective tissue directly over the vertebral column. Animals recovered from anaesthesia within one hour, and all recordings were made within four hours postanaesthesia. Immediately following each experiment, animals were killed by anaesthetic overdose and preserved in 10% formalin. Electrode position was confirmed by dissection.

EMGs were amplified 10000 times with Grass P511J preamplifiers with a 60 Hz notch filter and a bandpass of 100 to 3000 Hz before being recorded on a Teac XR5000 cassette data recorder operating at 9.5 cm/s. The 14 channels (13 electrodes plus synchronization pulse) were played back at one-eighth speed through a Keithley analog-to-digital converter sampling at 1000 Hz into a Dell 486 computer. The resulting digital file had an effective sampling rate of 8000 Hz, a frequency that has been previously shown (Jayne et al. 1990b) to capture faithfully high-frequency spikes in salamander muscles.

The digital data file for each stride sequence was then analyzed using a custom EMG analysis program (Data Manipulator, DataCrunch Software, San Clemente, CA). For each animal, visual comparison of all EMG channel recordings confirmed that each muscle's EMG profile was distinct from that of adjacent implanted muscles in onset and offset times, as well as the shape of the EMG burst. Thus, contamination of data due to muscle cross-talk effects was not deemed to be a problem. The Data Manipulator program allows the user to select the beginning and end of each EMG burst (by computer-mouse input), then records the corresponding time of onset and offset for the burst (in s), and calculates burst duration (in s) and rectified integrated area (in mV·s). Rectified integrated area (RIA) is often used as a measure of the intensity of muscle activity (Loeb and Gans 1986). It was calculated by integrating the area under the curve of the full-wave rectified EMG trace between the onset and offset points specified by the user. One potential confounding factor in interpreting RIA is that a long duration low amplitude burst may have the same RIA as a short duration high amplitude burst. A measure of the average *instantaneous* level of activity may be obtained by dividing the RIA by the duration of the burst.

All of the 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 is therefore expressed as a percentage of the step cycle duration. A *stride* begins with placement of the foot on the surface of the treadmill, and ends with touchdown of the foot for the beginning of the subsequent stride. The time during the stride in which the foot is in contact with the substrate is termed the *stance phase* or *contact interval*, while 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, the variables were further normalized by converting them into the corresponding values for a standardized stride consisting of 75% stance and 25% swing. This conversion was done according to the following formula: if the muscle onset/offset in question occurs during the stance phase of the stride, then

$$Variable_{adjusted} = \frac{Variable_{initial}}{Stance\%} \times 75\%$$

Essentially, this formula first converts the value of the onset/offset variable ($Variable_{initial}$) into a percentage of the *stance phase*, then the multiplication by 75% maps it to the appropriate value for the standardized stride. For example, if the stride in question was composed of 50% Stance/50% Swing and $Variable_{initial}$ occurred at 25% of the step cycle, then application of the conversion formula would first yield the percentage of the stance phase at which

$Variable_{initial}$ occurred ($0.25/0.50 = 0.50$; 50% of the way through the stance phase), and finally would result in a value of 37.5% for the standardized stride ($0.50 \times 0.75 = 0.375$; again, 50% of the way through the stance phase).

If the muscle onset/offset occurs during the swing phase, the appropriate transformation is

$$Variable_{adjusted} = \frac{Variable_{initial} - Stance\%}{Swing\%} \times 25\% + 75\%$$

This formula is slightly more complex, since before the value of $Variable_{initial}$ can be determined as a percentage of the swing phase, the length of the stance phase must be subtracted out. The resulting number is multiplied by the length of the swing phase in the standardized step cycle, and finally the length of the stance phase is added back to assure the correct position in the stride. For example, using the same 50%/50% stride in the example above, with a $Variable_{initial}$ this time of 75%, then the first operation would yield a value of 50% ($(0.75 - 0.50)/0.50 = 0.50$; half way through the swing phase). Multiplying by the length of the standardized swing phase gives the value of 12.5%, and addition of the stance phase length gives a final position for $Variable_{adjusted}$ within the standardized step cycle of 87.5%. This transformation assures that all variables are directly comparable in their relative positions during stance or swing phase. The ratio of 75%/25% was chosen because it is a convenient division of the step cycle that is close to the actual average Stance%/Swing% measured in this study.

Several literature sources provided electromyographic data for comparison with *Dicamptodon*. Though precise speed matching was not possible, all comparative literature data was selected from studies of animals moving using a walking gait. Data for lepidosaurs came from Jayne et al. (1990a; *Varanus*), and Jayne (personal communication; *Chamaeleo*). Crocodylian data came from Gatesy (1990, 1994; *Alligator*). Aves data was taken from Gatesy (1990, 1994; *Numida*) and Jacobson and Hollyday (1982; *Gallus*). Values for Mammalia came from Nicolopoulos-Stournaras and Iles (1984; *Rattus*) and Rasmussen et al. (1978; *Felis*). For all of these references, onset and offset (and their standard errors, if available) of muscle activity were obtained from tables or figures. To compare relative periods of activity of hind limb muscles of *Dicamptodon* with those of other tetrapod groups, I have performed the same transformation (to a standardized 75%/25% cycle) on the data taken from literature sources. Where available, I have transformed data presented in tabular form in the reference paper, but in several cases, I have derived the data from summary bar diagrams presented by the author(s). This procedure involved scanning the figure into a Macintosh IIci computer, and then using the measuring function of the public domain NIH Image 1.52 program (written by Wayne Rasband at the US National Institutes of Health and available from the Internet by anonymous ftp from zippy.nimh.nih.gov or on floppy disc from NTIS, 5285 Port Royal Rd., Springfield, VA 22161, part no. PB93-504868). I used the program to scale the entire step cycle to 100% and measure the resulting values for the ends of the EMG bars, which are thereby expressed in terms of percentage of step cycle. In this same way the percentages of the step cycle occupied by the stance and swing phases were measured, which allowed accurate scaling to the standard 75%/25% cycle. One caveat is that for two references (Gatesy 1990, 1994) the contact interval was not given in either summary tables or figures, and so this value was measured using NIH Image from figures of representative EMG traces, which may not be indicative of the true mean for these values.

