

## Thermal adaptations in lizard muscle function

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**Summary.** This study was undertaken to investigate thermal adaptations in muscle contractile properties in closely-related lizards with different preferred body temperatures (PBT). The species examined all belong to the *Sphenomorphus* group of Australian skinks (Scincidae: Lygosominae). Preferred body temperatures are conservative at the generic level as follows: *Ctenotus*, 35 °C; *Sphenomorphus*, 30 °C; *Eremiascincus*, 25 °C. Contractile properties of the fast glycolytic portion of the iliofibularis muscle were measured. Translational adaptations are evident in several isometric factors, including tetanic tension ( $P_o$ ), twitch tension ( $P_t$ ), twitch time to peak tension (TPT), and twitch half-relaxation time ( $1/2 RT$ ). Capacity adaptations are not evident in rates of tetanic tension development ( $dP_o/dt$ ) or in maximal velocities of isotonic shortening ( $V_{max}$ ). Rotational adaptations are not evident in any contractile properties. Thermal limits on upper response temperatures are about 5 °C warmer in *Ctenotus* than in the more cryophilic species, indicative of resistance adaptation in muscle performance. Despite these adaptive shifts, there is little indication that muscle functional capacities are optimized or equalized at PBT in these lizards.

### Introduction

Following the important discovery that many species of reptiles behaviorally regulate characteristic

*Abbreviations:* FG fast glycolytic; IF iliofibularis muscle; PBT preferred body temperature;  $P_o$  tetanic tension;  $P_t$  twitch tension;  $1/2 RT$  twitch half relaxation time; TPT twitch time to peak tension

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preferred body temperatures (Cowles and Bogert 1944), there has been considerable interest in the relationship between thermal preference (preferred body temperature = PBT) and thermal optima of physiological functions (Dawson 1975; Huey 1982). This is an issue of particular interest in lizards because PBTs in this group vary by as much as 20 °C intergenerically (Brattstrom 1965; Bennett and John-Alder 1986). It is now generally recognized that differences in thermal preference are correlated with differences in thermal optima of different physiological processes and in critical thermal limits (e.g. Huey 1982). However, it is not clear that physiological or behavioral processes are in fact optimized at PBTs. Optimality in this context is judged to be the most rapid rate or greatest quantity of which a physiological or behavioral process is capable at any temperature. The issue in question is whether animals are designed to function best at temperatures that they normally experience.

Locomotor capacities (e.g., burst speeds, endurance) are of particular relevance to this question, as they set limits on the entire behavioral repertoire of an animal (Bennett 1980b). The thermal dependence of these has been examined in several lizards (Bennett 1980a; Hertz et al. 1982, 1983; Bennett and John-Alder 1984). Lizards do not invariably run best at their PBTs, particularly if these are low; for example, PBT in *Gerrhonotus multicarinatus* is about 25 °C, but its burst speed is greatest at 37–38 °C (Bennett 1980a). In this case choice of a low body temperature results in sub-optimal behavioral capacity under field conditions. Does activity with low body temperature always result in submaximal performance, or do organisms adapt their physiological capacities in compensation for the effects of cold?

Thermal adaptations in behavioral or physiological processes of lizards with different PBTs can be identified by comparing thermal performance

curves. These are graphical presentations of the rate or capacity of a process over a broad range of temperatures (Huey 1982). Capacity adaptations indicate that functional levels of some process in a range of normally experienced body temperatures are compensated for differences in PBT (Prosser 1973, pp. 373–386). Rotational capacity adaptations are seen as differences in the slopes of thermal performance curves and can indicate a lower thermal dependence of some process in cryophilic species. Translational capacity adaptations are seen as proportional shifts in thermal performance over the entire thermal range and can indicate that constant functional levels of some process are attained at lower temperatures in cryophilic species than in thermophilic species (Precht et al. 1973, pp. 331–352; Prosser 1973, pp. 373–386). Resistance adaptations are seen as adjustments in the upper or lower limits of thermal performance and indicate differences in thermal ranges within which a biological process is responsive (Precht et al. 1973, pp. 440–442).

Thermal performance of muscle contractile properties has previously been compared among several species of lizards with dissimilar PBTs (Licht 1964; Licht et al. 1969; Putnam and Bennett 1982). These data demonstrate clear differences in upper thermal limits on contractile responses, indicative of pronounced resistance adaptations, and suggest that capacity adaptations may also be present. However, phylogenetic dissimilarity and differences in body size among species complicate an interpretation of the data as reflecting specific adaptations associated with PBTs.

In the present study, we examine interspecific correlations between PBTs and thermal performance for isometric and isotonic muscle contractile properties of some closely related Australian lizards. There are 8 species representing 3 genera in this study, and all are in the *Sphenomorphus* group of the scincid subfamily *Lygosominae* (Greer 1979). The close relatedness of these species gives a very high degree of phylogenetic similarity and strengthens the argument that differences in thermal performance reflect adaptations related to PBTs (Huey and Bennett 1986).

The thermal biology of the lizards examined in this study has been well-characterized under natural and laboratory conditions (Spellerberg 1972a, b, c, d; Bennett and John-Alder 1986), and their natural history has been described previously (Greer 1976; Cogger 1983). Their PBTs are conservative at the generic level within one degree Celsius (Bennett and John-Alder 1986). *Ctenotus* spp. are semi-arid adapted, relatively thermophilic skinks

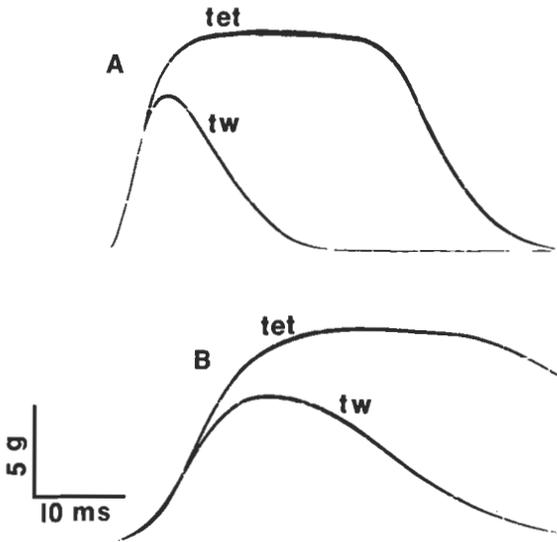
with PBTs of 35 °C (range of experimental species = 34.4 to 35.6 °C). *Ctenotus* spp. are diurnally active and are ecologically similar to small teiids and lacertids. *Sphenomorphus* spp. are large-bodied, aggressive water skinks. The common name of this genus derives from the moist habitats in which many are found and a common defense behavior involving submergence under water. Preferred body temperatures of *Sphenomorphus* spp. are about 30 °C (range of experimental species = 28.8 to 29.8 °C). *Eremiascincus fasciolatus* is a desert-dwelling, nocturnally-active sand swimmer. Despite its occurrence in some of Australia's hottest interior areas, its nocturnal PBT is only 24.4 °C for all individuals, both submerged and emerged. Emergent animals have an average PBT of 28.0 °C (Bennett and John-Alder 1986).

