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MUSCLE RECRUITMENT DURING TERRESTRIAL LOCOMOTION: HOW SPEED AND TEMPERATURE AFFECT FIBRE TYPE USE IN A LIZARD

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Summary

Synchronized electromyography and cinematography were used to study the activity of the red and white regions of the iliofibularis muscle in savannah monitor lizards, *Varanus exanthematicus* (Bosc), during locomotion. Analysis of variance on results from four individuals moving at speeds of up to 1.5 km h^{-1} at two body temperatures (25 and 35 °C) revealed that all kinematic variables were significantly affected by speed but none was affected by temperature. Hence, patterns of limb movement at any speed were similar at both temperatures. However, this similarity resulted from differences in muscle activity. Analysis of variance on electromyographic variables for activity in the red and white regions showed widespread significant effects of both temperature and speed. The red region was active at all speeds, and it displayed regular bursts of activity which usually occurred when the foot was above the ground, the femur was being abducted and the knee flexed. Variables measuring the intensity of red region activity generally increased with speed until a maximum was attained and no further change occurred with additional increases in speed. The speed at which maximum red activity was attained at 25 °C was less than that at 35 °C. For equal locomotor speeds, amplitudes of electromyograms (EMGs) from the red region at 25 °C were greater than those at 35 °C. In contrast to the red region, the white region was active only above some threshold speed, and activity was often rather irregular compared to that of the red region. At 25 °C the threshold speed for recruitment of the white region (0.9 km h^{-1}) was less than that at 35 °C (1.3 km h^{-1}). The relationship between locomotor speed and activity per minute for the red region was very similar to the relationship between speed and rate of oxygen consumption described in previous studies of lizards, and the threshold speed for recruitment of the white region was also similar to the maximal aerobic speed previously reported for this species. Hence, lizards increase speed and compensate for lower temperature by increasing intensity of activity within the red region and recruiting fibres in the white region. We suggest that compensation for muscle function at decreased body temperature may involve recruitment of greater numbers of motor units.

Key words: muscle, locomotion, lizard, electromyography, temperature.

Introduction

The wide ranges of speed and frequency of movement that occur during locomotion of animals is ideally suited for studying the mechanisms that constrain or modulate behaviour. The locomotion of ectothermic vertebrates is particularly interesting because the environmental constraint of temperature can drastically affect maximal performance (e.g. Marsh and Bennett, 1986*a*), yet many ectothermic vertebrates perform locomotor behaviours over both a large range in body temperatures (Brattstrom, 1963; Avery, 1982; Huey, 1982) and a wide range in speeds (Hertz *et al.* 1988). Because decreased temperature greatly slows the rate of force production and shortening in most vertebrate muscle (reviewed in Bennett, 1984), one would expect that ectotherms must use some compensatory pattern of muscle activity or alter kinematic patterns of limb use to attain equal speed as temperature decreases. As speed increases at a single temperature, one would also expect changing patterns of muscle recruitment that reflect the need for faster rates of force production and shortening. Thus, there could be similarities in the modulation of muscle activity that occur in response to both increased speed and decreased temperature, but limited information on this topic is available for vertebrate locomotion.

Another consequence of increasing locomotor speed is that metabolic support of muscle activity changes from almost exclusively aerobic to anaerobic. Because of the spatial segregation of different muscle fibre types in many species of fish (reviewed in Bone, 1978; Johnston, 1985), the relationship of muscle physiology to *in vivo* recruitment of locomotor muscle is understood best for this group. Muscle activity during slow locomotion is confined to the red aerobic region, and increasingly more anaerobic fibre types are recruited as speed increases (Bone, 1966; Rayner and Keenan, 1967; Hudson, 1973; Bone *et al.* 1978; Johnston and Moon, 1980). Furthermore, Rome *et al.* (1984) found that at lower temperatures muscle in the white region was active at slower swimming speeds than at higher temperatures. Hence, one expects that, in the red and white regions of vertebrate locomotor muscle, there will be differences in activity that depend on both temperature and locomotor speed, but the qualitative nature of the previous studies on fish limits our ability to predict precisely the expected differences in the duration and intensity of muscle activity. A variety of mechanisms could be used by animals to compensate for the demands of different speeds and temperature, and quantitative electromyography is a powerful method for clarifying how animals may modulate muscle activity in response to these variables.

In contrast to the situation in fish, the relationship of *in vivo* muscle activity to locomotor speed and fibre type is not known for terrestrial ectothermic vertebrates. The only previously published study on the muscle activity in the limbs of lizards is that of Jenkins and Goslow (1983), who determined the locomotor activity of forelimb muscles in *Varanus exanthematicus*, but they did not examine differences attributable to speed, temperature or fibre type. However, a thigh muscle of lizards, the iliofibularis, has been used extensively for *in vitro* studies of muscle contraction, in part because it has spatially distinct regions of different

fibre types, with white fibres located superficially and red fibres located deeper and nearer to the femur. For the distinct white and red regions of the iliofibularis in a variety of lizard species, the histochemistry (Gleeson *et al.* 1980*b*; Gleeson, 1983; Mutungi, 1990) and contractile properties and their thermal dependence (Gleeson *et al.* 1980*b*; Johnston and Gleeson, 1984; Gleeson and Johnston, 1987; Marsh and Bennett, 1986*a*; Adams, 1987) have been described. Like fish myotomal muscle, the red region of the iliofibularis muscle of lizards has higher mitochondrial and capillary densities compared to the white region, suggesting differential reliance of these two regions on anaerobic metabolism. The red regions of lizard locomotor muscles also have a significant proportion of tonic fibres (Gleeson *et al.* 1980*b*; Mutungi, 1990), leading Putnam *et al.* (1980) to suggest a postural role for these regions.

The purpose of this study was to determine the effects of speed, temperature and fibre type on *in vivo* activity of a single locomotor muscle of an ectothermic terrestrial vertebrate. We performed a quantitative electromyographic study of activity in the red and white regions of the iliofibularis muscle of the lizard *Varanus exanthematicus*. We specifically determined: (1) if the total amount and the intensity of muscle activity vary with speed when temperature and fibre type are held constant; (2) if temperature affects the amount and intensity of activity for a given fibre type when comparing equal locomotor speeds; (3) if regions of different fibre type show different patterns of activity as speed and temperature change; and (4) if variation in the amount and intensity of activity shows simple linear increases with speed or if there are threshold locomotor speeds below or above which no changes in muscle activity occur.

Materials and methods

Animals

Savannah monitor lizards, *Varanus exanthematicus*, were chosen for this study because they are active animals that can be readily induced to move with a wide range of speeds on a treadmill (Gleeson *et al.* 1980*a*; Rome, 1982; Jenkins and Goslow, 1983). Furthermore, the fibre type of the iliofibularis muscle in this (Mutungi, 1990) and another species of *Varanus* (Gleeson, 1983) has been studied, and the large size of animals facilitates implantation of electrodes within different regions of a single muscle. Animals were obtained from commercial sources. The lizards were maintained in a room with a 12 h:12 h light:dark cycle and photothermal gradients in cages that allowed daytime body temperatures to be selected between 25 and 35 °C; night temperatures varied from about 22 to 25 °C. We selected lizards with similar linear dimensions to minimize size effects. Snout–vent lengths of the four individuals used for statistical analysis ranged from 28.0 to 31.0 cm with a mean of 30.3 cm. The distance from toe-tip to toe-tip of the hindlimbs fully extended perpendicular to the body ranged from 22.0 to 24.7 cm with a mean of 23.7 cm. The mass of the lizards ranged from 368 to 497 g with a mean value of 436 g.

Motion analysis

Cinematography

A Redlake Locam camera operated at either 50 or 100 frames s^{-1} and respective exposure times of 1/500 or 1/1000 s was used to obtain 16 mm films of each lizard moving on a treadmill. The belt of the treadmill consisted of rubber-impregnated cloth and had a working area of 20 cm × 85 cm with the longer dimension parallel to the direction of the movement of the tread belt. A variable-speed motor allowed the speed of the tread surface to be varied from 0.1 to 4.0 km h^{-1}. The camera was positioned perpendicular to one side of the treadmill in order to obtain a lateral view of the lizard, and a front-surface mirror mounted at an angle to the tread surface allowed simultaneous filming of the dorsal view. Within view of the camera, a periodically blinking light simultaneously sent a signal to the tape recorder, allowing the movement and electromyographic records to be synchronized. Paint marks on the lizard facilitated recognition of features used to calculate kinematic variables.

For motion analysis, films were projected to half life size using a Lafayette stop-action projector. Tracings of the projected images of the paint marks on the leg containing an electrode were used to measure the distance travelled (±1 mm) during the stance phase (when the foot touches the substratum) and during the swing phase (when the foot moves above the substratum). The time of hind foot contact with the substratum was recorded to the nearest frame for films made at 100 frames s^{-1} and estimated to the nearest half-frame for films made at 50 frames s^{-1}.