Results

Anatomy and kinematics

The detailed hind limb myology and hind limb kinematics of *Dicamptodon* have been described elsewhere

(Ashley-Ross 1992, 1994a). Briefly, ventrally located muscles studied were puboischiotibialis (PIT; both proximal and distal portions; see also Abbreviations), pubotibialis (PTB), ischioflexorius (ISF; both proximal and distal portions), puboischiofemoralis externus (PIFE), caudalipuboischiotibialis (CPIT), and flexor primordialis communis (FPC). Dorsally located muscles studied were puboischiofemoralis internus (PIFI), extensor iliotibialis pars anterior (ILTA), extensor iliotibialis pars posterior (ILTP), and iliofibularis (ILFB). The caudofemoralis (CDF), located posterior to the hind limb, does not fit well into either dorsal or ventral category. Most of these muscles span multiple joints: PIT, PTB, ILTA, ILTP and ILFB cross both hip and knee joints; FPC crosses knee and ankle joints; CPIT and CDF cross the sacral joint and the hip joint; ISF is a unique three-joint muscle that crosses the hip, knee, and ankle. Only the PIFE and PIFI span a single joint (hip).

Salamanders typically use a diagonal-couplets lateral sequence walk (Hildebrand 1976; Ashley-Ross 1994a). Hind limb movements during the early part of the stance phase consist of femoral retraction and knee flexion as the salamander pulls its body toward the supporting foot. Once the hip joint has advanced in front of the ankle, the knee joint extends as the femur continues to retract, so that the salamander pushes forward off of the supporting foot. During the swing phase, initial movements are knee flexion and abduction of the femur as the foot is lifted clear of the substrate. These events are followed by extension of the knee joint as the femur is protracted so that the entire limb is swung forward while being held parallel to the treadmill surface. During this time the plantar surface of the foot faces posteriorly. Finally, the foot is repositioned for the beginning of the next stride by being supinated so that the plantar surface faces the substrate.

Electromyography

Table 1 lists mean values for all electromyographic variables measured, normalized to a 75% stance/25% swing cycle. Walking (0.75 SVL/s) *Dicamptodon* had an average step cycle duration of 1.14 s with a mean contact interval of 68%. Hind limb muscles fired in bursts that repeated each stride, with the bursts from contralateral limbs occurring out of phase with each other (Fig. 1). Ventrally located muscles typically become active in late swing phase, just before the foot contacts the substrate, and show extensive overlap of activity (Figs. 1, 2; Table 1). In the superficial muscle layer, activation tends to follow an anterior to posterior progression, beginning with PTB, followed by PIT, ISF, CPIT, and finally by the posteriorly located CDF (Fig. 2). Distal portions of the PIT and ISF tend to

Table 1 Summary of electromyographic variables measured for hind limb muscles of *Dicamptodon tenebrosus*. Onset, offset and duration are expressed in percent of step cycle, Rectified Integrated Area (RIA) has units of mV·(percent step cycle), and RIA/Duration

(*RIA Dur*) has units of mV. All values are given as mean (SD). The *rightmost column* gives the sample size for each muscle in total number of strides recorded from the total number of individuals. Means and SDs are based on $n =$ (number of strides)^a

Muscle	Onset	Offset	Duration	RIA	RIA/Dur	(Strides, Indivs)
Prox PIT 1°	97.7 (3.5)	32.9 (11.9)	33.0 (11.6)	10.7 (7.8)	0.35 (0.25)	34, 5
Prox PIT 2°	51.7 (11.9)	65.1 (19.6)	13.4 (9.8)	2.9 (2.2)	0.22 (0.07)	10, 2
Dist PIT 1°	96.5 (3.6)	32.0 (11.1)	33.8 (7.7)	13.4 (7.5)	0.40 (0.21)	70, 5
Dist PIT 2°	60.4 (21.9)	67.3 (22.5)	8.0 (4.6)	2.4 (1.9)	0.29 (0.10)	11, 3
PTB	94.6 (4.9)	28.4 (11.8)	29.3 (9.5)	13.6 (5.6)	0.46 (0.14)	20, 3
Prox ISF	0.0 (2.1)	45.0 (12.8)	34.1 (7.8)	12.2 (7.0)	0.37 (0.20)	14, 3
Distal ISF	99.2 (3.4)	22.7 (8.3)	23.3 (8.2)	7.8 (5.0)	0.31 (0.14)	41, 5
PIFE 1°	93.8 (14.1)	60.7 (12.0)	41.6 (18.8)	13.3 (9.7)	0.30 (0.12)	27, 4
PIFE 2°	76.0 (12.0)	87.4 (7.0)	11.7 (10.9)	1.7 (1.1)	0.20 (0.11)	11, 2
CPIT	4.4 (6.9)	38.4 (18.2)	28.5 (11.6)	8.3 (5.7)	0.27 (0.17)	24, 4
CDF	11.3 (13.3)	46.6 (12.0)	34.8 (22.0)	11.3 (12.6)	0.27 (0.17)	47, 4
FPC 1°	97.4 (5.7)	55.4 (19.1)	53.2 (21.1)	23.5 (19.7)	0.39 (0.24)	33, 4
FPC 2°	60.3 (17.0)	69.7 (19.6)	16.6 (8.5)	9.1 (10.5)	0.49 (0.50)	17, 3
PIFI 1°	75.6 (12.6)	85.2 (21.3)	16.8 (6.8)	11.2 (5.5)	0.72 (0.31)	39, 5
PIFI 2°	27.5 (21.5)	32.1 (21.8)	4.6 (2.2)	1.6 (0.9)	0.40 (0.28)	12, 2
ILTA	79.8 (5.4)	94.0 (2.4)	23.4 (7.6)	10.8 (3.7)	0.49 (0.19)	32, 3
ILTP 1°	76.6 (6.2)	86.6 (6.3)	13.2 (8.3)	3.5 (2.1)	0.27 (0.10)	31, 5
ILTP 2°	35.7 (18.9)	48.2 (17.4)	14.2 (9.1)	3.0 (2.3)	0.22 (0.13)	28, 4
ILFB 1°	74.6 (4.9)	81.4 (4.1)	7.5 (5.7)	2.1 (1.3)	0.33 (0.24)	22, 4
ILFB 2°	41.6 (10.3)	55.9 (9.3)	13.2 (8.9)	3.4 (2.4)	0.28 (0.12)	10, 2
Cycle Duration (s)	1.14 (0.19)					83, 8
Contact Interval (%)	67.96 (12.34)					