## Materials and methods

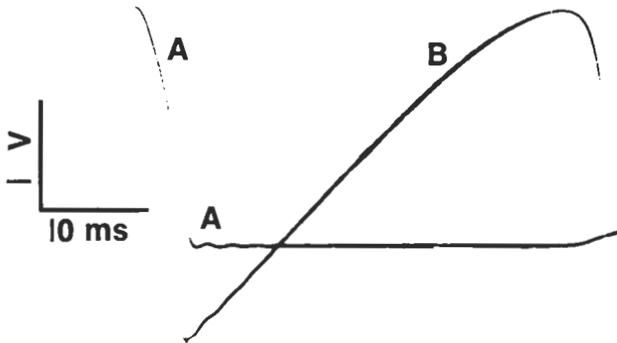
**Animal procurement and maintenance.** All animals were collected during the austral summer of 1983–4 and were housed at the University of Adelaide in South Australia under conditions allowing daily behavioral thermoregulation on a natural photoperiod. Details of collection locations and dates have been reported previously (Bennett and John-Alder 1986). Animals were collected under South Australian National Parks and Wildlife Permit No. 918 and New South Wales National Parks and Wildlife Permit No. A216 to AFB. Average body masses (grams) of animals were as follows (mean  $\pm$  1 SE [n]): *Ctenotus regius*: 6.1  $\pm$  0.1 (6); *C. robustus*: 13.1  $\pm$  1.5 (6); *C. taeniolatus*: 6.0  $\pm$  0.5 (6); *C. uber*: 6.2  $\pm$  0.2 (7); *Eremiascincus fasciolatus*: 16.0  $\pm$  0.8 (8); *Sphenomorphus kosciuskoi*: 10.3  $\pm$  1.0 (7); *S. quoyi*: 26.9  $\pm$  1.5 (6); *S. tympanum*: 17.9  $\pm$  1.2 (6). Animals were maintained primarily on twice-weekly feedings of mealworms that were occasionally supplemented with locally-caught termites. Water was available at all times. *Sphenomorphus* spp. were provided with a moist substrate enabling them to submerge themselves or to remain dry. Animals were held in the laboratory for no longer than 5 months. Voucher specimens have been deposited at the South Australian Museum.

**Muscle preparation.** Animals were confined in collecting bags overnight before experimentation and were weighed before being sacrificed by decapitation. Muscles were prepared by a procedure adapted from Marsh and Bennett (1985). The resting length of the iliofibularis muscle (IF) was measured in situ in legs bent 90 degrees at the knee. The distal tendon of the IF was tied with 5-0 silk and the muscle was removed along with half of the pelvic girdle (the proximal insertion). All muscles other than the IF were removed from the pelvic girdle, and a stainless steel chain was connected to the distal tie. Only the fast-glycolytic (FG) region (Gleeson et al. 1980) was used in experiments; the remaining fibers of the IF were cut using iridectomy scissors. The isolated muscle was kept moist with oxygenated Ringer's at all times.

The muscle was mounted in a chamber similar to that described in Putnam and Bennett (1982). The pelvis was attached to a stationary steel bar using 3-0 or 1-0 silk, depending on the size of the preparation. The distal end was attached via the stainless steel chain to the motor arm of a Cambridge Instruments Model 300H servo-controlled muscle ergometer. The force output of the ergometer was checked with an independent



**Fig. 1 A, B.** Isometric tetanic (tet) and post-tetanic twitch (tw) contractions at 40 °C (A) and 25 °C (B) of the white (FG) iliofibularis from a *Ctenotus robustus*. A:  $P_o = 11.55$  g (1505 g/cm<sup>2</sup>),  $dP_o/dt = 2273$  g/s [29.05 kN/(m<sup>2</sup>·ms)],  $P_t = 8.2$  g,  $TPT = 6.25$  ms,  $1/2 RT = 6.50$  ms; B:  $P_o = 11.55$  g,  $dP_o/dt = 1135$  g/s [14.52 kN/(m<sup>2</sup>·ms)],  $P_t = 7.9$  g,  $TPT = 15.5$  ms, and  $1/2 RT = 15.75$  ms



**Fig. 2 A, B.** Isotonic contraction at 30 °C of the white (FG) iliofibularis from a *Sphenomorphus quoyi*. A Force; 5 g/V, 10.85 g, 50.0%  $P_o$ . B Position; 0.5 mm/V. Force development (A) is limited to a pre-determined level by the ergometer. Shortening of the muscle, as indicated by the change in position (B), occurs when this force level is achieved by the contracting muscle

force transducer (Pixie, model 8101) and was found to monitor force displacements accurately at least up to 500 Hz. The transducer was mounted on a stand that eliminated vibration and allowed accurate adjustments of muscle length. Muscle temperature was maintained by submersion in a recirculating flow of oxygenated Ringer's solution from a 500 ml reservoir in a water bath. The Ringer's solution contained 145 mM NaCl, 4 mM KCl, 20 mM imidazole, 2.5 mM CaCl<sub>2</sub> and 11 mM glucose. The pH of this solution was adjusted to 7.5 at room temperature using HCl. Variations in pH were reported previously to be 7.4 at 44 °C to 7.8 at 15 °C (Marsh and Bennett 1985). One muscle preparation from each species was submerged in Ringer's solution containing acetylcholine (1 mg/ml). The absence of any sustained contractile response was taken

as verification that all functional fibers were twitch fibers (see Gleeson et al. 1980).

**Stimulation protocol.** Muscles were stimulated via two platinum plate electrodes placed on opposite sides of the muscle. Supramaximal stimuli consisted of 0.5 ms pulses produced by a Grass S-48 stimulator and amplified with a D.C.-coupled power amplifier from Southwest Technology Inc. The wave-form of the square pulses was not modified by the amplifier. Stimulation frequencies during tetanic contractions were 300/s. This frequency produced smooth tetani at all temperatures. Lower frequencies at low temperatures did not change the tetanic responses. Stimulus train duration was sufficient to elicit maximal tetanic tension. Train durations varied from about 20 ms at the highest temperatures to about 200 ms at the lowest temperatures. Twitch parameters were recorded post-tetanicly. All isometric observations were displayed on a Tektronix Model 5111 storage oscilloscope and were photographed with Polaroid film. Examples of typical isometric responses of one preparation at two temperatures are shown in Fig. 1. Isotonic contractions were recorded using tetanic stimuli (40 ms at 25 °C, 30 ms at 30 °C and 35 °C). During isotonic measurements, stimuli were applied at intervals of 1–2 min until a series of 19 observations had been made. An example of transducer outputs during an isotonic contraction at an intermediate force is given in Fig. 2. Preliminary observations indicated no deleterious effects of tetanic stimuli applied 1/min for more than 30 min.