Kinematic variables

The resulting records of time and displacement were used to calculate the kinematic variables listed in Table 1. For the hindlimb containing the electrode, the durations of the stance (DOWNDUR) and swing phases (UPDUR) were recorded. Stride duration (STRDUR=DOWNDUR+UPDUR) was measured as the time that elapsed between successive footfalls, and stride length (STRLEN) was the distance of the line segment joining the two points of displacement at the times of successive footfalls. Step length (STPLEN) was the projection of distance travelled during the stance phase along the line segment representing stride length. The proportion of time for one cycle of movement that was taken up by the swing phase (PUP) was calculated by dividing UPDUR by STRDUR, and the ratio of step length to stride length (STPSTR) was found by dividing STEPLEN by STRLEN. Mean forward velocity (VX) was calculated for each stride by dividing STRLEN by STRDUR. A more detailed kinematic description of hindlimb movements is beyond the scope of this study, but the present variables permitted us: (1) to describe grossly the amplitude and frequency of hindlimb movement, (2) to partition the electromyographic record into cycles of limb movement, and (3) to determine VX more accurately than by assuming that the animals' speed precisely

Table 1. *Summary of* F*-tests for significance of effects in separate three-way ANOVAs performed on each kinematic variable*

	Effect						
Variable	Speed (4, 12)	Temp (1, 3)	Indiv (3, 116)	Speed ×Temp (4, 11)	Speed ×Indiv (12, 116)	Temp× Indiv (3, 116)	Speed× Temp× Indiv (11, 116)
VX	612.2***	1.2	0.5	0.4	2.5**	0.7	1.0
STRDUR	106.8***	0.9	10.5***	0.9	4.4***	3.3*	2.9**
DOWNDUR	73.1***	0.0	27.8***	0.2	7.6***	1.2	4.5***
UPDUR	30.0***	2.2	7.8***	5.5*	1.0	4.4**	0.4
PUP	17.0***	2.2	15.5***	0.3	1.3	2.3	1.0
STRLEN	21.8***	0.0	17.9***	0.9	2.5**	7.0***	1.4
STEPLEN	8.5**	0.7	36.5***	0.4	1.7	1.9	2.0*
STEPSTR	6.7**	0.6	12.7***	0.5	1.0	5.0**	2.0*

Degrees of freedom associated with each F statistic are given in parentheses below each effect.
Significance levels of $F<0.05$, <0.01 and <0.001 are indicated by *, ** and ***, respectively. See Materials and methods for explanation of kinematic variables.
Temp, temperature; Indiv, individual.

matched that of the treadmill. A more detailed study of the kinematics will be presented elsewhere.

Muscle activity

Electromyography

Bipolar electrodes (similar to those of Jayne, 1988) used to record muscle activity consisted of 0.051 mm diameter stainless-steel wire with an uninsulated portion of about 0.6 mm. Prior to implantation of electrodes into the iliofibularis muscle, the lizards were anaesthetized with halothane administered by inhalation as necessary, and an incision through the skin parallel to the femur was made on the dorsal surface of the thigh. Reflecting the skin and separating some of the fascia anterior to the iliofibularis muscle facilitated implantation of each electrode directly into the red and white portions of the iliofibularis muscle *via* a 26 gauge hypodermic needle. After implantation of the electrodes, the incision was closed with three sutures. To maximize the chances of obtaining recordings from both the red and white regions of a single muscle, usually three electrodes were implanted in both the right and left muscles. At the conclusion of an experiment, animals were killed with an overdose of anaesthetic and electromyograms (EMGs) were analyzed statistically only from electrodes whose position had been confirmed by dissection.

After allowing from 8 to 24 h of recovery from anaesthesia, lizards were filmed moving on the treadmill at increments of tread speed of 0.3 km h^{-1} from 0.3 to 1.5 km h^{-1} for each of two temperatures, and more limited data were gathered at

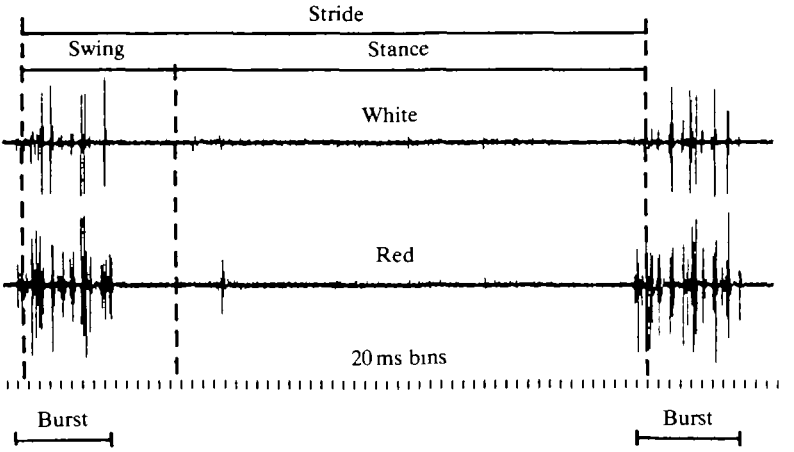
faster speeds. A thermocouple implanted in the cloaca of the lizard continuously monitored body temperature during the experiments, which were conducted at body temperatures of either 25 or 35 °C (±0.5 °C). The latter temperature is near the preferred body temperature of this species and, for individuals with mass about equal to 1 kg, a maximal aerobic speed of 1.2 km h^{-1} has been reported for this temperature (Gleeson *et al.* 1980*a*). Hence, to determine the effects of speed and temperature on muscle activity, we chose a range of speeds and temperatures to encompass a presumed range of both aerobically supported and anaerobically supported activity. For two lizards, the trials at the lower temperature were conducted on the first day after surgery and the high-temperature trials were on the second day; for the other two individuals the order of temperatures was reversed.

The EMGs were amplified 10 000 times with Grass P511J preamplifiers with a 60 Hz notch filter and half-amplitude low and half-amplitude high filter settings at 100 Hz and 3 kHz, respectively. A supplemental RC filter with a cutoff frequency of 50 Hz was also used before the analog EMG signal was stored on magnetic tape using a Bell & Howell 4020A operated at a speed of 19.05 cm s^{-1}. For visual inspection, copies of the EMGs were made by playing the signal from the tape recorder to a Gould 260 chart recorder operating at 16 cm s^{-1} and these copies were used to measure EMG burst duration to the nearest 0.01 s. To generate a digital file of the EMGs, the analog signal was played back at one-quarter recording speed to a 12-bit Kiethley analog-to-digital converter operated at an effective sampling frequency of 8400 Hz. The digital EMG was then analyzed using customized software that counted spikes (using the algorithm of Beach *et al.* 1982) and determined other measures of activity per 20 ms bin for the entire duration of the EMG file. A peak voltage in the EMG was only counted as a spike if it differed by more than 12 μV (in units of the unamplified EMG) from the baseline voltage. Files containing the bin-wise listing of EMG activity were then imported into a spreadsheet program for further analysis.

Electromyographic variables

As shown in Fig. 1 and Tables 3 and 4, several variables describing the numbers and amplitudes of spikes and rectified integrated areas of the EMG were used to describe muscle activity. The endings for the names of EMG variables indicate the following: (1) DUR=duration (in seconds), (2) S=numbers of spikes, (3) SXA=number of spikes times mean amplitude of spikes (in units of voltage), (4) AR=rectified integrated area (in units of voltage times time), and (5) AMP=spike amplitude (in mV). The red region of the iliofibularis muscle showed a distinct pattern of bursts of electrical activity at all speeds (Figs 1–3), and variables indicating activity during this burst begin with the letters BUR. For burst variables, TL=total activity during the red burst, MN=mean activity per 20 ms bin for all bins within a burst, and MX=the maximum activity observed for a single bin among all of the bins occurring during the burst. Variables indicating the total activity during an entire stride begin with STR, and variables indicating activity

per minute (=STR activity times strides per minute) begin with MIN. For example, in Fig. 1 and Table 3, the number of EMG spikes that occur per minute of muscle activity is abbreviated MINS, whereas BURMXAR indicates the maximum rectified integrated area observed for a 20 ms bin during the burst of red



Burst total	Burst mean/bin	Burst max/bin
(1) BURTLS	(4) BURMNS	(8) BURMXS
(2) BURTLSXA	(5) BURMNSXA	(9) BURMXSXA
(3) BURTLAR	(6) BURMNAR	(10) BURMXAR
(17) BURDUR	(7) BURMNAMP	

Stride total	Minute total (=stride total×strides min^{-1})
(11) STRS	(14) MINS
(12) STRSXA	(15) MINSXA
(13) STRAR	(16) MINAR

Fig. 1. Diagram summarizing the 17 EMG variables measured in this study. EMGs shown are from a single iliofibularis muscle of a lizard moving at 0.9 km h^{-1} at 25 °C. The horizontal lines indicate the time intervals of a single stride (with its swing and stance phases) and the bursts of red region activity. The five general categories of electromyographic variables are indicated and are based on the time intervals used to sum the bins of muscle activity. Variable names are entirely in capital letters and are numbered in order of their appearance in Tables 3 and 4. Variables 1–16 were calculated for both the red and white regions, whereas variable 17, the duration of the burst (BURDUR), was only determined for the red region. To deal with the irregular nature of white region activity at slow speeds, white region 'burst' variables were calculated over the same time interval as the red burst. Variables ending with S, SXA or AR are numbers of spikes, spike number times mean spike amplitude, and rectified integrated area, respectively. In contrast to other variables describing mean activity per bin within the burst (burst total/number of bins), mean spike amplitude (BURMNAMP) was determined as BURTLSXA/BURTLS. Maximum activity within a burst was the greatest amount of activity contained within a single bin. See Materials and methods for more details.