^a Complete data set is available from the author upon request

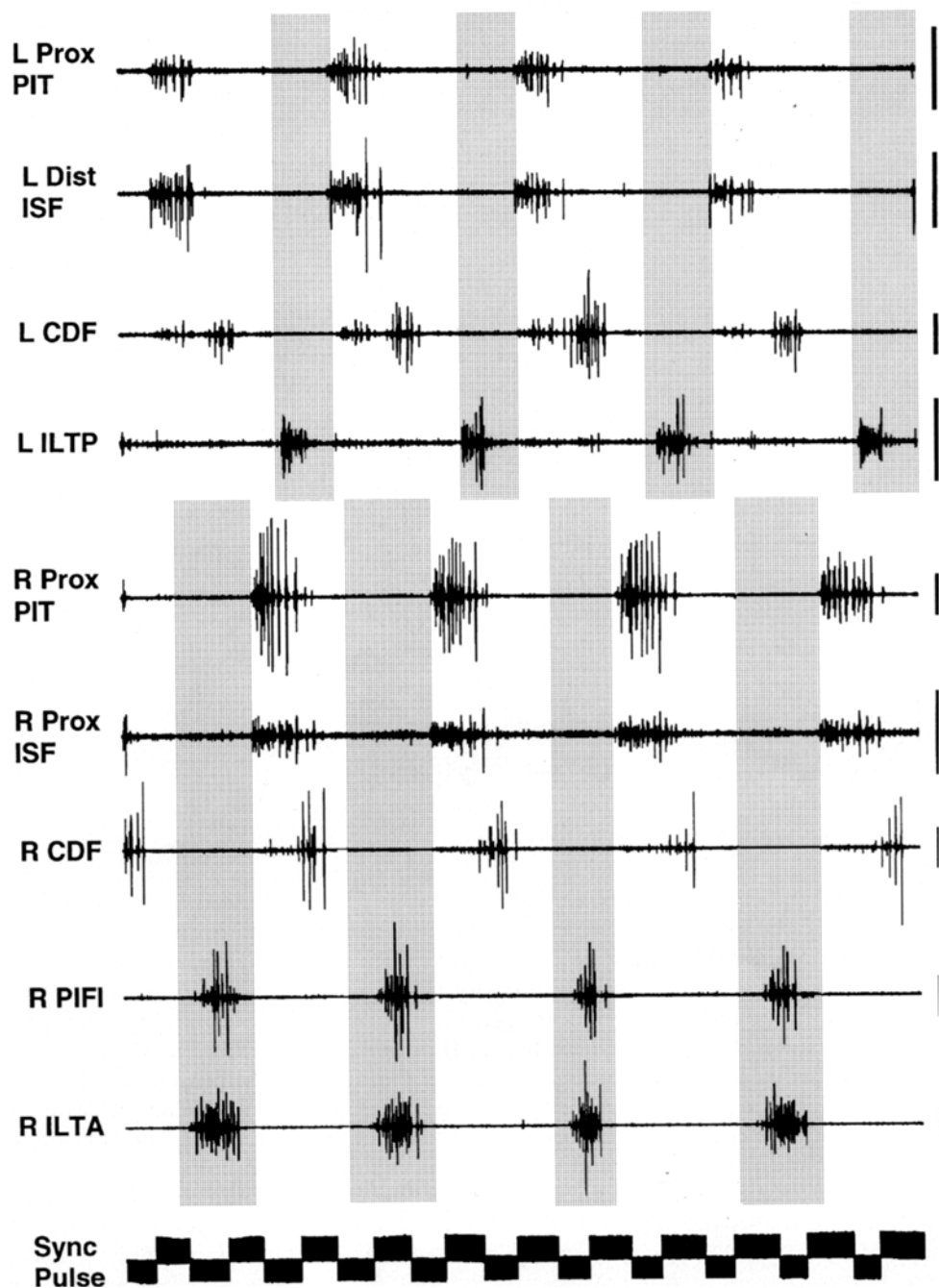
become active before the proximal sections. With the exception of the proximal ISF, all of these muscles cease their electrical activity at or before the midpoint of the stance phase. PIFE, a deep muscle originating on a large portion of the ventral surface of the puboischiac plate and inserting on the ventral surface of the femoral shaft, becomes active in concert with the PTB and persists through most of the stance. FPC, the major urodele calf muscle, likewise shows activity throughout much of the stance phase. In addition to these consistent bursts of electrical activity (the muscle's "primary burst"), several ventral muscles showed "variable secondary bursts." These were bursts that were not consistently present from animal to animal, nor from stride to stride within a single animal, but when present, were distinct from the primary burst within a stride. When present, secondary bursts in the PIT (both proximal and distal portions) and the FPC occurred during the stance, while secondary bursts in the PIFE occurred during the early swing phase.

Dorsal hind limb muscles all showed their primary burst during the swing phase, and had considerable overlap in their periods of activity (Fig. 1, 2; Table 1). The superficial group of strap-like muscles (ILTA, ILTP, and ILFB) also shows a progression of activation, this time in a posterior to anterior direction, with ILFB activating just before the start of swing phase,

followed by ILTP and then ILTA (Fig. 2). The single-joint, deeply located PIFI activates in concert with the ILFB and continues through the first half of the swing phase. All dorsal muscles except the ILTA show a variable secondary burst of activity. Again, not all animals showed the secondary burst, which also was not regularly present from stride to stride within a single animal. When present, the secondary burst always occurred during the middle portion of the stance phase (Fig. 2).