Muscles were first equilibrated at the animal's PBT rounded to the nearest 5 °C (25 °C: *Eremiascincus*; 30 °C: *Sphenomorphus* spp.; 35 °C: *Ctenotus* spp.). Muscle length and stimulus intensity were adjusted to give maximal tetanic tension. Isometric measurements were recorded initially at PBT and then at 5 °C intervals down to 10 °C. Subsequently, the temperature was returned to PBT and was then increased by intervals of 5 °C up to 35 °C and by intervals of 2.5 °C up to the highest temperature at which a muscle would respond. Temperatures within 0.9 °C of the target were accepted. Muscles were allowed 5 min for equilibration at each temperature prior to an observation. It generally took 2–3 min for intermediate temperature adjustments and 10–15 min in returning from 10 °C to PBT (Table 2). Resting tension was not observed to change at non-damaging temperatures. In the isotonic series, measurements were made using the quick-release method initially at PBT. Subsequently, a second isotonic series was made at 25 °C for *Ctenotus* spp. and *Sphenomorphus* spp. and at 35 °C for *Eremiascincus*. A third isotonic series at 35 °C was finally made for *Sphenomorphus* spp. Tetanic tension was recorded initially, finally, and after every 5 isotonic contractions. The force during isotonic series is expressed as a fraction of  $P_o$  assuming linear changes in  $P_o$  between measurements. Shortening velocities were measured at fractions of  $P_o$  as close as possible to and in the order of the following: 1.0, 0.90, 0.70, 0.50, 0.30, 0.075, 1.0, 0.80, 0.60, 0.40, 0.20, 0.025, 1.0, 0.95, 0.85, 0.125, 0.050, 0.010, 1.0. At the end of a series of measurements, muscle length was measured, and this length was taken as  $L_o$ . Lengths measured in situ were within 5% of those measured in vitro. The muscle was removed from the chamber, damaged fibers were removed, and the pelvis and distal tendon were removed. The muscle was blotted dry and weighed to the nearest 0.01 mg. Cross-sectional area was calculated by dividing the mass of the muscle by the muscle length. Individual fibers in the IF extend at least 95% of the total muscle length (Gleeson et al. 1980). The density of the muscle was assumed to be 1.0 g/ml.

**Statistics and curve-fitting.** All calculations were done using a Hewlett-Packard 41 CX calculator with a statistics module. Sta-

**Table 1.** Tetanic tension (Po), maximal rate of tetanic tension development ( $dPo/dt$ ), and twitch tension (Pt) at the initial measurement temperature of each species. Units are  $kN/m^2$  for tension and  $kN/(m^2 \cdot ms)$  for  $dPo/dt$ . Values are means  $\pm$  SE. Sample sizes are in parentheses<sup>a</sup>

Species	Initial temperature	Po <sup>b</sup>	$dPo/dt$ <sup>c</sup>	Pt <sup>d</sup>
<i>C. regius</i>	35 °C	251 $\pm$ 4.4 (6)	30.6 $\pm$ 2.08 (6)	91.3 $\pm$ 7.47 (6)
<i>C. robustus</i>	35 °C	223 $\pm$ 22.0 (7)	36.0 $\pm$ 5.68 (6)	145.5 $\pm$ 28.22 (6)
<i>C. taeniolatus</i>	35 °C	215 $\pm$ 20.8 (6)	31.8 $\pm$ 1.74 (6)	88.9 $\pm$ 7.20 (6)
<i>C. uber</i>	35 °C	207 $\pm$ 24.9 (6)	27.3 $\pm$ 3.05 (6)	90.4 $\pm$ 9.70 (6)
<i>E. fasciolatus</i>	25 °C	238 $\pm$ 25.3 (7)	18.8 $\pm$ 3.19 (7)	93.1 $\pm$ 14.82 (7)
<i>S. kosciuskoi</i>	30 °C	187 $\pm$ 20.4 (7)	21.3 $\pm$ 3.42 (7)	63.8 $\pm$ 7.42 (6)
<i>S. quoyi</i>	30 °C	264 $\pm$ 26.8 (6)	35.8 $\pm$ 5.51 (6)	128.8 $\pm$ 19.27 (6)
<i>S. tympanum</i>	30 °C	193 $\pm$ 11.8 (6)	27.5 $\pm$ 2.78 (6)	95.8 $\pm$ 3.31 (6)

<sup>a</sup> Means were calculated using the higher of two measurements when both IF muscles from an individual were used

<sup>b</sup> Significant differences only among *Sphenomorphus* spp. (ANOVA;  $P < 0.05$ ). Minimum significant differences between *Sphenomorphus* means = 58.4  $kN/m^2$  by the Tukey-Kramer method

<sup>c</sup> Significant differences only among *Sphenomorphus* spp. ( $P < 0.05$ ). Minimum significant difference between *Sphenomorphus* means = 11.3  $kN/(m^2 \cdot ms)$

<sup>d</sup> Significant differences only among *Sphenomorphus* spp. ( $P < 0.05$ ). Minimum significant difference between *Sphenomorphus* means = 40.5  $kN/cm^2$

**Table 2.** Elapsed time (min) between initial and final measurements and per cent changes between initial and final measurements of Po,  $dPo/dt$ , and Pt at original temperatures. Negative values indicate decremental changes. Values are means  $\pm$  1 SE. Sample sizes are in parentheses. Original data were analyzed by paired *t*-tests. Significance is judged with one-tailed tests; significant decline in performance is marked by an asterisk

Species	Elapsed time	% Changes from initial to final measurement		
		Po	$dPo/dt$	Pt
<i>C. taeniolatus</i>	64 $\pm$ 3 min	-10.8 $\pm$ 3.1*	2.2 $\pm$ 5.8	-0.2 $\pm$ 7.7
<i>C. uber</i>	61 $\pm$ 5	-10.2 $\pm$ 3.8*	-1.6 $\pm$ 0.9*	-4.8 $\pm$ 10.7
<i>C. regius</i>	64 $\pm$ 7	-17.0 $\pm$ 3.0*	-10.0 $\pm$ 4.1*	-18.6 $\pm$ 5.0*
<i>C. robustus</i>	67 $\pm$ 3	-6.2 $\pm$ 5.7	-8.1 $\pm$ 4.3*	-1.6 $\pm$ 5.1
<i>E. fasciolatus</i>	51 $\pm$ 4	-3.5 $\pm$ 2.7	-6.5 $\pm$ 2.8*	-8.5 $\pm$ 3.7*
<i>S. quoyi</i>	47 $\pm$ 3	-2.8 $\pm$ 2.1	-2.3 $\pm$ 2.6	-2.1 $\pm$ 6.6
<i>S. tympanum</i>	46 $\pm$ 3	-6.7 $\pm$ 2.6*	-4.0 $\pm$ 4.0	-8.6 $\pm$ 5.5*
<i>S. kosciuskoi</i>	63 $\pm$ 2	-16.5 $\pm$ 3.6*	-11.4 $\pm$ 3.2*	-15.3 $\pm$ 5.6*

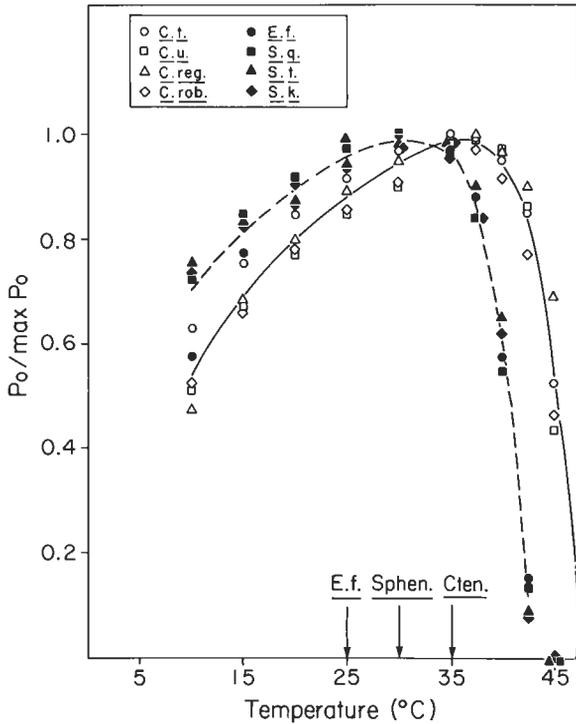
tistical analyses are described in Sokal and Rohlf (1981) as referenced. Isotonic force-velocity data were fit to an exponential-linear equation developed by Marsh and Bennett (1985, 1986) using NLLSQ (CET Research Group, Ltd., Norman, Oklahoma), a non-linear least squares curve fitting program for Apple II micro-computers. The exponential-linear equation was as follows:  $V = \alpha e^{-\beta P/Po} + \gamma(1 - P/Po)$ , where  $\alpha$  and  $\gamma$  are constants with dimensions of velocity and  $\beta$  is a dimensionless constant,  $V$  is the velocity of shortening, and  $P/Po$  is the fraction of tetanic tension (Po) at which shortening velocity was measured. Significance was judged at  $P < 0.05$ .