activity. The mean amplitude of spikes within a burst (BURMNAMP) was calculated as the mean of the amplitude of all spikes within a burst and this differs slightly from the use of mean values per bin within a burst for number of spikes (BURMNS), rectified integrated area (BURMNAR) and spike number times

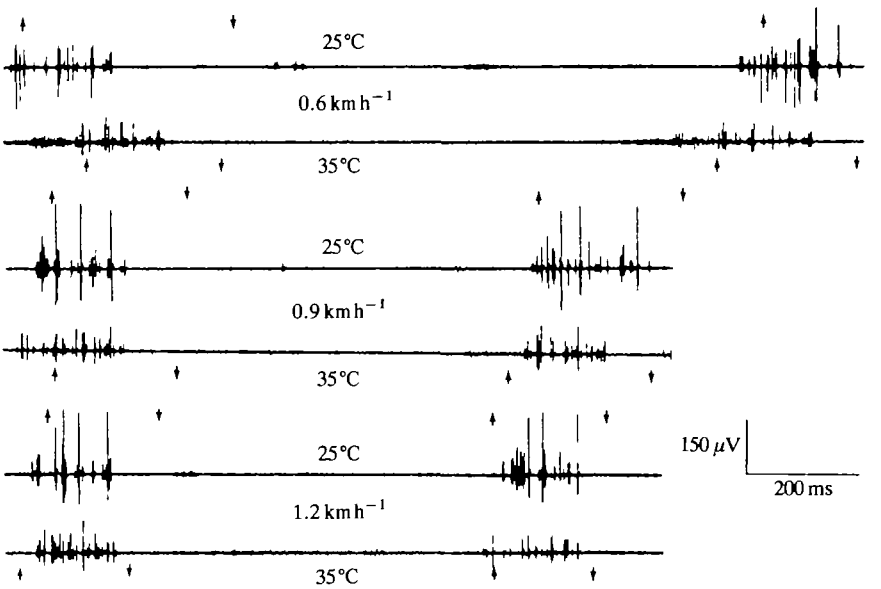


Fig. 2. Representative EMGs from a single electrode in the red region of the iliofibularis muscle at three speeds and two different temperatures. Arrows pointing up and down indicate the beginning and end of the swing phase of limb movement, respectively. The vertical and horizontal lines indicate the voltage and time scales, respectively.

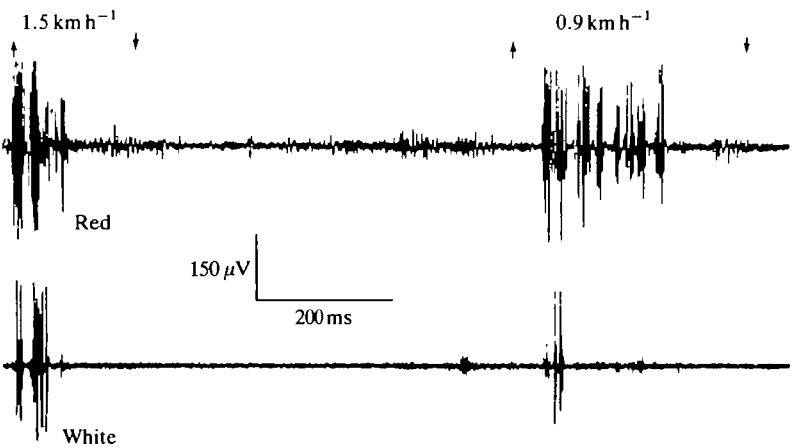


Fig. 3. Simultaneous EMGs from the red and white regions in a single iliofibularis muscle. Notation is as in Fig. 1. Note the greater similarity between activity in the red and white regions at the faster speed. These recordings are from a different individual from the one used for Fig. 2.

mean amplitude (BURMNSXA). It is helpful to note at this point that variables ending in SXA and AR are complex measures of muscle activity depending on *both* spike number *and* amplitude of spikes. In addition, rectified integrated area (AR) of the signal will be sensitive to the frequency characteristics of individual spikes, whereas spike number times mean amplitude (SXA) will not change with this aspect of individual spikes of voltage.

Statistical analysis

All statistical analyses were performed using a microcomputer version (SPSSPC+) of the Statistical Package for Social Sciences (SPSS). The primary method of testing for the effects of speed and temperature on kinematic and EMG variables involved a three-way analysis of variance using data from four individuals. In these three-way ANOVAs, we considered speed (five levels: 0.3, 0.6, 0.9, 1.2 and 1.5 km h^{-1}) and temperature (two levels: 25 and 35 °C) as fixed factors and individuals as a random factor. Following the guidelines in Zar (1984), the *F*-tests for significant effects of speed and temperature involved dividing the mean squares (MS) of the fixed effect by the two-way interaction term of the fixed and random effect. The MS of each two-way interaction term involving the random factor was divided by the residual MS to calculate the *F* value. In contrast, the speed×temperature interaction MS was divided by the three-way interaction term to test for significance. Identical sample sizes were used for the analysis of kinematic variables (Table 1) and EMG variables for the red region of the iliofibularis muscle. However, noise on one data channel recording white activity at the high temperature for one lizard resulted in missing values of EMG variables for speeds of 0.6–1.5 km h^{-1}; hence, the different degrees of freedom comparing Tables 3 and 4.

In addition to the use of analysis of variance for overall tests of significant temperature and speed effects, the cell means were examined to clarify the direction of change among the treatments. To facilitate explanation of these effects, graphs of variables are presented for a single lizard, including observations for faster speeds not included in the three-way ANOVA (see Figs 4–9).

Results

Kinematics

Table 1 summarizes the results of the three-way ANOVAs on kinematic variables, and Table 2 shows representative mean values for a single lizard. For each combination of temperature and treadmill speed, usually 3–5 strides per individual were used for the statistical analysis, and the ANOVA on mean forward speed (VX) confirmed that there were no significant differences for this variable between trials at different temperatures or among the different individuals (Table 1). Hence, the effects of temperature can be assessed without a confounding influence of speed. All the remaining seven kinematic variables had significant variation attributable to locomotor speed and to different individuals. Examin-

Table 2. *Means of kinematic variables for a single lizard at 25 and 35°C*

Speed ($km\ h^{-1}$)	Kinematic variable			
	DOWNDUR (s)	UPDUR (s)	STRLEN (cm)	STEPLEN (cm)
0.3	1.13/1.17	0.53/0.39	15.2/14.7	9.3/11.8
0.6	0.79/0.81	0.42/0.36	20.3/20.0	13.9/14.0
0.9	0.55/0.50	0.35/0.28	22.0/20.4	14.4/14.3
1.2	0.51/0.43	0.30/0.24	24.0/21.1	13.9/14.1
1.5	0.33/0.38	0.24/0.19	23.6/23.2	14.9/15.1

Sample sizes range from 2 to 6.
Values to the left and right of the slash are for 25 and 35°C, respectively.

ation of the cell means indicated that the individual differences in kinematic variables were consistent with the minor variation in size among the different lizards. For example, the smallest lizard had slightly smaller values for stride length and duration than the larger lizards. In contrast to the variation attributable to speed and different individuals, temperature had no significant effects on any kinematic variable (Table 1).

As shown in Fig. 4A, the duration of the swing phase (UPDUR) decreases curvilinearly as speed increases and both stride and stance phase duration (STRDUR, DOWNDUR) showed a similar pattern of decrease with increased speed. Speed significantly affected both step and stride length (Table 1). The shortest step length observed for each individual occurred at the slowest speed ($0.3\ km\ h^{-1}$, Table 2), but there were no systematic differences in step length among the remaining cell means of speeds ($0.6–1.5\ km\ h^{-1}$, Table 2) used in the analysis of variance. In contrast to step length, stride length increased over a wider range of increasing speed (from $0.3–1.5\ km\ h^{-1}$, Table 2). The step corresponds to the propulsive phase of a stride and, as indicated by the significant effect of speed on PUP, the proportion of time per cycle spent in the propulsive and recovery phases does not remain constant for all speeds. Instead, at the slowest speeds relatively more time per cycle is spent in the propulsive phase (low value of PUP). In addition, at the slowest speed the relative contribution of step length to stride length (STPSTR) is at its greatest. Thus, speed (from 0.3 to $1.5\ km\ h^{-1}$) at a single body temperature was modulated by changing both the frequency and the amplitude of limb excursions.