To explore possible differences in intensity of activation among muscles, Rectified Integrated Area/Duration (Table 1) was used as a measure of average instantaneous activity. Graphing RIA/Duration vs. Duration (Fig. 3) gives a visual representation of the relationship between intensity of activity and the duration of that activity. Figure 3 shows that instantaneous activation intensity is generally similar for all muscles, with the notable exception of the primary burst of the PIFI (uppermost black square in Fig. 3; Table 1), whose activation intensity is considerably higher than all other muscles. Fig. 3 also shows that ventral muscles demonstrate longer durations of activity than dorsal muscles (which is independent of the conversion of timing variables to conform to a 75% stance/25% swing step cycle). Primary bursts also tend to be longer and more intense than secondary bursts (Fig. 3, Table 1).

Fig. 1 Representative EMG traces from four strides of *Dicamptodon* no. 6. Grey boxes indicate swing phase for the appropriate hind leg. Synchronizing pulse shown at bottom. Vertical scale bar = 0.5 mV for each trace. Horizontal scale bar = 1 s. L = left limb, R = right limb. See list of abbreviations for muscle names



Discussion

Motor patterns in *Dicamptodon*

Most of the superficial muscles of the salamander hind limb span more than one joint (Table 2). Each of these muscles therefore has multiple possible actions, potentially causing movement at both (or more, in the case of ISF) joints simultaneously, or at only one joint at any given time. Because of this capacity to effect different combinations of joint movements, it is difficult to pre-

dict with certainty what their functions and activity patterns are during locomotion (proposed activity periods given in Table 2 are based strictly on the hypothesized functions given by previous authors). Additionally, the contraction characteristics of salamander limb muscles are almost entirely unknown, which makes predicting the electrical activity period required to produce given contraction kinetics difficult.

In the present study, while many of the activity patterns proved to conform to the general predictions, several were quite different from what was expected. All of the ventral muscles, with the exception of the CPIT,

had been predicted to be active starting in late swing or the beginning of the stance phase, and all do show this pattern. The PIFE and FPC come close to being active for the entire stance phase as predicted, but the ISF does not. The ISF is unique in that it spans three joints, with long lever arms about two of the joints (hip and knee). Because it is composed of distinct proximal and distal portions, which are separately innervated

(Francis 1934), it has the potential for complex activity patterns. With its unusual position, the ISF was initially hypothesized to (in order) flex the knee, retract the limb, and roll the foot up onto the toes during the propulsive phase of the step cycle, and therefore to be active during the entire stance phase (Table 2). However, the ISF only bursts in phase with the PIT and PTB, and never was observed to show secondary bursts at other times during the stride. It therefore appears to be functioning only in knee flexion and possibly femoral retraction.

Three ventrally located muscles showed a variable secondary period of activity during the stride. For PIT and FPC, the additional burst occurred during the stance phase, when an additional input of muscle force to help support the pelvic girdle or maintain traction of the foot on the treadmill surface is mechanically reasonable. However, the secondary burst of PIFE, when present, occurred during the early part of the swing phase, a time when a femoral adductor would be predicted to be inactive.

On the basis of Francis' (1934) proposed function for the CPIT as a flexor of the tail (Table 2), this muscle was predicted to be active during the swing phase, as the tail moves toward that side of the body while the leg is being protracted. However, the present data clearly show that the CPIT is active during the stance phase, with an onset following that of the other ventral muscles. Hence, the most probable action of the CPIT is to tense the PIT, "taking up the slack" in the latter muscle as the limb retracts.

Onset of activity in the caudofemoralis follows that of the CPIT, and this muscle is consistently the last to be activated during the stance phase. Barclay (1946) proposed, based on the "double crank" arrangement of the CDF, femur, and crus (see Ashley-Ross 1994a, for

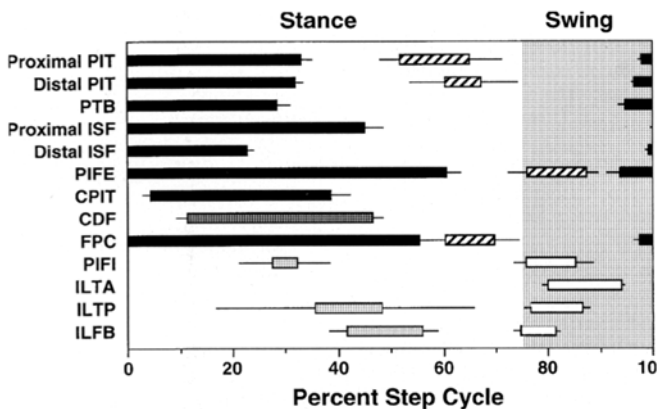


Fig. 2 Summary bar diagram of EMG activity in hind limb muscles relative to kinematic events of the stride, which were determined by video analysis. The stance phase begins with the placement of the foot on the treadmill surface. The swing phase begins with the lifting of the foot from the surface of the treadmill and continues until the foot again contacts the substrate at the start of the next stride. The ends of the thick bars represent the mean onset and offset of muscle activity, and the thin bars show one standard error of the mean. Black bars represent ventrally located muscles, white bars represent dorsally located muscles. Hatched and stippled bars indicate variable secondary bursts of activity from the corresponding muscle. Numerical values and sample size for each muscle given in Table 1. All values have been calculated as a percentage of the step cycle duration, normalized to 75% stance/25% swing. See text for details

Fig. 3 Plot of (Rectified Integrated Area)/Duration (in percent of step cycle) versus duration for all hind limb muscles studied. Circles represent ventral muscles, squares represent dorsal muscles, and the triangle indicates the caudofemoralis. Filled symbols indicate the primary activity burst of the muscle, while open symbols indicate variable secondary bursts. Numerical values given in Table 1