## Results

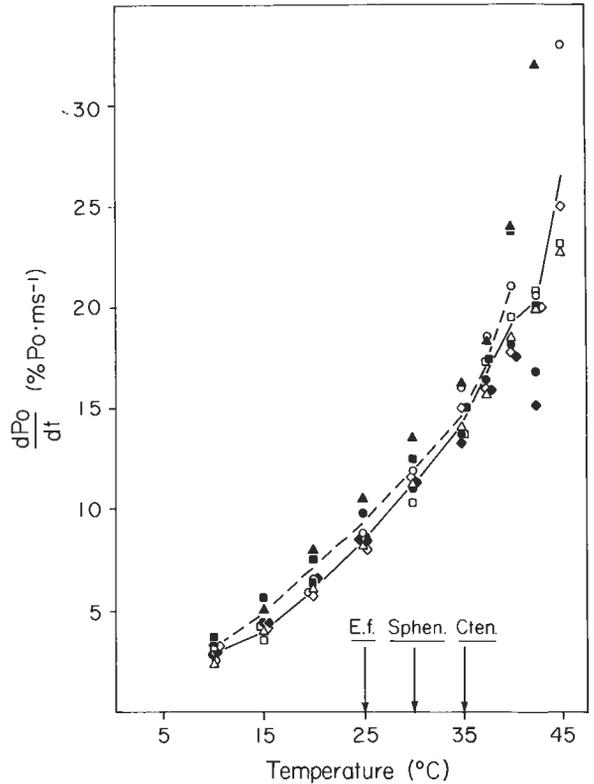
Muscle preparations were first equilibrated at the respective PBT of each species. Initial values for tetanic tension (Po), maximal rates of tetanic tension development ( $dPo/dt$ ), and twitch tension (Pt) at PBT are presented in Table 1. Among the *Sphenomorphus* spp., *S. quoyi* had significantly greater Po than both other species ( $P < 0.05$ ;

Tukey-Kramer) and significantly greater  $dPo/dt$  and Pt than *S. kosciuskoi* ( $P < 0.05$ ; Tukey-Kramer). There were no significant differences in these factors among *Ctenotus* spp. With the exception of *S. quoyii*, Po's and Pt's at respective PBTs are not significantly different among all other species (ANOVA,  $P > 0.05$ ). The initial observations at the original temperature were the most accurate measurements of contractile performance, and an index of the stability of a preparation is obtained by comparing the initial and final measurements at the original temperature. Approximately half of the species showed significant decremental changes in Po,  $dPo/dt$ , and/or Pt measured at PBT before and after the series of measurements at lower temperatures (Table 2). However, these decrements are relatively small, with the mean decrement for each factor averaging less than 10%.

Analyses of thermal effects on Po and Pt were



**Fig. 3.** Normalized tetanic tension as a function of muscle temperature. Points represent species means. The continuous line connects the averages of mean values for *Ctenotus* spp.; the broken line connects the averages of mean values for *Sphenomorphus* spp. and *Eremiascincus*. Generic PBTs are indicated on the x-axis. Values at each temperature were analyzed by ANOVA and Scheffe's method for contrasting means. *Ctenotus* spp. are significantly different than the other species at all temperatures other than 35 °C ( $P < 0.05$ )



**Fig. 4.** Maximal rates of tetanic tension development ( $dPo/dt = \%Po/ms$ ) as a function of temperature. Symbols, lines, and PBTs as in Fig. 1. *Sphenomorphus* spp. are significantly greater than *Ctenotus* spp. at 15 °C ( $P < 0.05$ ; ANOVA and Scheffe's method)

**Table 3.** Preferred body temperature and the thermal range of maximal  $Po$ . See text for method of analysis

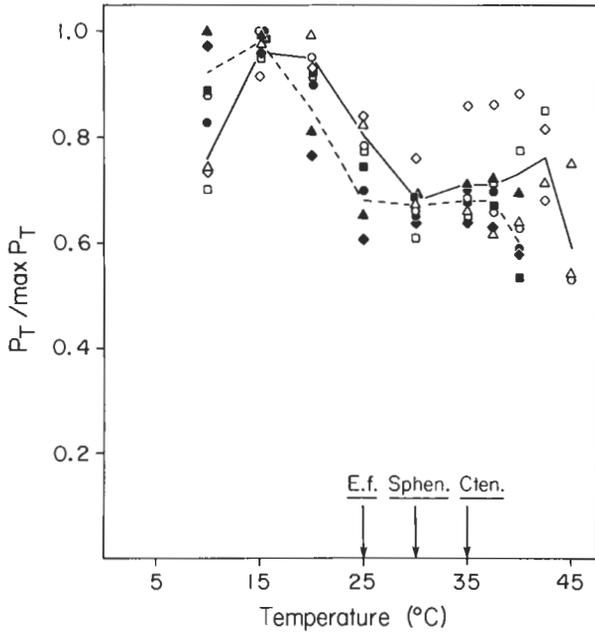
Species	PBT <sup>a</sup> (°C)	Thermal range of maximal $Po$
<i>C. taeniolatus</i>	35.3	35–37.5 °C
<i>C. uber</i>	35.5	35–40 °C
<i>C. regius</i>	35.6	35–37.5 °C
<i>C. robustus</i>	34.4	35–37.5 °C
<i>E. fasciolatus</i>	24.4	30–35 °C
<i>S. quoyi</i>	28.8	30 °C
<i>S. tympanum</i>	29.6	30–35 °C
<i>S. kosciuskoi</i>	29.8	25–35 °C

<sup>a</sup> PBT's from Bennett and John-Alder (1986)

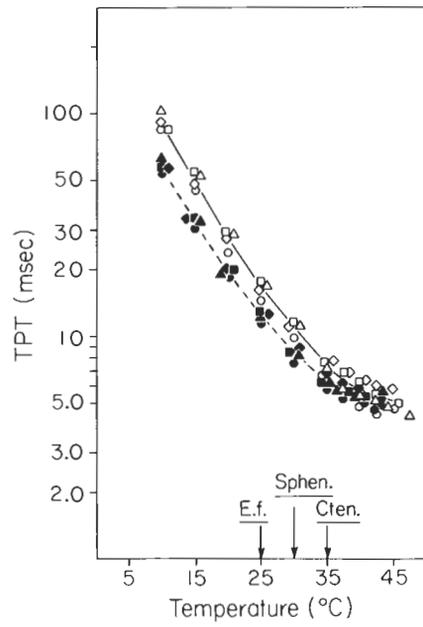
made using the following procedure. First, a value obtained at any temperature was expressed as a fraction of the tension attained at PBT. For calculations at temperatures below PBT, estimated tension at PBT was interpolated as a function of time between the initial and final measurements at PBT. For values at temperatures above PBT, the final

tension at PBT was used. These fractional performance values were then normalized to a maximum of 1.0 by dividing all values in a series by the highest value. This procedure adjusted for non-thermal changes in tension over time. Comparisons of  $Po$  and  $Pt$  among species are based on these normalized values. Interspecific differences in tension capacities not related to thermal biologies of species are thereby eliminated from consideration. Thermal ratios ( $R_{10s}$ ) (Bennett 1984) for tension capacities were calculated using these normalized values. Temperature coefficients and ratios were calculated for individuals using the temperatures recorded during a measurement. Mean  $Q_{10s}$  and  $R_{10s}$  for a species were then calculated from individual values. Temperature intervals were rounded to the nearest 2.5 °C.