General pattern of muscle activity

As shown in Figs 1–3, the red region of the iliofibularis muscle displayed a periodic pattern of bursts that mostly occurred during the swing phase of limb movement. Visual inspection of the films indicated that the femur was being abducted and the knee was being flexed during these bursts of red region activity. Sometimes the red region also displayed a very short burst (<40 ms) of activity at

the moment of footfall, but the irregular occurrence of this activity did not lend itself to quantification. For the slowest speeds, often no activity was detected in the white region. As locomotor speed increased, the white region began to show an

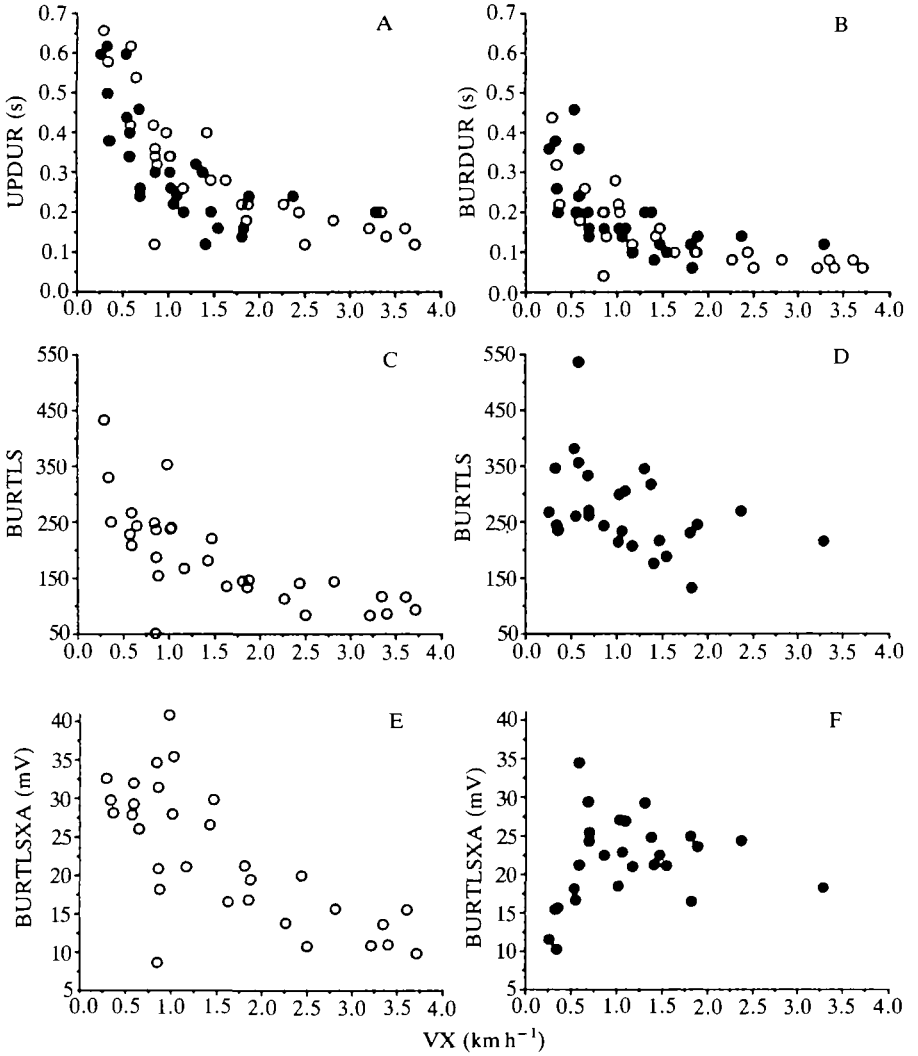


Fig. 4. Plots of kinematic and electromyographic variables *versus* mean forward velocity (VX) for 31 cycles of hindlimb movement at 25°C (open symbols) and 27 cycles of movement at 35°C (closed symbols) for a single individual of *Varanus exanthematicus*. (A) Duration of the swing phase of the left hindlimb *versus* VX. Electromyographic variables are for muscle activity recorded from a single electrode in the red region of the iliofibularis of the left leg. (B) Duration of the burst of red region activity (BURDUR). (C,D) Total numbers of spikes during the red burst (BURTLS). (E,F) Total spike number times mean spike amplitude during the red burst (BURTLSXA). See Fig. 1 and Materials and methods for a complete explanation of electromyographic variables.

increasingly clearer pattern of bursting activity that occurred within the time of the red region burst during the swing phase (Fig. 3). At intermediate speeds, although the burst of the white region overlapped with the burst of the red region, the duration of the white burst was often less than that of the red region (Fig. 3). It was also not uncommon for the white region to show some irregular low-level activity not corresponding to the time of the red burst of activity.

No activity was detected for the red or white regions of the iliofibularis when lizards were stationary with the abdomen either contacting or elevated above the substratum. In addition, when two lizards were placed on inclined surfaces with slopes varying from 0 to 90°, no postural muscle activity was detected.

As shown in Tables 3 and 4 many descriptors of the precise nature of muscle activity varied significantly with speed and temperature. With only one exception (duration of the red burst, BURDUR), all EMG variables had highly significant variation among different individuals (Tables 3 and 4). A MANOVA on the first and second principal components (which accounted for 87 % of the variance) of a

Table 3. *Summary of* F*-tests for significance of effects in separate three-way ANOVAs performed on each electromyographic variable for electrodes in the red region of the iliofibularis muscle*

	Effect						
Variable	Speed (4, 12)	Temp (1, 3)	Indiv (3, 116)	Speed ×Temp (4, 11)	Speed ×Indiv (12, 116)	Temp× Indiv (3, 116)	Speed× Temp× Indiv (11, 116)
BURTLS	0.5	1.7	69.7***	1.4	3.6***	2.5	1.2
BURTLSXA	1.9	12.4*	252.5***	0.8	0.8	2.8*	2.0*
BURTLAR	2.3	4.3	215.9***	1.4	0.5	29.0***	1.0
BURMNS	9.4**	0.3	69.5***	0.6	2.1**	10.3***	2.0*
BURMNSXA	5.6*	58.1**	180.5***	0.6	3.6***	0.4	1.7
BURMNAR	7.2**	8.6	287.9***	3.1	3.4***	20.7***	0.8
BURMNAMP	3.4*	18.8*	232.0***	1.4	4.2***	9.7***	1.3
BURMXS	5.7**	1.9	42.6***	0.9	2.0*	13.2***	1.9*
BURMXSXA	4.0*	150.1***	169.0***	0.5	2.8**	0.3	3.3***
BURMXAR	8.3**	9.2	325.3***	2.0	2.0*	39.4***	1.9*
STRS	0.2	1.6	107.5***	0.7	4.6***	13.7***	4.3***
STRSXA	2.0	16.4*	299.8***	0.9	1.4	3.3*	3.3***
STRAR	2.8	8.6	235.6***	1.0	0.7	9.1***	1.7
MINS	9.4**	0.7	202.9***	1.1	5.5***	55.6***	5.6***
MINSXA	11.1***	6.4	238.2***	1.1	5.7***	10.2***	1.9*
MINAR	10.3***	6.9	213.5***	3.2	4.1***	24.4***	1.0
BURDUR	12.3***	1.0	1.0	0.9	1.2	0.9	0.5

Degrees of freedom associated with each F statistic are given in parentheses below each effect. Significance levels of $F<0.05$, <0.01 and <0.001 are indicated by *, ** and ***, respectively. See Materials and methods and Fig. 1 for explanation of electromyographic variables. Temp, temperature; Indiv, individual.

Table 4. *Summary of* F*-tests for significance of effects in separate three-way ANOVAs performed on each electromyographic variable for electrodes in the white region of the iliofibularis muscle*

	Effect						
Variable	Speed (4, 12)	Temp (1, 3)	Indiv (3, 105)	Speed ×Temp (4, 71)	Speed ×Indiv (12, 105)	Temp× Indiv (3, 105)	Speed× Temp× Indiv (7, 105)
BURTLS	9.9***	12.2*	35.0***	2.3	1.8	3.3*	2.2
BURTLSXA	10.0***	39.0**	60.9***	2.8	3.0**	1.0	1.3
BURTLAR	10.7***	41.9**	41.5***	3.4	2.1*	0.8	1.2
BURMNS	24.5***	23.5*	24.0***	1.3	1.0	1.4	3.0*
BURMNSXA	5.7**	64.8**	28.8***	1.2	3.6***	0.3	1.4
BURMNAR	6.2**	57.0**	191.5***	1.6	24.4***	2.7	11.2***
BURMNAMP	4.6*	59.1**	31.4***	3.0	2.9**	0.5	1.3
BURMXS	26.0***	41.8**	33.3***	3.1	0.8	1.1	2.5
BURMXSXA	3.8*	112.8**	44.4***	4.3*	5.5***	0.2	0.6
BURMXAR	5.4**	129.3**	25.8***	5.3*	3.2***	0.2	0.6
STRS	4.4*	45.7**	13.2***	2.3	2.4**	0.6*	1.4
STRSXA	10.3***	57.9**	12.5***	2.6	1.1	0.4	1.0
STRAR	10.7***	40.4**	9.9***	3.0	0.8	0.4	0.7
MINS	8.1***	40.7**	103.6***	2.4	4.9***	0.9	0.9
MINSXA	4.4**	141.6**	100.5***	2.8	10.1***	0.3	0.6
MINAR	5.2*	150.4**	55.6***	3.2	6.2***	0.2	0.6

Degrees of freedom associated with each F statistic are given in parentheses below each effect. Significance levels of $F<0.05$, <0.01 and <0.001 are indicated by *, ** and ***, respectively. See Materials and methods and Fig. 1 for explanation of electromyographic variables. Temp, temperature; Indiv, individual.

set of 11 EMG variables indicated that overall there were significant differences attributable to fibre type (Wilks' lambda=0.012, F=80.5, P=0.012); however, to facilitate interpretation of the patterns of fibre recruitment, results for each fibre type were analyzed separately.

Analysis of electromyographic variables

We analyzed several different EMG variables because there were few precedents indicating which type of EMG variables are most useful for detecting the effects of temperature and speed on patterns of recruitment. The following descriptions of muscle activity are grouped into five categories of EMG variables that are based on the time interval used to measure activity (Fig. 1).