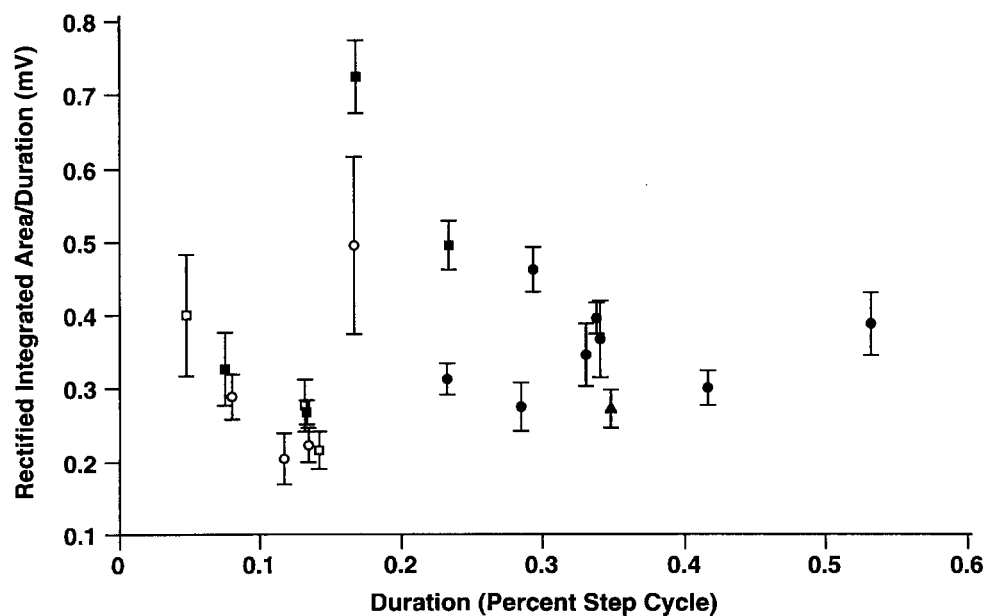


Table 2 Hypothesized function of major salamander hind limb muscles, taken from Francis (1934) and Peters and Goslow (1983). *Names in boldface* indicate electromyographic data was obtained for the muscle in the present study. See Abbreviations and text for details

Muscle	Joints spanned	Hypothesized function	Proposed activity period
ISC	2 ^a	Flex the tail; compress the cloacal gland (male)	Swing
CPIT	2 ^a	Flex the tail	Swing
CDF	2 ^a	Flex the tail or retract the femur	Mid-stance ^b
PTB	2	Adduct the hind limb, flex the knee	Early stance
PIT	2	Flex the knee, adduct the femur	Late swing through mid-stance ^b
ISF	3	Retract the femur, flex the knee and toes	Entire stance
PFM	1	Protract the femur	Late swing
PIFE	1	Adduct the femur	Entire stance
PIFI	1	Protract the femur	Entire swing
ILTA	2	Extend the knee	Late stance, mid-swing
ILTP	2	Extend the knee	Late stance, mid-swing
ILFB	2	Flex the knee	Early stance, early swing
ILFM	1	Retract the femur	Stance
ISFM	1	Depress and retract the femur, or support and project the pelvis	Late stance
FMFB	1	Flex the knee	Early stance
FPC	2	Flex the digits and tarsus, pronate the foot	Entire stance
EXT	1, 2	Extend the crus	Late stance, early to mid-swing
EDC	3 ^a	Extend the foot	Early to mid-swing
EXF	1, 2	Extend the crus and tarsus	Late stance, early to mid-swing

^aThe various vertebrae and tarsals spanned by these muscles are considered for the present purpose as one functional joint

^bPeriod of activity determined by Peters and Goslow (1983)

further discussion of the double crank mechanism), that the lower leg acts as a spoke on a wheel as the femur is rotated by the CDF, producing backward movement of the foot throughout the propulsive phase of the step cycle. It was therefore hypothesized that the CDF would show activity throughout the propulsive (stance) phase. The electromyographic results of the present study agree with those of Peters and Goslow (1983), who found that femoral rotation was not always present, and the CDF did not show activity until the middle of the stance phase (when femoral retraction and rotation commenced). It has been argued elsewhere (Ashley-Ross 1994a), based on mechanical considerations and the actual positioning of the limb throughout the stance phase, that the double crank mechanism can only function during the middle portion of the stance phase when the femur and crus are close to being at right angles with one another. The timing of activity in the CDF is appropriate for contributing to the operation of the double crank mechanism of femoral rotation and retraction.

All of the dorsal muscles examined showed consistent bursts of activity in early to mid-swing. Again, this pattern is only partially consistent with predictions of

muscle function. The ILFB becomes active first, just before the foot is lifted from the substrate, followed in the early part of the swing phase by the ILTP and ILTA. This pattern is expected as the knee is first flexed, then extended as the swing phase progresses. The PIFI is active during only the first half of the swing, not the entire phase as predicted. While the PIFI undoubtedly causes femoral protraction during the early to mid-swing, it may be that inertial forces or slow relaxation kinetics of the muscle (long-lasting force production) is responsible for continued protraction of the femur during the late swing. Alternatively, the PFM may provide the force for protraction of the femur during late stance, though this muscle's attachments would also be expected to cause depression of the femur if it were contracted strongly (Ashley-Ross 1992). ILTA, ILTP and ILFB were predicted to show additional bursts during the stance phase. No such secondary bursts were recorded from ILTA, indicating that it does not play a role in knee extension during the second half of the stance. ILTP sometimes does have a secondary burst of activity in its predicted place, mid-way through the stance, which suggests that this muscle is likely to play a role in knee extension during

stance. The secondary burst of ILFB and the unpredicted secondary burst of PIFI occurred during times of the step cycle when they would seem to have no obvious function. For these seemingly anomalously timed bursts, as well as that of the PIFE, the most reasonable hypothesis is that they are coactive with antagonists in order to better direct the motion of the limb (Basmajian and De Luca 1985).

Comparisons with other tetrapods

Table 3 lists the current hypotheses of hind limb muscle homologies among major tetrapod groups. It must be noted that the assignment of homologies to particular muscles across these different groups still has not been definitively resolved, and therefore the conclusions drawn here may have to be re-evaluated at a later date.