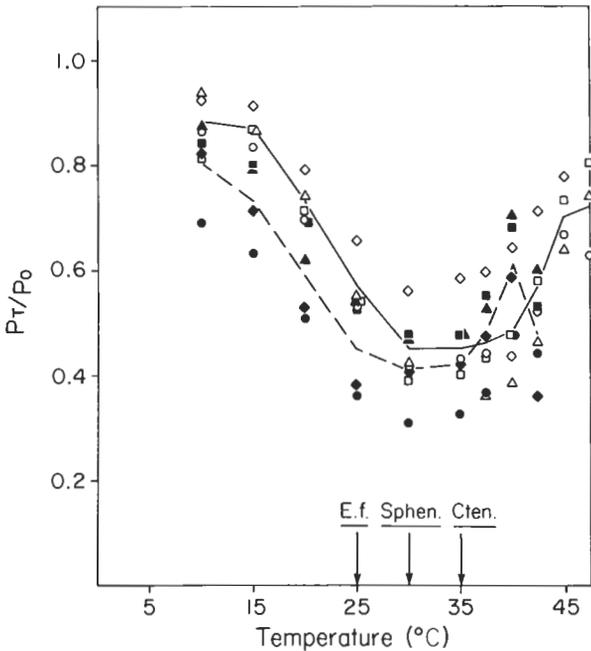
Normalized tetanic tension as a function of muscle temperature is presented for each species in Fig. 3. In all species,  $Po$  is above 80% of maximal values over at least a 20 °C range of temperatures, and in all except *Eremiascincus*, maximal  $Po$



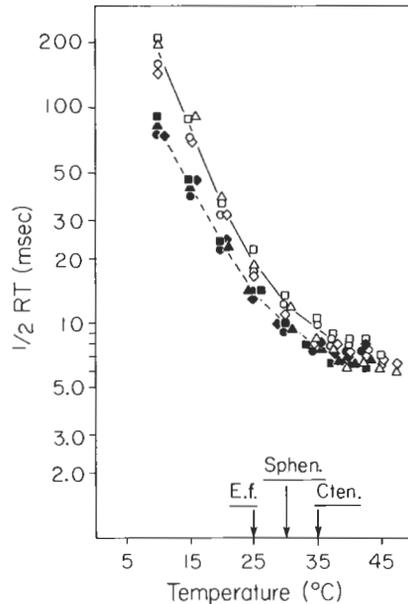
**Fig. 5.** Normalized twitch tension as a function of muscle temperature. Symbols, lines, and PBTs as in Fig. 1



**Fig. 7.** Twitch time to peak tension as a function of temperature. Symbols, lines, and PBTs as in Fig. 1. *Ctenotus* spp. are significantly slower than the other species at all temperatures below 35 °C ( $P < 0.05$ ; ANOVA and Scheffe's method)



**Fig. 6.** Twitch to tetanic tension ratios as a function of temperature. Symbols, lines, and PBTs as in Fig. 1. *Ctenotus* spp. are significantly higher than the other species at 15 °C and 20 °C ( $P < 0.05$ ; ANOVA and Scheffe's method)



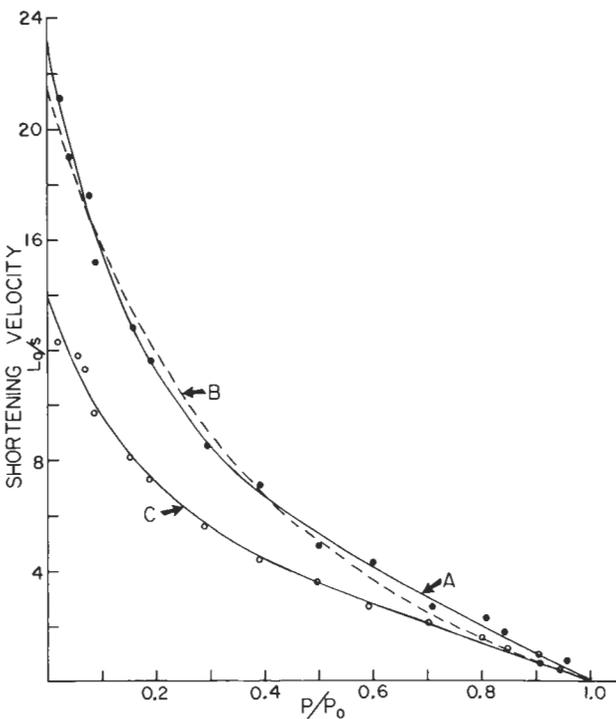
**Fig. 8.** Twitch half-relaxation time as a function of temperature. Symbols, lines, and PBTs as in Fig. 1. *Ctenotus* spp. are significantly slower than the other species at all temperatures below 37.5 °C ( $P < 0.05$ ; ANOVA and Scheffe's method)

is attained at PBT. The range of temperatures over which  $P_0$  is maximal was determined using paired  $t$ -tests between  $P_0$ 's at adjacent temperatures. The results of this analysis are presented in Table 3. The highest temperatures at which responses were

obtained were 42.5 °C in *Sphenomorphus* spp. and *Eremiascincus* and 47.5 °C in *Ctenotus* spp. Mean normalized  $P_0$ 's of *Ctenotus* spp. as a group are significantly lower than those of *Sphenomorphus* spp. and *Eremiascincus* as a group at temperatures

**Table 4.** Iliofibularis muscle lengths (mm), maximal velocities of isotonic shortening ( $V_{\max} = l_0/S$ ), maximal power output ( $\dot{W}_{\max} = \text{watts/kg}$ ), and power ratios ( $\dot{W}_{\max}/VoPo$ ; Marsh and Bennett 1985). Values are means  $\pm 1$  SE. Power ratios were calculated from mean data. Sample sizes are given in parentheses. See text for details of calculations