Total activity during the red burst

Table 3 shows that for the ANOVA on activity of the red region for forward speeds (VX) from 0.3 to 1.5 km h^{-1} none of the three EMG variables indicating the total activity per burst (BURTLS, BURTLSXA and BURTLAR) was affected

significantly by speed. However, the duration of bursts did vary significantly with speed (BURDUR, Table 3), decreasing curvilinearly with increasing speed (Fig. 4B) in a manner similar to that of the duration of the swing phase (Fig. 4A). Because burst duration decreased with increased speed, it is not too surprising that some of the total amounts of activity per burst at high speeds ($VX>1.5\,km\,h^{-1}$) were less than those at lower speeds (e.g. Fig. 4C,D,E). Furthermore, at 25°C the relationship between burst total activity and speed closely resembled that between burst duration and speed.

For the red region, the only total burst variable that showed a significant effect of temperature was spike number times mean amplitude (BURTLSXA, Table 3). Comparing the pattern of change in BURTLSXA with speed for the two temperatures (Fig. 4E *vs* 4F) reveals that the BURTLSXA initially increased steeply with speed at 35°C (Fig. 4F), whereas there was little change in this variable at 25°C for the same range in speed ($VX<0.9\,km\,h^{-1}$, Fig. 4E). Temperature did not significantly affect the duration of bursts in red region activity (Fig. 4B and Table 3).

Fig. 5 shows that different measures of total burst activity of the red region have significant positive correlations with each other at a single temperature, but temperature affects the relationship between any two variables. For example, for equal numbers of spikes per burst (BURTLS), the total rectified integrated area (BURTLAR) at 25°C was greater than that at 35°C (Fig. 5A). Similarly, BURTLAR at 25°C was greater than at 35°C when comparing bursts with similar BURTLSXA (Fig. 5B). Furthermore, using *t*-tests to compare the slopes of the regressions for the high- and low-temperature data revealed a significant effect of temperature on this statistic. The nature of these differences was consistent with the interpretation that the EMG bursts of the red region at lower temperatures had lower frequencies and greater amplitudes of individual spikes of voltage.

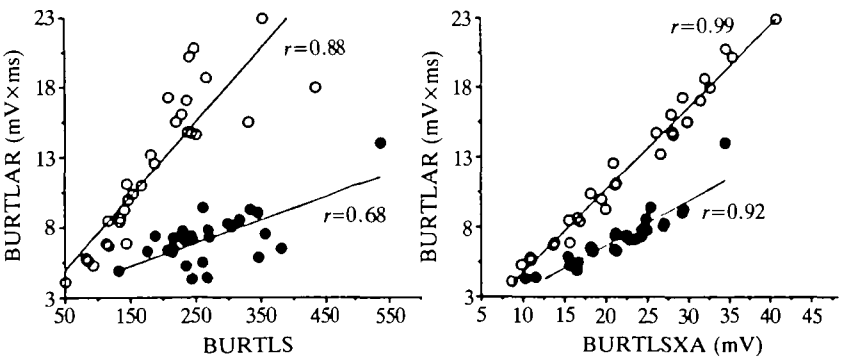


Fig. 5. For a single electrode, the correlations between different electromyographic variables measuring total activity of the burst for the red region of the iliofibularis muscle, for 31 cycles of hindlimb movement at 25°C (open symbols) and 27 cycles of movement at 35°C (closed symbols). (A) Rectified integrated area (BURTLAR) *versus* number of spikes (BURTLS). (B) BURTLAR *versus* spike number times mean spike amplitude (BURTLSXA).

As shown in Table 4, all measures of the total amount of white activity occurring during the red burst were significantly affected by both speed and temperature. For both temperatures these amounts of white activity were negligible at the slowest speeds and then increased with increased speed. For equal speed, the activity of the white region at 25°C was nearly always greater than that at 35°C, and the speed at which activity became detectable at 35°C exceeded that at 25°C.

Mean activity during the red muscle burst

Compared to the total amounts of activity during the red burst, the mean amounts of activity per bin within the time of the red burst give a better indication of the intensity of muscle activity. For the activity of the red region, all four measures of mean activity (BURMNS, BURMNSXA, BURMNAR and BURMNAMP) varied significantly with speed (Table 3). Mean spike amplitude (BURMNAMP) for the red region (Fig. 6A) showed an initial steep increase with speed up to a maximum, after which little change occurred with further increases in speed; this pattern of change with speed was similar to that for the other three measures of mean activity of the red region. Instead of mean spike amplitude changing simply as the mean of different normal distributions, Fig. 7 shows that increased mean spike amplitude resulted mainly from a greater proportion of large-amplitude spikes and from greater maximum amplitudes.

Mean spike amplitude (BURMNAMP) and the mean of spike number times mean spike amplitude per bin (BURMNSXA) were the only two measures of mean red region activity that were significantly affected by temperature (Table 3). The speed at which the largest values of BURMNAMP and BURMNSXA were attained was less at 25°C than at 35°C (Fig. 6A,C), and values at 25°C were usually greater than those at 35°C when comparing equal speeds of less than $1.5\,km\,h^{-1}$ (Figs 6A,C and 7).

As indicated in Table 4, all four variables measuring mean activity of the white region during the red burst were significantly affected by both speed and temperature. The mean amplitude for the spikes of activity from the white region generally increased with increasing speed (Fig. 6B), but the mean amplitude of white activity was always small compared to that of the red region (Fig. 6A *vs* 6B). At a given speed when mean measures of activity of the white region at 25°C and 35°C differed, values at 25°C exceeded those at 35°C.

In summary, for the red region, some mean measures of activity indicated that the intensity of activity increased with locomotor speed up to a maximum, and more intense activity was also associated with lower temperature. For the white region, mean measures of activity indicated greater intensity associated with both increased speed and decreased temperature.

Maximum activity during the red burst

Trends in the intensity of activity indicated by maximum per bin were generally similar to those of mean values per bin. Tables 3 and 4 show significant effects of speed on all three variables measuring maximal amount of activity per bin

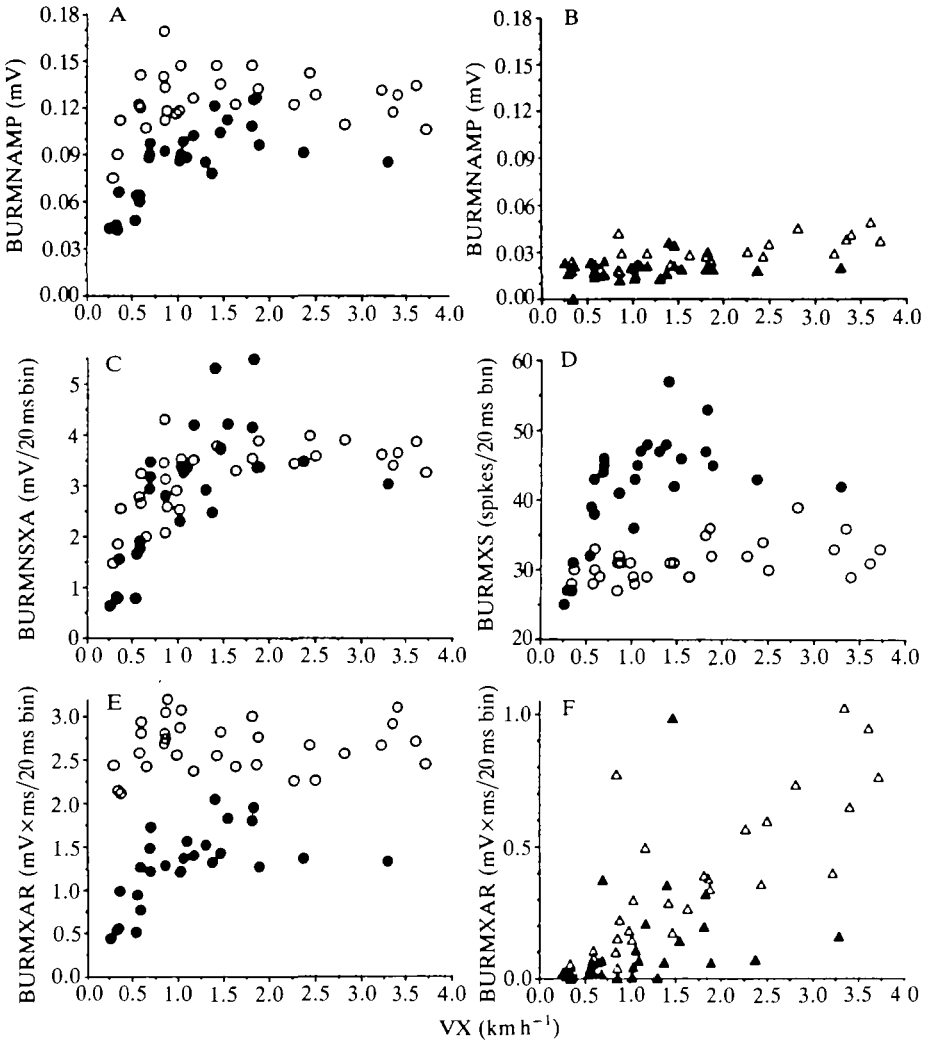


Fig. 6. For a single electrode in the red region (circles) and another electrode in the white region (triangles) of the same iliofibularis muscle, mean (A,B) and maximum (C–F) activities during the red region burst *versus* mean forward velocity (VX) for 31 cycles of hindlimb movement at 25°C (open symbols) and 27 cycles of movement at 35°C (closed symbols). (A,B) Mean spike amplitude (BURMNAMP). (C) Mean spike number times mean spike amplitude per bin (BURMNSXA). (D) Maximum number of spikes per bin (BURMXS). (E,F) Maximum rectified integrated area per bin (BURMXAR).