Figures 4 and 5 show summary bar diagrams of activity patterns for the homologous ventral and dorsal (respectively) hind limb muscles of representatives of the major tetrapod taxa. Only those muscles for which comparative data could be found in the literature have been diagrammed, and all data has been transformed to a 75% stance/25% swing step cycle as described in Materials and methods.

The ventral muscles from all taxa show at least one burst during the stance phase, beginning either in late swing or early stance (Fig. 4). The FPC and its homologues, which show the most similarity in anatomical orientation among taxa, also show the highest degree of overlap, with all bursts beginning during the swing phase and persisting for large portions of the stance phase. The PIT and ISF, both of which contribute to early knee flexion and femoral retraction during the stance phase (plesiomorphic step cycle features), show overlapping activity with their homologues during the early portion of the stance phase. Posture does not seem to affect the timing of activity in the PIT; in both *Chamaeleo* (which holds its limbs in a more sagittal

orientation than other lizards, a derived condition among lepidosaurs; Peterson 1984) and the mammalian species (*Rattus* and *Felis*), the homologous muscle has a vertical orientation, yet the general period of activity is the same as in *Dicamptodon*, though in the mammals shown the variable secondary burst is absent. The mammalian homologues of the horizontally oriented three-joint salamander ISF likewise retain a similarity of timing, in that all become active during the late swing and persist in activity through early stance. Semitendinosus (ST), a two-joint muscle that shares the same dual innervation pattern as the ISF (Bodine et al. 1982; Windhorst et al. 1989), seems to have acquired a second burst, occurring during the transition from stance to swing, which may be associated with flexing the knee of the sagittally oriented leg at the beginning of swing to raise the foot clear of the ground. Wentink's (1976) recordings of the semimembranosus of dogs (data not shown) indicate a yet more complex pattern for this ISF homologue: the muscle contains two distinct compartments, one of which fires a single burst during early to mid-stance, while the other shows two bursts of activity, one during early to mid-stance, and the other at the beginning of the swing phase (see also discussion of mammalian sartorius, below).

The posteriorly located CDF (Fig. 4) also shows a high degree of similarity of timing among the tetrapod taxa, with the exception of its activity period in the Guinea fowl (*Numida*). It is interesting to note that the form of the CDF is rather different in caudates from that in lizards and in crocodylians. The salamander CDF is a simple straplike muscle originating from as few as one caudal vertebra and inserting on the posterior surface of the femoral trochanter; in lizards and in crocodylians, the CDF originates from as many as a dozen caudal vertebrae, curls around the trochanter to insert at the base of the *anterior* side of the trochanter (Snyder 1954; Gatesy 1990), and also has a long accessory tendon extending to the knee capsule (Snyder 1954). Snyder (1962) argues that this arrangement makes it possible for the CDF to generate large

Table 3 Homologies of tetrapod pelvic limb muscles taken from Romer and Parsons (1977), Rowe (1986), and Walker and Hemberger (1992). Only muscles for which literature data exists for

reptiles, birds, or mammals are listed. *Muscle names in italics* indicates that data was not available for that muscle in that taxon

Caudata	Lepidosauria	Crocodylia	Aves	Mammalia
PIT	<i>Puboischiotibialis</i>	<i>Puboischiotibialis</i>	<i>Not present</i>	<i>Gracilis</i>
ISF	<i>Flexor tibialis internus</i>	<i>Flexor tibialis internus</i>	<i>Ischioflexorius</i>	<i>Semimembranosus</i> , <i>semitendinosus</i> , <i>biceps femoris</i>
FPC	<i>Medial gastrocnemius</i>	<i>Medial gastrocnemius</i>	<i>Medial gastrocnemius</i>	<i>Medial gastrocnemius</i> , <i>soleus</i>
CDF	<i>Caudofemoralis</i>	<i>Caudofemoralis</i>	<i>Caudofemoralis</i>	<i>Piriformis</i>
PIFI	<i>Puboischiofemoralis internus</i>	<i>Puboischiofemoralis internus 1</i> <i>Puboischiofemoralis internus 2</i>	<i>Iliofemoralis internus</i>	<i>Iliopsoas</i>
ILFB	<i>Iliofibularis</i>	<i>Iliofibularis</i>	<i>Iliotrochantericus cranialis</i> and <i>medius</i> <i>Iliofibularis</i>	<i>Gluteus maximus</i>
ILTA	<i>Ambiens</i>	<i>Ambiens</i>	<i>Ambiens</i> (<i>Sartorius</i>)	<i>Sartorius</i>
ILTP	<i>Iliotibialis</i>	<i>Iliotibialis</i>	<i>Iliotibialis posterior</i>	<i>Rectus femoris</i>