Species	Muscle length	$V_{\max}$			$\dot{W}_{\max}$			$\dot{W}_{\max}/VoPo$		
		25 °C	30 °C	35 °C	25 °C	30 °C	35 °C	25 °C	30 °C	35 °C
<i>C. regius</i> (4)	9.1 $\pm$ 0.09	10.8 $\pm$ 0.28		19.1 $\pm$ 0.77	321		586 $\pm$ 35	0.131		0.126
<i>C. robustus</i> (2)	10.2 $\pm$ 0.27	11.1 $\pm$ 0.66		20.1 $\pm$ 0.93	325		638 $\pm$ 248	0.156		0.136
<i>C. taeniolatus</i> (5)	7.4 $\pm$ 0.12	11.4 $\pm$ 0.70		19.7 $\pm$ 1.04	331		540 $\pm$ 76	0.150		0.137
<i>C. uber</i> (6)	8.9 $\pm$ 0.13	11.2 $\pm$ 0.92		19.8 $\pm$ 0.42	271		480 $\pm$ 59	0.137		0.125
<i>E. fasciolatus</i> (6)	9.6 $\pm$ 0.22	10.2 $\pm$ 0.85		16.2 $\pm$ 1.09	293 $\pm$ 35		530	0.129		0.136
<i>S. kosciuskoi</i> (4)	7.9 $\pm$ 0.20	12.3 $\pm$ 0.29	16.0 $\pm$ 0.35	19.6 $\pm$ 0.86	294	417 $\pm$ 32	451	0.132	0.131	0.132
<i>S. quoyi</i> (3)	11.9 $\pm$ 0.28	13.5 $\pm$ 0.48	18.2 $\pm$ 1.03	22.5 $\pm$ 0.79	460	623 $\pm$ 81	747	0.132	0.125	0.129
<i>S. tympanum</i> (5)	10.9 $\pm$ 0.36	12.5 $\pm$ 0.72	16.2 $\pm$ 0.95	20.8 $\pm$ 1.21	328	416 $\pm$ 40	522	0.140	0.141	0.131



**Fig. 9.** Force-velocity curves at 35 °C (A and B) and 25 °C (C) for a white (FG) iliofibularis from a *Ctenotus taeniolatus*. Original data are given by closed (35 °C) and open (25 °C) symbols. Curves A and C were fit using the exponential-linear equation (see text), and curve B was fit using the Hill equation with fixed  $P_0$ . For curves A and B, the residual sums of squares (RSS) are 2.976 and 7.212, respectively, and this difference is typical of all force-velocity curve fits. By the criterion of the minimum RSS, the exponential-linear equation is substantially better than the Hill equation. The most significant deviations of the Hill equation from the data occur at high and low fractions of  $P_0$ .

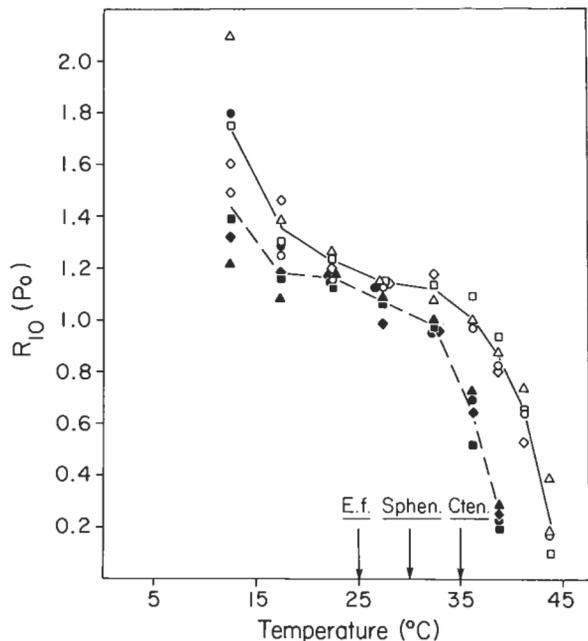
below 35 °C and are significantly higher at temperatures above 35 °C (ANOVA and Scheffe's method of contrasting means;  $P < 0.05$ ).

Maximal rates of tetanic tension development ( $dPo/dt$ ) are presented as a function of temperature

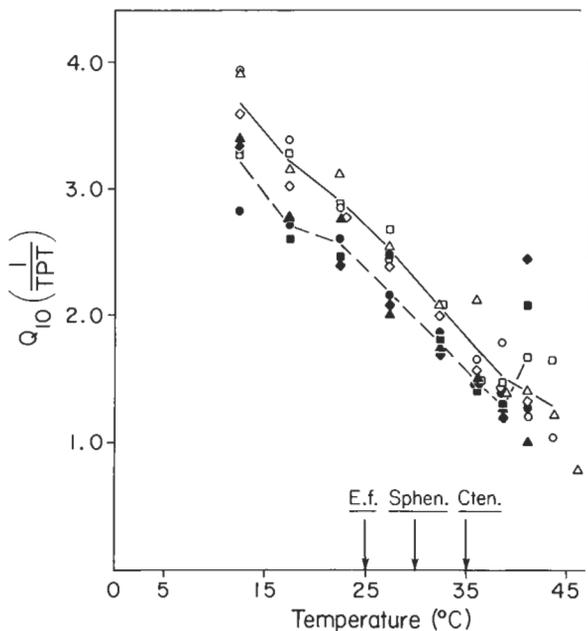
in Fig. 4. Rates of tension development are presented as %Po/ms to standardize levels of Po. In all species,  $dPo/dt$  shows a very pronounced thermal dependence over a broad range of temperatures. Maximal  $dPo/dt$ 's (%Po/ms) are attained at temperatures above PBT in all cases and at the highest temperature in a series in most cases. It should be noted that absolute rates of tension development, expressed as  $\text{kN}/(\text{m}^2 \cdot \text{ms})$ , are maximal at 35 to 37.5 °C in *Sphenomorphus* spp. and *Eremiascincus* and at 40 °C in *Ctenotus* spp. At most temperatures, there are no significant differences in  $dPo/dt$  (%Po/ms) among species, except at 15 °C, at which  $dPo/dt$ 's of *Sphenomorphus* spp. are significantly greater than those of *Ctenotus* spp. ( $P < 0.05$ ; ANOVA and Scheffe's method of contrasting means).

Twitch tension is presented as a function of temperature in Fig. 5. In all species, twitch tension is optimized, i.e. maximal, at temperatures considerably below PBT. Twitch tension is optimized at 15–20 °C in *Ctenotus* spp. and *Eremiascincus* and at 10–15 °C in *Sphenomorphus* spp. While twitch tension decreases below 15 °C in *Ctenotus* spp. and in *Eremiascincus*, the ratio of twitch to tetanic tension is generally as high or higher at 10 than at 15 °C in these two genera (Fig. 6). In *Sphenomorphus* spp., the ratio of twitch to tetanic tension is higher at 10 than at 15 °C in all species.

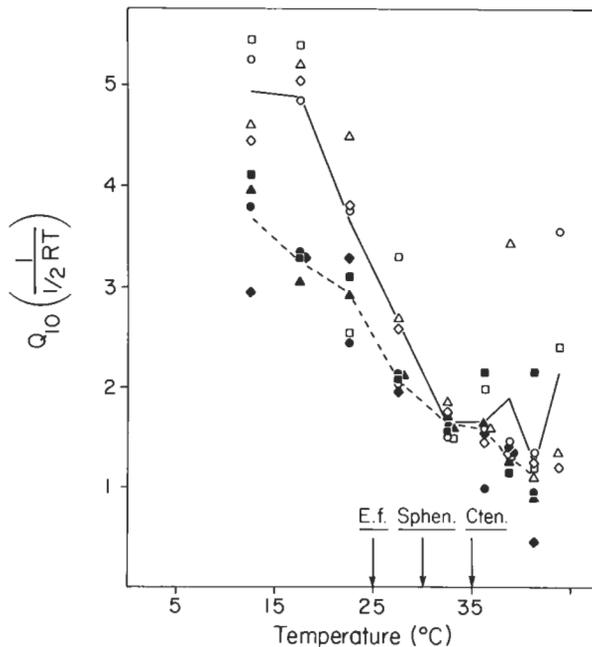
Twitch time to peak tension (TPT) is presented as a function of temperature in Fig. 7. At all temperatures below 35 °C, TPTs of *Ctenotus* spp. are significantly longer than TPTs of *Sphenomorphus* spp. and *Eremiascincus* ( $P < 0.05$ ; Scheffe's method). Twitch half-relaxation times ( $1/2$  RT) are presented as a function of temperature in Fig. 8. At all temperatures below 37.5 °C,  $1/2$  RTs of *Ctenotus* spp. are significantly longer than those of *Sphenomorphus* spp. and *Eremiascincus* ( $P < 0.05$ ;