(BURMXS, BURMXSXA and BURMXAR) for both the red and white regions in addition to some significant effects of temperature on these three variables. For the red region, most of these variables initially increased with speed but showed little change past some threshold speed (Fig. 6D, 35°C and 6E). Maximal activity per bin for the white region generally increased with speed over a range of speeds

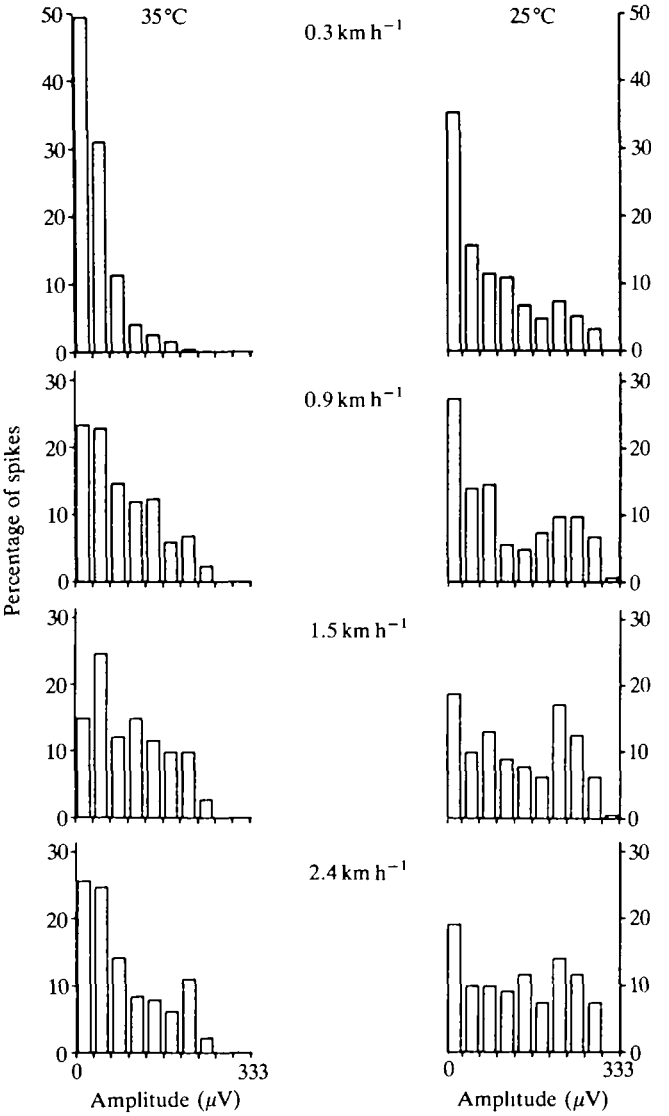


Fig. 7. For a single electrode in the red region of the iliofibularis muscle, the distributions of spike amplitudes for one burst of activity at four different speeds for both of the experimental temperatures. Note the higher proportion of large-amplitude spikes at the lower temperature and over certain ranges of increasing speed.

greater than that for which maximal activity of the red region increased (Fig. 6F *vs* 6E). Other than maximum numbers of spikes per bin for the red region (Fig. 6D), the measures of maximal activity per bin for both the red and the white regions at 25°C were usually greater than those at 35°C (Fig. 6E,F). For the red region in all the lizards, the greatest values of maximum number of spikes per bin (BURMXS) were observed at the higher of the two temperatures.

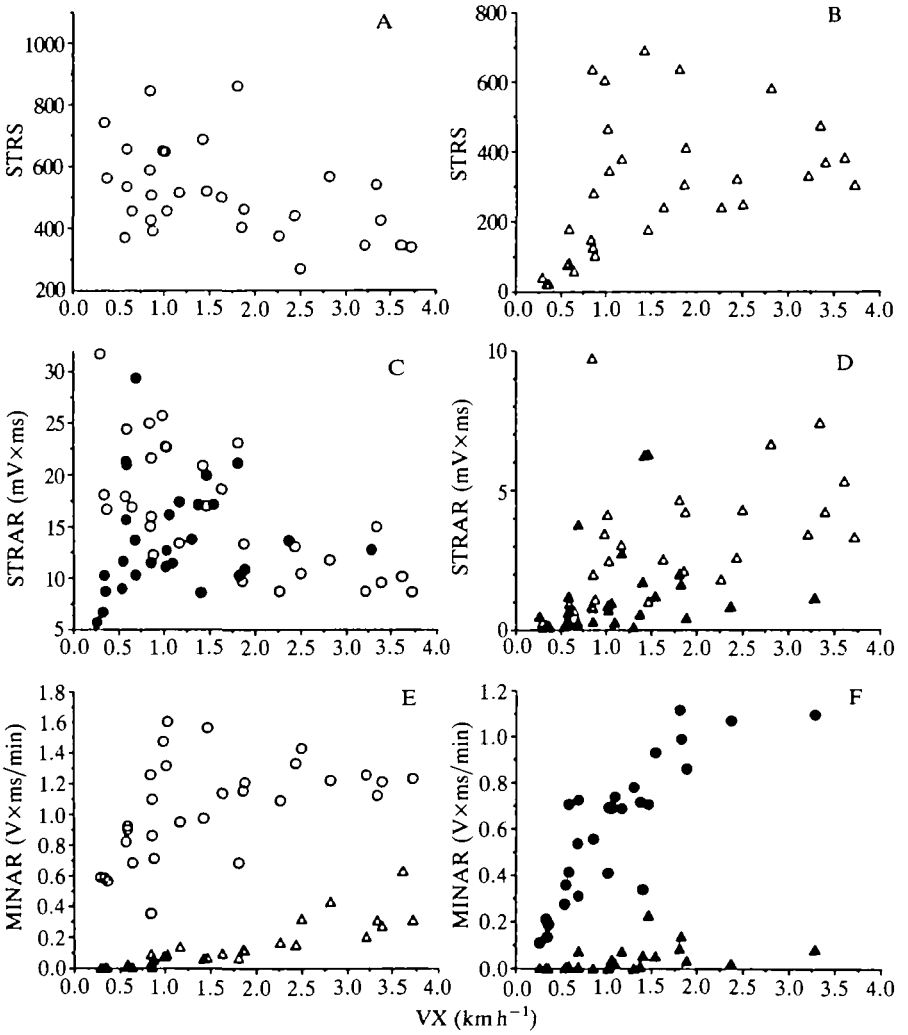


Fig. 8. For a single electrode in the red region (circles) and another electrode in the white region (triangles) of the same iliofibularis muscle, muscle activity per stride (A–D) and activity per minute (E,F) *versus* mean forward velocity (VX) for 31 cycles of hindlimb movement at 25°C (open symbols) and 27 cycles of movement at 35°C (closed symbols). (A,B) Spikes per stride (STRS). (C,D) Rectified integrated area per stride (STRAR). (E,F) Rectified integrated area per minute (MINAR).

Activity per stride

Although most of the muscle activity occurred during the burst of the red region, some additional irregular activity of both the red and white regions did occur at other times during the cycle of limb movement (Fig. 3). To account for this activity, the total activity per stride was determined for each of the EMG variables (STRS, STRSXA and STRAR). Despite the activity that occurred outside the time of the red burst, changes with speed and temperature in stride

total variables (Fig. 8A–D; Tables 3, 4) were similar to those of burst totals (Fig. 4C–F). Over a wide range in speeds at 25°C the activity per stride of the white region increased with increasing speed (Fig. 8B,D), whereas the same variables for the red region often decreased with increased speed because of decreased duration of activity (Fig. 8A,C). By accounting for all muscle activity per stride and multiplying by stride frequency we estimated total muscle activity per minute (Fig. 8, C *vs* E and D *vs* F).

Activity per minute

Because the energetic cost of locomotion is commonly measured per unit time, it is useful to calculate the amount of muscle activity per unit time since duration, intensity and frequency of muscle activity all may vary with speed. As indicated in Table 3, all measures of activity per minute (MINS, MINSXA and MINAR) of the red region varied significantly with speed. At both temperatures, MINAR of the red region increased over a wide range of increasing speed (Fig. 8E,F), and a similar pattern was shown by MINS and MINSXA. Although there was not a significant main effect of temperature for the ANOVA on any of the minute totals of red region activity for the data analyzed from 0.3 to 1.5 $km\,h^{-1}$, the plots of minute activity *versus* speed showed some consistent differences with temperature. For example, at 25°C the minute total activity of the red region showed little change as speed increased above about 0.9 $km\,h^{-1}$, whereas at 35°C these variables continued to increase for a greater range in values of speed (Fig. 8E *vs* 8F).