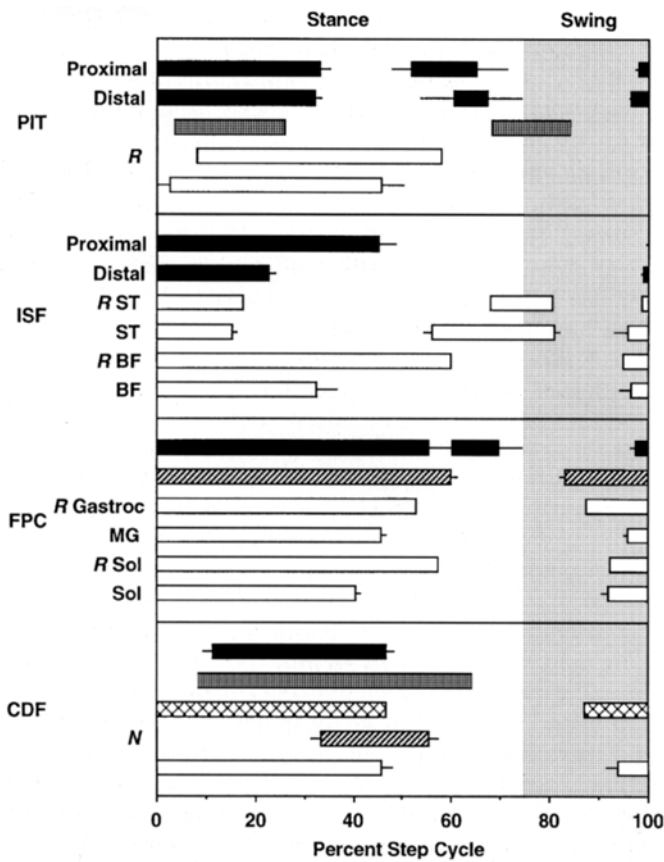


Fig. 4 Summary bar diagram comparing activity periods of *Dicamptodon* ventral hind limb muscles with those of other tetrapod taxa (culled from the literature). All values have been normalized to 75% stance/25% swing. See text for details. *Black bars* represent *Dicamptodon*, *shaded bars* represent lepidosaurs, *cross-hatched bars* represent crocodilians, *diagonally hatched bars* represent birds, and *open bars* represent mammals. Homologous muscles (from Table 3) have been grouped together. Where more than one homologue exists for a salamander muscle, the bars have been labeled appropriately (see Abbreviations table). *Italic labels* indicate the taxon the data was taken from (*V* = *Varanus*; *N* = *Numida*; *R* = *Rattus*). *Unlabeled bars* are from the "standard" species for that group (Lepidosauria = *Chamaeleo*; Crocodilia = *Alligator*; Aves = *Gallus*; Mammalia = *Felis*). *Shaded region* indicates the swing phase of the step cycle

amounts of force over a wide range of pelvic girdle-femur angles. In crocodilians, the CDF is active from late swing phase until the latter portion of the stance phase during the high walk (Gatesy 1990), with active femoral retraction occurring during this time. This difference in CDF activity patterns between salamanders and crocodilians is likely due to the differing postures of the leg at the beginning of the propulsive phase. In salamanders, the foot is placed far forward of the hip, and the leg is completely extended; the knee must be flexed (by active contraction of PIT; Peters and Goslow 1983) before femoral retraction/rotation occurs. In contrast, both crocodilians and lizards place the foot only slightly forward of the hip, with the knee already flexed, so that femoral retraction by the CDF can commence immediately (Brinkman 1981). The relative

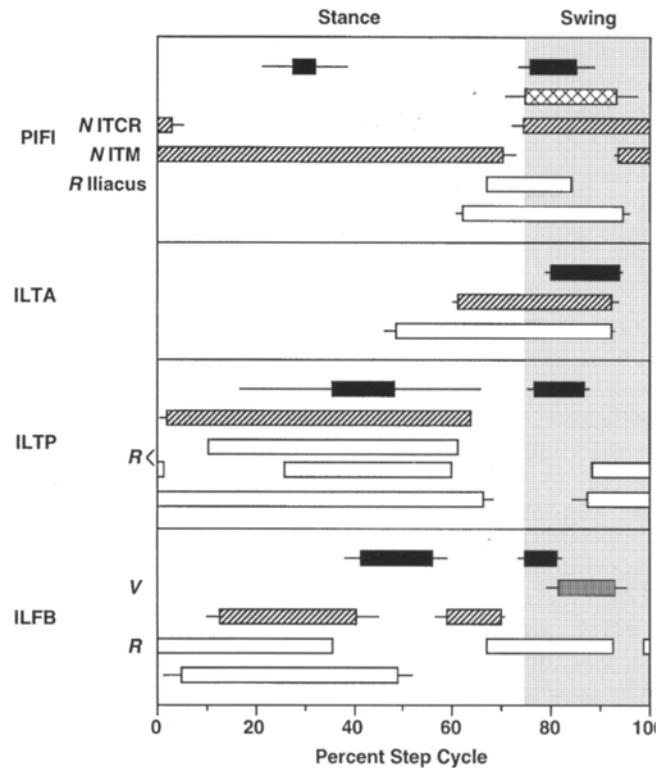


Fig. 5 Summary bar diagram comparing activity periods of *Dicamptodon* dorsal hind limb muscles with those of other tetrapod taxa (culled from the literature). Same conventions as Fig. 4

timing of the burst of activity in *Numida* is ascribed by Gatesy (1990) to the pattern of femur movement typical of birds: femoral retraction only occurs after the foot has passed under the acetabulum, during the final two-thirds of the stance phase. Femoral retraction and CDF activity in this taxon also show speed-dependence, often being absent at slow walking speeds but appearing more regularly as speed increases (Gatesy 1990).

The dorsally located muscles tend to show a lesser degree of overlap among tetrapod taxa (Fig. 5). The greatest similarity in this group of muscles is found in the PIFI and ILTA. The PIFI and its homologues all show activity at the start of the swing phase, in spite of differing origins for this muscle in crocodilians (from lumbar vertebrae) versus all other taxa (from the internal surface of the pelvic girdle). The sole exception to this conserved pattern is demonstrated by one of the two homologues of PIFI in *Numida* (iliotrochantericus medius), which shows activity associated with the stance phase. The homologues of the ILTA in birds and mammals (sartorius in both groups) show extensive overlap with the activity period of this muscle in *Dicamptodon*, but also extend their activity back into the late stance phase, where they may be involved with knee extension during this time. The orientation of the homologues of the ILTA differs according to limb posture in the different taxa. In *Dicamptodon* (sprawling posture) the ILTA is a simple straplike muscle lying

along the anterodorsal surface of the femur. In birds and mammals, the upright posture of these groups is associated with anterior rotation of the limb, resulting in the sartorius curving medially over the anterior aspect of the limb as it passes from origin to insertion. In addition, recent studies of the mammalian sartorius have shown it to have a very complex architecture (Scott et al. 1992; Heron and Richmond 1993). In *Felis*, the sartorius is composed of two distinct compartments, anterior and medial sartorius, which demonstrate different activity patterns during locomotion (Hoffer et al. 1987a, b). The medial compartment is characterized by a single burst of activity during the step cycle that occurs during the swing phase, while the anterior compartment shows two bursts of activity, one during swing and one during stance (Hoffer et al. 1987b). Interestingly, single motoneurons of the double-bursting anterior sartorius show activity during only one of the two bursts (Hoffer et al. 1987b).