**Fig. 10.** Thermal ratios for tetanic tension as a function of temperature. Symbols, lines, and PBTs as in Fig. 1. Points are plotted at the mid-point of temperature intervals. An  $R_{10}$  value of 1.0 indicates thermal independence. Thermal ratios for *Ctenotus* spp. are significantly greater than for other species from 15 to 20 °C and at all intervals above 30 °C ( $P < 0.05$ ). From 10 to 15 °C,  $R_{10}$ s for *Ctenotus* spp. are significantly greater than for *Sphenomorphus* spp. ( $P < 0.05$ ; ANOVA and Scheffe's method)



**Fig. 11.** Temperature coefficients for twitch contraction rates as a function of temperature. Symbols, lines, and PBTs as in Figs. 1 and 7. In this and the following figures, a  $Q_{10}$  value of 1.0 indicates thermal independence. Temperature coefficients of *Ctenotus* spp. from 20 to 35 °C are significantly greater than for other species (ANOVA;  $P < 0.01$ )



**Fig. 12.** Temperature coefficients for twitch half-relaxation rates as a function of temperature. Symbols, lines, and PBTs as in Figs. 1 and 6

Scheffe's method). Twitch kinetics of *Eremiascincus* are not significantly different from those of *Sphenomorphus* spp. (ANOVA;  $P > 0.05$ ). Both TPT and  $1/2$  RT attain their lowest (shortest) values at the highest temperature at which a muscle responded, not at PBT. The reciprocals of TPT and  $1/2$  RT are rates with units of  $1/s$ . These rates were used in calculating  $Q_{10}$ s for twitch kinetics.

Maximal velocities of isotonic shortening ( $V_{max}$ ) and maximal power output ( $\dot{W}_{max}$ ) during shortening at the PBTs of each species are presented in Table 4. Shortening velocities at 30 °C were measured only in *Sphenomorphus* spp. The estimates of  $V_{max}$  are extrapolated at zero force from curves fit to the force-velocity data as described. The exponential-linear equation used for this purpose produced curves that fit the data substantially better than the Hill equation (Hill 1938) in all cases (see Fig. 9). Typical force-velocity curves for *C. taeniolatus* are shown in Fig. 9. There are no significant differences in  $V_{max}$  among species at 25 °C or at 30 °C among *Sphenomorphus* spp. At 35 °C, *Eremiascincus* has a significantly lower  $V_{max}$  than *C. uber*, *S. quoyi*, and *S. tympanum* ( $P < 0.05$ ; Tukey-Kramer). Maximal power output during isotonic shortening was attained at 0.4 to 0.5  $P_o$ , usually at 0.45  $P_o$ . Among *Sphenomorphus* spp., *S. quoyi* had a significantly greater maximal power output than either of the other two species.

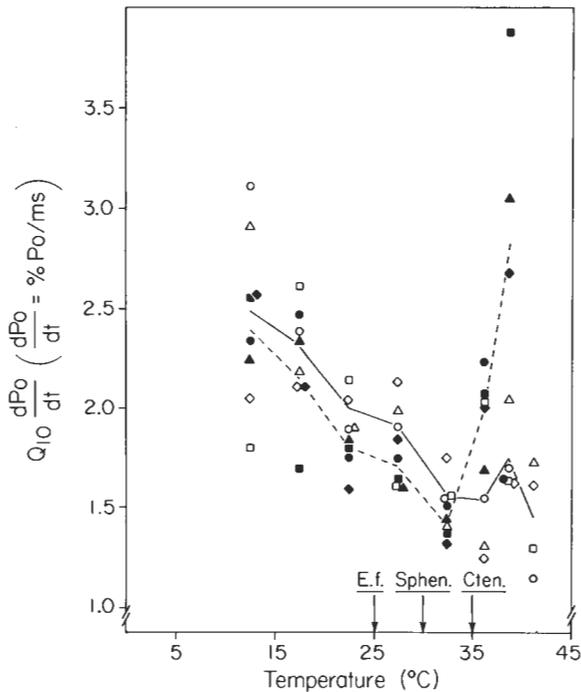


Fig. 13. Temperature coefficients of rates of tetanic tension development ( $dPo/dt = \%Po/ms$ ) as a function of temperature. Symbols, lines, and PBTs as in Figs. 1 and 6

**Table 5.** Temperature coefficients for maximal velocities of isotonic shortening ( $V_{max} = l/s$ ) and for maximal power output ( $\dot{W}_{max}$ ). Values for  $Q_{10}$ s of  $V_{max}$  are means  $\pm 1$  SE, and for  $\dot{W}_{max}$ , values are calculated from pooled data. For  $V_{max}$ ,  $Q_{10}$ s of *Ctenotus* spp. are higher than for the more cryophilic species ( $P=0.014$ ; Man-Whitney U test). For  $\dot{W}_{max}$ , differences among  $Q_{10}$ s just fail to attain significance ( $P=0.057$ )

Species	$Q_{10}$ from 25 °C to 35 °C	
	$V_{max}$	$\dot{W}_{max}$
<i>C. regius</i>	1.77 $\pm$ 0.06	1.83
<i>C. robustus</i>	1.95 $\pm$ 0.23	1.96
<i>C. taeniolatus</i>	1.71 $\pm$ 0.04	1.63
<i>C. uber</i>	1.84 $\pm$ 0.11	1.77
<i>E. fasciolatus</i>	1.62 $\pm$ 0.04	1.81
<i>S. kosciuskoi</i>	1.63 $\pm$ 0.06	1.53
<i>S. quoyi</i>	1.67 $\pm$ 0.06	1.62
<i>S. tympanum</i>	1.70 $\pm$ 0.08	1.59

To calculate  $\dot{W}_{max}$  at temperatures other than PBT for which isotonic data are available,  $V_{max}$  was taken as the average for a species and  $Po$  at the desired temperature was calculated as the product of a species' mean  $Po$  at PBT (Table 1) and the normalized values in Fig. 1. This method of calculating maximal power output gives an estimate based on the best determinations of muscle performance but precludes statistical treatment. It is clear

that *S. quoyi* is superior to all other species in terms of maximal power output at 25, 30, and 35 °C. There are no other significant differences in  $\dot{W}_{max}$  among species that correspond to differences in PBT. *Sphenomorphus* spp. and *Eremiascincus* do not attain maximal values of  $V_{max}$  or  $\dot{W}_{max}$  at PBT; such a determination cannot be made for *Ctenotus* spp. Power ratios ( $\dot{W}_{max}/VoPo$ ) (Table 4) are calculated as an index of curvature of the force-velocity relationship (Marsh and Bennett 1985, 1986). The degree of curvature is correlated with efficiency of work output (Woledge 1968). The constancy of the power ratios calculated for the muscles indicates that this efficiency is temperature-independent.