For the white region, all three measures of activity per minute showed significant main effects of both speed and temperature (Table 4). Fig. 8E,F illustrates the general pattern of change with speed and temperature that occurred for these variables. At low speeds, activity per minute of the white region was negligible and, above a certain threshold speed, white region activity increased over the entire observed range of increasing speed. In addition, the speed at which activity of the white region began to increase was greater at 35°C than at 25°C (Fig. 8E *vs* 8F).

Discussion

Evaluation of electromyographic variables

Intuitively one would expect that increases in speed result from 'increased muscle activity'. However, a finding of central importance in our study is that clearer insight into patterns of recruitment is gained when 'increased muscle activity' is differentiated more precisely into: (1) variables describing the total amount of muscle activity per burst or cycle of movement, (2) the cumulative amount of activity per unit time, and (3) the intensity of muscle activity within a single burst.

In our study, variables describing total activity per burst (BURTLS, BURTLSXA and BURTLAR) were not very useful because of simultaneous

decreases in burst duration and increases in intensity that occurred as speed increased. Similarly, variables describing total activity per stride (STRS, STRSXA and STRAR) were minimally informative by themselves, but when these quantities were multiplied by strides min^{-1}, the resulting variables describing activity min^{-1} (MINS, MINSXA and MINAR) did reveal some interesting relationships with speed. Determining the amount of activity per stride was also helpful for quantifying the amount of activity in the white region, which was often irregular compared to the discrete bursts shown by the red region (Fig. 3). Variables quantifying mean (BURMNS, BURMNSXA, BURMNAR and BURMNAMP) and maximum (BURMXS, BURMXSXA and BURMXAR) activity were the most useful indicators of the intensity of muscle activity. To some extent mean and maximum measures of activity were redundant. However, simultaneously accounting for mean and maximum activity per bin within a burst revealed that the bursts of activity for our particular preparation were often not of uniform intensity.

As discussed in recent reviews of methodology for electromyography (Basmajian and De Luca, 1985; Loeb and Gans, 1986), rectified integrated area and spike number times mean spike amplitude are composite measures of activity that both involve the amplitude and number of spikes in a signal. In practice, it would appear to make little difference which one of these two variables was used because of the highly significant correlation that we found between these two quantities at a single temperature (Fig. 5). However, because we wanted to document thoroughly the effects of body temperature on EMGs, we analyzed both these variables and found that the relationship between them was temperature dependent. In our study, we found that the use of a variable with numbers of spikes in combination with either spike number times mean amplitude or rectified integrated area was a powerful combination of variables for determining different patterns of activity.

Expected patterns of recruitment

Like the force–velocity curves for other vertebrate muscles (Hill, 1970), the iliofibularis muscle of lizards has a decreasing speed of shortening as load increases, and the speed of shortening at a given load decreases with decreasing muscle temperature (Marsh and Bennett, 1986*a*). The activity observed for the iliofibularis muscle of *Varanus exanthematicus* in this study occurred during flexion of the knee and abduction of the femur, suggesting that the muscle shortens when it is active. Because no kinematic variables of *Varanus exanthematicus* were significantly affected by temperature (Table 1), the speed of limb movement and iliofibularis shortening at a single locomotor speed appear not to have been affected by temperature. Therefore, at lower body temperatures, some functional compensation must occur to overcome the inhibiting effect of low temperature on muscle mechanics. If a muscle fibre shortens at equal rates at two temperatures, its force output is less at the lower temperature. To achieve equal levels of force in the entire muscle at both temperatures, one compensatory mechanism would be the

activation of more motor units at the lower body temperature (reviewed in Rome, 1986). This pattern of recruitment would permit temperature-independent force production in a muscle and equal speeds of contraction, albeit with a lower force per fibre at the lower temperature. What evidence do we have for such a pattern?

In view of the widespread effects of temperature and speed on the EMG variables measured in this study, it is useful to consider some mechanisms that could affect these variables. Two broad categories of mechanisms expected to influence the EMG variables are passive changes in the electrical properties of the muscle and patterns of recruitment used by the animal to activate the muscle. Basmajian and DeLuca (1985) provide a useful review of patterns of muscle recruitment that affect the EMGs of vertebrate twitch muscle, which are a complex pattern of voltage generally resulting from both repetitive firing of individual motor unit action potentials (MUAPs) and several MUAPs from different motor units. Mechanisms to compensate for the lower rates of force production and shortening at lower temperature could involve: (1) a higher rate of repetitive activation of an individual motor unit, (2) increased numbers of motor units, (3) increased synchronization of motor units, or (4) increased duration of activity using a particular pattern of recruitment. The first of these mechanisms should be detected as an increased number of spikes in the EMG, the second mechanism could increase both the numbers and amplitudes of spikes, the third mechanism could increase EMG amplitude and decrease the frequency characteristics of the signal, and the fourth mechanism should increase the total activity per burst. For the passive electrical properties of single fibres from the iliofibularis muscle of lizards, Adams (1987) found that decreasing temperature increases the time constant and apparent membrane resistance. Consequently, one would expect that a MUAP at decreased temperature would have a longer duration and lower frequency characteristics than at high temperature, and these changes in individual MUAPs should cause an increase in amplitude and a decrease in the numbers of spikes observed in EMGs at a lower temperature. These changes in the EMGs predicted from changes in passive electrical properties should have a similar effect across all speeds when comparing two temperatures. Hence, to distinguish compensatory patterns of recruitment at a lower temperature from passive effects, one should concentrate on differences in EMGs that cannot be attributed simply to passive properties.

Observed patterns of recruitment

At the level of the whole muscle, the fact that white plus red region activity was observed at a lower speed at 25°C than at 35°C (Figs 8 and 9) suggests that a greater number of fibres were recruited to compensate for decreased temperature. However, because only the red region was used during slow locomotion, it is worthwhile to examine in more detail whether this region also displayed compensatory recruitment for the lower temperature during slow speeds.

For equal locomotor speed, the EMGs of the red region observed at 25°C consistently had greater spike amplitude than those at 35°C (Figs 2, 6A and 7) and

the relationships, shown in Fig. 5, of total rectified integrated area per burst (BURTLAR) to numbers of spikes per burst (BURTLS) and total spike number times mean spike amplitude (BURTLSXA) indicated that individual spikes of voltage in the EMG had lower frequency components. These results are consistent with changes in EMG variables that could be caused simply by differences in the passive electrical properties.

Other aspects of the increase in amplitude variables, such as the observed increase with speed within each of the experimental temperatures (Figs 2, 6A and 7), do suggest compensatory recruitment within the red region for lower temperature. As shown in Fig. 6A, the relationship between mean amplitude of spikes in a burst (BURMNAMP) and speed at 25 °C cannot be obtained simply by applying a constant correction to the curve for 35 °C and for the different amplitude attributable to passive electrical properties. Instead, mean spike amplitude (BURMNAMP) at 25 °C increased with speed to a maximum at about $1\,km\,h^{-1}$, whereas at 35 °C this variable increased with speed to about $2\,km\,h^{-1}$. Hence, based on mean spike amplitude (BURMNAMP), one mechanism that compensated for low temperature involved recruiting the red region maximally at slower speed than at higher temperature.

For speeds of less than $1\,km\,h^{-1}$, based only on mean spike amplitude (BURMNAMP) of the red region, it is not clear whether the greater values at 25 °C reflect passive electrical properties, different patterns of recruitment, or both. However, examining other variables helps to clarify whether there was detectable compensatory recruitment for low temperature when speed is less than $1\,km\,h^{-1}$. In three of the four individuals, the cell mean of mean number of spikes per bin within a burst (BURMNS) for $0.9\,km\,h^{-1}$ at 25 °C was greater than that at 35 °C, a result opposite to that expected for the influence of temperature on the passive electrical properties. Consequently, mean number of spikes (BURMNS) suggests that recruitment of the red region at some lower speeds was more intense at 25 °C than at 35 °C as a result of either greater numbers of active motor units or greater frequency of motor unit stimulation.

Because there was no *a priori* reason to assume that the stimulation of muscle is uniform during a burst of activity, maximal activity per bin was determined in addition to the mean activity per bin. Different patterns of change between maximum and mean variables suggest that stimulation of the muscle during a burst is heterogeneous. For maximum number of spikes per bin within the burst (BURMXS) of the red region at 25 °C (Fig. 6D) there was effectively no increase with speed, whereas mean number of spikes per bin (BURMNS) clearly increased with speed to about $1\,km\,h^{-1}$. Similarly, for each lizard at 25 °C the two other measures of mean activity (BURMNSXA, Fig. 6C and BURMNAR) generally increased over a greater range of speed than the respective maximum measures (BURMXSXA and BURMXAR) of the red region. These relationships suggest that at 25 °C and slower speeds the burst of red region activity is heterogeneous, with a brief period of maximal muscle stimulation. However, as speed increases above about $1\,km\,h^{-1}$, the red region is maximally stimulated for the entire

duration of the burst, causing the burst total activity (e.g. Fig. 4E) to change with speed in the same fashion as does burst duration (Fig. 4B). This finding of maximal stimulation of a region of a muscle at submaximal speed has not been reported previously for the locomotion of ectothermic vertebrates.