The ILTP and ILFB show the least amount of overlapping activity with their respective homologues. Both of these muscles show differences in orientation and origin between groups with sprawling postures and groups with upright posture. The transition to upright posture in birds and mammals is associated with a change in orientation from horizontal to vertical for both muscles, and a migration of muscle origin on the pelvic girdle (anterior for the ILTP homologues and posterior for the ILFB homologues). The double bursting pattern of the ILTP has seemingly been lost in all taxa other than *Dicamptodon*, with the exception of the rat. However, Nicolopoulos-Stournaras and Iles (1984) report that the ILTP homologue in the rat (rectus femoris) sometimes shows a single bursting pattern and sometimes a double burst, with the secondary burst appearing during the late swing phase (see Fig. 5, double bars marked with *R*). It is as yet unclear whether two separate populations of motoneurons are responsible for producing the two bursts, as in the cat sartorius (see above). The homologues of the ILFB show a variety of activity patterns during the step cycle, with little consistent overlap as one moves from salamanders and reptiles to birds and mammals. Birds have a double bursting pattern in their ILFB, but it is shifted in the step cycle relative to salamanders. The mammalian homologue of the ILFB, gluteus maximus, is very different in its orientation compared to the ILFB of salamanders and reptiles. The attachments of the gluteus maximus are such that this muscle is in a position only to extend the hip, which may account for its activity primarily during the early to mid-stance phase in mammals.

Do conservative neuromuscular patterns drive plesiomorphic step cycle features?

The features of the step cycle previously identified as plesiomorphic, and accessible to electromyographic

investigation in this study, were: (1) femoral retraction throughout the stance phase (caused by PIT, ISF, and CDF); (2) knee flexion in early stance (caused by PIT and ISF); and (3) knee joint extension in late stance (by ILTA and ILTP). All of these muscles were predicted to have activity periods similar to their homologues in other taxa, as they are responsible for producing a common sequence of motion of the limb during the stance phase. However, only the ventrally located muscles, PIT and ISF, together with CDF, showed the expected pattern of conservatism in activity period. The dorsally located ILTA and ILTP were characterized by a variety of activity periods in the different tetrapod taxa.

Taken as a whole, these comparisons suggest that neuromuscular conservatism is present for some muscles, but not others. Specifically, the ventral muscles in the different tetrapod taxa do show extensive overlap in activity periods in spite of anatomical rearrangements. These muscles fulfill the criteria of Smith (1994), who has argued that only muscles that show anatomical differences among homologues provide a rigorous test of the hypothesis of neuromuscular conservatism. All of these muscles function in movements that were proposed to be plesiomorphic for the tetrapod step cycle (Ashley-Ross 1994a), so it is perhaps not unexpected that the motor patterns controlling these movements should be retained among the various tetrapod groups. Cohen (1988) has argued that the ventral muscles all arose phylogenetically from one precursor, are all driven by one class of oscillator in the central nervous system, and therefore should show conservatism in their activity periods.

Dorsal muscles show the greatest departure from a conserved pattern. In comparison with ventral muscles, across taxa the dorsal muscles show more variability in their pattern of activity, as would be expected if they are controlled by both swing and stance oscillators, as proposed by Cohen (1988). However, there are regions of overlap in activity periods of dorsal muscles, particularly for muscles spanning the same joints, though with differences in anatomical orientation (e.g., PIFI and ILTA). An additional complication is the capability of some muscles to switch between single- and double-bursting patterns from stride to stride (this study, Nicolopoulos-Stournaras and Iles 1984). Various authors (Goslow et al. 1989a, b) have suggested that muscles capable of showing biphasic activity patterns may represent an intrinsic source of plasticity in the motor pattern, with different taxa emphasizing one burst over the other depending on the functional requirements of the limb's posture and kinematic pattern.

Future research

On the basis of the comparative data presented above, it appears that ventrally located muscles of the hind

limb retain similar patterns across the tetrapod taxa. However, a final determination of the validity of the hypothesis of neuromuscular conservatism must await rigorous statistical analysis. Smith (1994) has pointed out that most studies which hypothesize neuromuscular conservatism in a given musculoskeletal system (such as the vertebrate feeding apparatus) are lax in their definition of characters being tested. I therefore suggest that the relative timing of muscle onset or offset within a stride be tested for statistically significant difference among vertebrate taxa; a statistically significant difference among vertebrate groups falsifies the hypothesis of neuromuscular conservatism for that muscle. The data currently available in the literature are inadequate to achieve this objective, with many studies (with the notable exception of Rasmussen et al. 1978) failing to report means, standard deviations/errors and sample sizes for the electromyographic variables measured. Also, our knowledge of motor patterns in lepidosaurs and crocodylians is limited to very few muscles. In order to have a true estimate of the variability of each major taxon and to avoid the pitfall of pseudoreplication (Hurlbert 1984), data from many more species of all the tetrapod groups are required. When such a representative sampling of electromyographic data from various taxa is available, the hypothesis of neuromuscular conservatism in specific muscles may be statistically tested by a nested analysis of variance (Zar 1984), with individuals nested within species which in turn are nested within major taxon. Because measurements on multiple species within a monophyletic lineage are not independent of one another (Felsenstein 1985), significance levels should be extremely conservative. As this hypothesis is concerned with the preservation of relative timing activity within the stride, it should be tested on only the timing variables (onset and offset) for the tested muscles.

To allow such statistical comparisons to be made more accurately, methods of data presentation need to be standardized. I therefore propose that researchers carrying out electromyographic studies of vertebrate locomotion transform their activity-timing data to conform to a standardized step cycle, such as the 75% stance/25% swing step cycle used here. The precise proportions of stance/swing are unimportant, as normalized data for one standard can easily be transformed to fit any other. Data should be presented in tabular as well as bar diagram form, in order to facilitate comparisons (which may require numerical transformations) by other researchers. Especially important is the inclusion of mean, standard deviation/standard error and sample size for each variable (onset, offset, etc.) from each muscle. Only when data from a number of representative species for each main group are available in a standardized, easily accessible format will we be in a position to track the evolution of the neural control of locomotion accurately through the tetrapod lineages.

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