Temperature coefficients for isometric data are presented graphically in Figs. 10, 11, 12, and 13 and for  $V_{max}$  in Table 5. *Ctenotus* spp. as a group have slightly higher temperature coefficients for isometric properties than *Sphenomorphus* spp. and *Eremiascincus*. Significant differences are as indicated in the figure legends. These differences are a consequence of translational adaptations in thermal performance (Precht et al. 1973, pp. 331–352; Prosser 1973, pp. 373–386). To ascertain the possible presence of rotational adaptations,  $Q_{10}$ s for *Ctenotus* were compared to those of *Sphenomorphus* and *Eremiascincus*. In these comparisons, temperature intervals for *Ctenotus* were 5 °C higher than for the more cryophilic species. There are no significant differences among  $Q_{10}$ s for any of the isometric contractile factors at temperatures lower than 37.5 °C. Significant differences in these comparisons would have been evidence of rotational adaptation (Prosser 1973, pp. 373–386). Mean temperature coefficients for  $V_{max}$  (Table 5) of *Sphenomorphus* and *Eremiascincus* are significantly lower than those of *Ctenotus* ( $P=0.014$ , Mann-Whitney U test). A similar comparison for  $\dot{W}_{max}$  just fails to attain significance ( $P=0.057$ ).

## Discussion

The experimental results show clearly that there has been some adjustment in the thermal dependence of muscle contractile performance among these closely related genera of lizards. These shifts occur both in the level of function attained at any single temperature and in the thermal range of contractile activity. However, the thermal dependence of these contractile processes is still pronounced, even in species with lower PBT. Compensation of the retarding effects of colder temperatures is far from complete. For subsequent discussion, the more thermophilic *Ctenotus* spp. are compared to

the more cryophilic *Sphenomorphus* spp. and *Eremiascincus*.

Resistance adaptations, alterations in the thermal range over which functional capacity is maintained (Precht et al. 1973, pp. 442–440), are apparent in all isometric factors examined: Po, Pt,  $dPo/dt$ , TPT, and 1/2 RT. *Ctenotus* maintain contractile capacity at higher temperatures than do *Sphenomorphus* or *Eremiascincus* (Figs. 3–5, 7, 8). The thermal performance breadths (Huey 1982) of these factors are nearly the same for the three genera, e.g., 80% of maximal Po is attained over a range of 21–25 °C for all species (Fig. 3).

Capacity adaptations to PBT are also apparent in most isometric factors measured. In Po, Pt, TPT, and 1/2 RT (Figs. 3, 5, 7, 8), there are pronounced differences in the rate or capacity curves of genera with warm and cool thermal preferenda: those of the more cryophilic animals are left-shifted with respect to the more thermophilic. At cool temperatures, lizards with lower PBT thus have a greater force generating capacity and shorter twitch contraction time when compared to thermophilic species. The differences observed can be accounted for almost exclusively by translational rather than rotational adaptations (e.g., Figs. 10–13). No capacity adaptation is apparent in  $dPo/dt$  (Fig. 4). The isotonic factors tend to show a lower thermal dependence in cryophilic species between 25 and 35 °C. Whether these differences are due to translational or rotational adaptations cannot be determined from these data.

In spite of these adaptive shifts, there is little indication that muscle functional capacities are optimized at PBT in these lizards. Only in Po (Fig. 3) is such optimization apparent, and even in this case it is not optimized in *Eremiascincus*. Maximal Pt is always attained at temperatures well below PBT (Fig. 5). Rates of tetanic tension development (Fig. 4), twitch contraction (Fig. 7), and twitch relaxation (Fig. 8) are most rapid at the highest temperature tolerated by the muscles, which always exceeds PBT. The isotonic data are too limited for a similar determination, except that it is clear that  $V_{max}$  is not optimized at PBT in either *Sphenomorphus* or *Eremiascincus* (Table 4). It is apparent, therefore, that even though some adjustment of muscle function to PBT has been accomplished in this group of species, this adjustment has not been completely effective at equalizing performance at PBT among species, nor has it resulted in optimal function at PBT.

Even though adaptation has not been effective

at optimizing muscle functional capacities at PBT, it is of interest whether they have been compensated for equivalent levels of function at PBT among the species. In other words, is a functional parameter equivalent in *Eremiascincus* at 25 °C, *Sphenomorphus* spp. at 30 °C, and *Ctenotus* spp. at 35 °C? Tension capacities, both Po and Pt, are not generally different among all species at their respective PBTs (Table 1). In light of the low thermal dependence of these factors over a broad range of temperatures, this is not surprising. Twitch time to peak tension is partially, not completely, compensated for interspecific differences in PBT. Times to peak twitch tension at any single temperature are longer in *Ctenotus* spp. than in the more cryophilic species. However, at respective PBTs, TPTs of *Ctenotus* spp. are shorter than those of *Sphenomorphus* spp., and those of *Sphenomorphus* spp. shorter than those of *Eremiascincus* ( $P < 0.05$ ; ANOVA; Scheffe's method of contrasting means). Thus, for example, TPTs of *Sphenomorphus* spp. at 30 °C are intermediate between those of *Ctenotus* spp. at 30 °C and 35 °C. Twitch half-relaxation time is nearly completely compensated for differences in PBT between *Ctenotus* spp. and *Sphenomorphus* spp., as Scheffe's method fails to attain significance in a contrast of these two groups. However, 1/2 RT of *Eremiascincus* at its PBT is significantly longer (slower) than that of the other species ( $P < 0.05$ ; ANOVA; Scheffe's method).

The thermal effects on contractile properties reported here are qualitatively similar to those reported for other lizards (Putnam and Bennett 1982; Johnston and Gleeson 1985; Marsh and Bennett 1985) and for other groups of vertebrates (Bennett 1985), and most of these effects need no further explanation. However, some observations are worthy of mention. At all temperature intervals below 25 °C,  $Q_{10}$ s for TPT are significantly less than those of 1/2 RT (paired  $t$ -test on species means;  $P < 0.025$ ), an observation that has been reported previously (Bennett 1984, 1985). Thus, relaxation times are shortened with increasing temperature to a greater extent than are contraction times. At temperature intervals above 25 °C, there are no systematic differences in the  $Q_{10}$ s of these twitch rate functions. The relatively greater thermal dependence of 1/2 RT than of TPT can help to explain the thermal dependence of Pt up to 25 °C (Hill 1951; Bressler 1981; Bennett 1985).

*Sphenomorphus quoyi* is superior to its congeners in terms of Po and  $dPo/dt$ , and when initial values of these factors for all species (Table 1) are

adjusted to 35 °C by using  $Q_{10}$ s reported in Figs. 10 and 11, *S. quoyi* appears to be superior in these respects to all of the other species examined in this study (Table 1). Differences in isometric contractile properties between toad and frog sartorius muscle were recently reported in the context of differences in organismal behavioral patterns (Renaud and Stevens 1983). Natural behavioral correlates of superior muscle performance in *S. quoyi* cannot presently be identified.

Several conclusions can be drawn from the foregoing analysis of thermal effects on muscle contractile properties among these closely related skinks. It is clear that there have been adaptive adjustments associated with differences in PBT. Resistance adaptations are clearly indicated by differences in upper thermal limits, and most of the isometric contractile properties show translational adaptations in their thermal performance curves. Optimization or equalization of function at PBT has generally not occurred. Because of the very close relatedness of the species examined (Greer 1979), we argue that the thermal adaptations demonstrated in this study reflect specific adaptations associated with PBTs. These adaptations have been only marginally effective in compensating for the depressing effects of low preferred temperatures.

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