To summarize, the observed patterns of activity suggest the following sequence of muscle recruitment as speed increases at a single temperature. At the slowest speed only the red region is used, and the intensity of its activity is sub-maximal. Speed is increased by increasing the intensity of the bursts of red activity. Near the speed at which the maximal intensity of red activity is attained, white activity begins. The intensity of white activity then continues to increase with further increases in speed, while the red region continues to be used with maximal intensity. Decreasing temperature appears simply to decrease the speed at which any of the events described above occurs (=compression of recruitment order, Rome, 1986). If a lizard were cold enough, patterns of recruitment listed first in this sequence might not be observed, and decreasing temperature further would probably increase the number of initial steps in the sequence of recruitment that were not used by the animal. Of the four mechanisms for compensating for decreased temperature mentioned earlier, only increased duration of activity per burst can be ruled out definitively. However, whenever the red region displays maximal intensity of activity, the addition of white activity must indicate the recruitment of additional fibres (motor units). Hence, it is tempting to conclude that the increased intensity of activity detected from a single electrode also mainly reflects the recruitment of additional motor units, and that this is the primary mechanism for increasing speed and compensating for the effects of temperature.

Comparisons with other studies

Mutungi (1990) recently characterized the muscle fibre types in the iliofibularis muscle of *Varanus exanthematicus* by examining the histochemistry, innervation, capillary density and mitochondrial volume. The muscle can be divided grossly into an inner red region and an outer white region, which comprise 75 % and 25 % of the cross-sectional area of the muscle, respectively. The outer white region has 80 % fast glycolytic (FG) fibres, whereas the red region has 45 % fast oxidative glycolytic (FOG), 40 % slow oxidative (SO) and 15 % tonic fibres. The tonic fibres in the red region tend to be concentrated nearest the femur (G. Mutungi, personal communication), and this very deep location near the bone was the site of implantation for the electrodes used in our study. On average, the red region has six times the mitochondrial volume and 4.5 times the capillary density of the white region (Mutungi, 1990). The proportion of the iliofibularis muscle consisting of the red region reported for *Varanus exanthematicus* is about three times the values reported for the lizards *Dipsosaurus dorsalis* (Gleeson *et al.* 1980*b*) and *Varanus salvator* (Gleeson, 1983). The differing compositions of the iliofibularis between these species correspond well to relative differences in their aerobic scopes.

Although the electrodes in the red region of the iliofibularis in our study appeared to be in a location with a high proportion of tonic fibres, no postural

activity was detected for the iliofibularis muscle, contrary to the predictions of Putnam *et al.* (1980). It is possible, however, that tonic fibres do play some postural role. Carrier (1989) recently performed an electromyographic study of the activity of tonic fibres of the intercostal muscles in lizards, and he found that most of the power of the signal was below 100 Hz. To reduce motion artefacts and 60 Hz noise in our study, we used a 60 Hz notch filter and high band pass filters which severely reduced the amplitude of such low-frequency signals; this may explain the lack of postural activity observed for the deep red region of the iliofibularis.

Like the iliofibularis muscle of lizards, the red region of fish myotomal muscle is characterized by having a greater capacity for aerobic activity than the white region (Bone, 1978; Johnston, 1985). Several electromyographic studies on the myotomal muscle activity during the swimming of fish have shown clearly that the red region is active at all swimming speeds, whereas there is a complete absence of activity in the white region until a certain threshold speed is attained (Bone, 1966; Rayner and Keenan, 1967; Hudson, 1973; Bone *et al.* 1978; Johnston and Moon, 1980; Rome *et al.* 1984). Johnston and Moon (1980) specifically reported an increase in EMG amplitude with speed for the red region. Other studies of fish swimming (Bone, 1966; Rayner and Keenan, 1967; Hudson, 1973; Bone *et al.* 1978; Rome *et al.* 1984) included illustrations of EMGs that also indicated that the amplitude and numbers of spikes commonly increased with a wide range of increase in speed and, at intermediate speeds, the EMGs of white muscle were often irregular compared to those of the red muscle. Rome *et al.* (1984) found that decreased temperature decreased the threshold speed at which white muscle was recruited; however, the extent of change in the intensity of red activity with temperature was not clear. Hence, the findings of our study are very similar to those on fish locomotion, although our quantitative approach to EMG analysis permits finer differentiation of the pattern of fibre type utilization.

Based mainly on studies of fish locomotion, Rome (1986) suggested a general pattern of muscle recruitment for ectotherms moving over a range of different speeds and temperatures. Three key points in this scheme of muscle recruitment are: (1) at a single temperature, animals increase locomotor speed by using all of the motor units that are used at a slower speed plus additional motor units necessary for increasing power, (2) all the aerobic fibres are recruited before any anaerobic fibres as speed increases, and (3) with decreased temperature the speed at which a particular motor unit is recruited is lower, but the order in which motor units are recruited with increased speed is preserved (compression of recruitment order). Points 1 and 3 are generally supported by our experimental observations on the activity of the iliofibularis in *Varanus*. Our observations for *Varanus* also suggest that increased intensity of activity in the red region alone can be sufficient to compensate for decreased temperature at slow speeds, but we cannot clearly distinguish increased rate of use of motor units from increased numbers of motor units. An implication from point 2 is that the red region would show no increase in activity above some speed, and our electromyographic observations included a range of speeds over which no further increases in red activity occurred (Figs 6, 8,

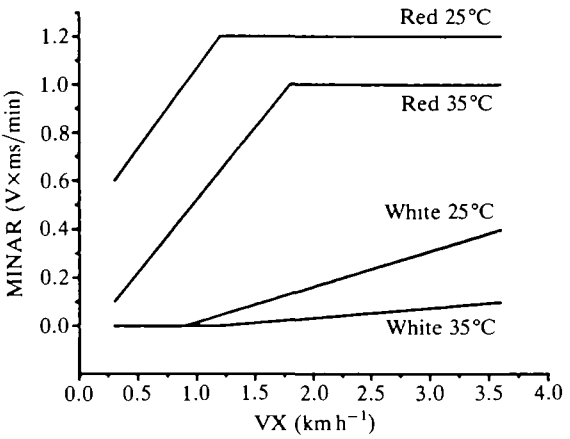


Fig. 9. Schematic summary of activity (rectified integrated area) of the iliofibularis muscle per minute for the red and white regions at 25 and 35 °C. Note ranges of speed over which activity is constant.

9). Finally, our observation that white region activity occasionally occurred during submaximal intensity of red activity (Fig. 8F) suggests that point 2 may be too simplistic.

The amount of muscle activity per unit time is particularly relevant for relating the activity of aerobic and anaerobic fibres to the aerobic cost of transport. Fig. 9 gives a schematic summary of the amounts of red and white region activity per minute at the two temperatures used in this study. At both temperatures, red activity increases with speed until a maximum is attained, and white activity begins at a lower speed than that of maximal red activity and continues to increase with further increases in speed. At the lower temperature there is more activity of both the white and red regions. In part, the greater activity observed at the lower temperature results from changing passive electrical properties, but, as discussed above, this increase also appears to reflect more intense recruitment. The aerobic cost of transport has been documented for a variety of lizard species, including *Varanus exanthematicus* (e.g. Moberly, 1968; Gleeson *et al.* 1980*a*; John-Alder *et al.* 1983; Bennett and John-Alder, 1984), and without exception oxygen consumption increases with speed until a maximum is attained, at which point (=maximum aerobic speed) oxygen consumption remains constant ($V_{O_2,max}$) as speed increases further. This apparent saturation of oxygen transport capacities agrees well with the patterns of muscle activity observed in our study (Figs 8 and 9), which have threshold speed above (red) or below (white) which no change in the amount of activity occurred. For *Varanus exanthematicus* which were about twice the mass of those used in our study, Gleeson *et al.* (1980*a*) reported a maximal aerobic speed (MAS) of 1.2 km h^{-1} at 35 °C. This value of MAS is slightly less than the speed (about 1.7 km h^{-1}) at which maximum activity per minute of the red region occurred at 35 °C, and it is nearly identical to the threshold speed (about 1.3 km h^{-1}) for recruitment of the white region (Figs 8F and 9). The MAS

of lizards decreases with decreasing body temperature (Bennett and John-Alder, 1984), and in our study the threshold speed for white recruitment and maximal red recruitment also decreased with decreased temperature.

For *Varanus exanthematicus* data on such diverse topics as the energetic cost (Gleeson *et al.* 1980*a*), the muscle physiology (Mutungi, 1990), the influence of temperature on physiology (Rome, 1982) and the neuromuscular modulation (this study) involved in a single behaviour generally form a very cohesive framework for understanding the physiology of terrestrial ectothermic locomotion. One seemingly puzzling result discussed by Rome (1982) was that the energetic cost of locomotion in *V. exanthematicus* was independent of temperature, whereas Rome (1982) expected that a higher energetic cost of producing isometric force in isolated muscle at higher temperature could increase the cost of locomotion. Our experimental observations of greater *in vivo* muscle activity in *Varanus* at a lower temperature suggest a factor that could actually increase the cost of locomotion at lower temperature or at least offset the effect of temperature on the cost of isometric force production. It is presently difficult to predict the effects of temperature on the energetic cost of locomotion based on its effects on isolated, stimulated muscle, and the unknown nature of length changes during muscle activity further complicates matters. Future studies should clarify if the influence of temperature on the energetic cost of locomotion in terrestrial ectotherms is minor, hence difficult to detect experimentally, and precisely how the cost of simultaneously using more motor units at low temperatures compares with other effects of temperature on the energetics of muscle contraction.

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