

## Locomotor performance of closely related *Tropidurus* species: relationships with physiological parameters and ecological divergence

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### Summary

Tropidurid lizards have colonized a variety of Brazilian open environments without remarkable morphological variation, despite ecological and structural differences among habitats used. This study focuses on two *Tropidurus* sister-species that, despite systematic proximity and similar morphology, exhibit great ecological divergence and a third ecologically generalist congeneric species providing an outgroup comparison. We quantified jumping capacity and sprint speed of each species on sand and rock to test whether ecological divergence was also accompanied by differences in locomotor performance. Relevant physiological traits possibly associated with locomotor performance – metabolic scopes and fiber type composition, power output and activity of the enzymes citrate synthase, pyruvate kinase and lactate dehydrogenase of the iliofibularis muscle – were also compared among the three *Tropidurus* species. We found that the two sister-species exhibited

remarkable differences in jumping performance, while *Tropidurus oreadicus*, the more distantly related species, exhibited intermediate values. *Tropidurus psamonastes*, a species endemic to sand dunes, exhibited high absolute sprint speeds on sand, jumped rarely and possessed a high proportion of glycolytic fibers and low activity of citrate synthase. The sister-species *Tropidurus itambere*, endemic to rocky outcrops, performed a large number of jumps and achieved lower absolute sprint speed than *T. psamonastes*. This study provides evidence of rapid divergence of locomotor parameters between sister-species that use different substrates, which is only partially explained by variation in physiological parameters of the iliofibularis muscle.

Key words: locomotion, *Tropidurus*, muscle physiology, metabolism, enzyme activity, habitat divergence, evolution.

### Introduction

The performance of individuals, and consequently their fitness, is affected by morphological and physiological traits (Arnold, 1983; Garland and Losos, 1994). Because different suites of morphological and physiological characters increase behavioral performance in specific ecological contexts, one can expect that natural selection favors traits that improve performance in an individual's current habitat (Garland and Losos, 1994). The hypothesis of a relationship between structural habitat, morphology and locomotor performance is supported by studies with *Anolis* lizards (Losos, 1990, 1992; Losos et al., 1998; Beutell and Losos, 1999) but is not unambiguously supported by data from other lizard taxa (Miles, 1994; Vanhooydonck and Van Damme, 1999). For example, the sub-family Tropidurinae (including genera *Plica*, *Strobilururs*, *Eurolophosaurus* and *Tropidurus*) exhibits remarkable ecological diversity but minimal morphological

differentiation compared with that reported for *Anolis*. Species from forested and more open environments differ in limb and tail morphology, but species from sand dunes, rock outcrops, semiarid grasslands and other habitats exhibit similar body shape and size (Kohlsdorf et al., 2001).

The limited morphological diversity among Tropidurinae lizards from non-forested environments is surprising given the striking ecological diversity that exists among these species. For example, rock and sand are common substrates for *Tropidurus* but contrast dramatically in friction and force restitution coefficients. Sand involves a higher energetic cost of locomotion and limits acceleration in comparison with non-granular surfaces (Hawkey, 1991; Lejeune et al., 1998; Kerdork et al., 2002). In addition, the greater exposure to predators and increased risk of overheating in sand dunes environments (Rocha, 1998) may favor high sprint speeds. In

the context of Arnold's paradigm (Arnold, 1983), therefore, sand species should exhibit traits that improve performance on this demanding substrate. However, given the absence of remarkable morphological differentiation within this taxon, physiological divergence may be responsible for relative changes in the locomotor performance of tropidurines from sandy habitats. This hypothesis is the focus of the present paper.

Various aspects of muscle physiology and energy metabolism are known to enhance the locomotor performance of lizards exhibiting contrasting behaviors and ecologies. For example, an increase in proportion of fast-glycolytic fibers associated with high limb cycle frequency appears to enhance sprint speed in phrynosomatid species that locomote on sand (Bonine et al., 2001). Improved endurance capacity, involved in the large distances covered by lizards that forage actively, is associated with high proportions of oxidative fibers in the leg muscles (Mutungi, 1992), high field metabolic rates (Anderson and Karasov, 1981) and high metabolic scopes (Frappell et al., 2002). Muscle fibers of a glycolytic nature contract quickly but fatigue rapidly, whereas slow-oxidative fibers, aerobic in nature, exhibit high endurance (Brooks et al., 1996) but require improved oxygen delivery to the muscle. Because of this functional link, positive correlations are expected among the proportion of aerobic fibers in leg muscles, the activity of oxidative enzymes, the muscle fatigue resistance, the aerobic scope and the degree of sustained activity of lizard species. The daily activity patterns demonstrated by a species should be related to the ecological pressures affecting that species (Irschick and Garland, 2001). The metabolic design of animal muscle tissue varies according to daily locomotor activity patterns (Kernell et al., 1998) and would consequently affect muscle power output and fatigue resistance (Rome, 1998). Therefore, species that use different habitats and locomote on substrates with distinct mechanical and energetic demands should exhibit variation in locomotor performance supported by differences in organismal aerobic capacity and muscle functioning, which is influenced by the proportion of fiber types and the activity of specific enzymes from glycolytic and oxidative pathways.

In the present study, we predict that (1) *Tropidurus* species that inhabit sandy environments will exhibit higher sprint speeds but will be less prone to jump, since their specific substrate does not favor activities that demand very high propulsive forces, (2) physiological differentiation of the leg muscles, specifically in the proportion of fiber types and enzyme activity, will be associated with the improved sprint speed performance in sand species and (3) variation in aerobic capacity (indicated by metabolic scopes) to sustain activity will be associated with an increased capacity to sustain jumping activity in rock species. We focus on two sister-species in the genus *Tropidurus* – *T. itambere* and *T. psamonastes* – that, despite systematic proximity and overall morphological similarity, exploit the two contrasting habitats in question. *Tropidurus itambere* is a rock-outcrop specialist found in Brazilian Cerrados (Fig. 1A; a habitat characterized by



Fig. 1. Pictures of Cerrado (A), the Brazilian habitat typical of *T. itambere* and *T. oreadicus*, which is characterized by rock outcrops and spaced trees, and Caatinga (B), the Brazilian habitat typical of *T. psamonastes*, which is characterized by loose sand dunes and scattered vegetation.

scattered trees and bushes and exposed rocks; for a description, see Van Sluys, 1991), while *T. psamonastes* exclusively utilizes the sand dunes of the Caatinga (Fig. 1B; a northeastern Brazilian habitat characterized by sandy soil, scattered shrubs, high substrate temperatures and seasonal and limited rainfall; for a description, see Rocha, 1998). As an outgroup for additional comparison (see Garland and Adolph, 1994), and to represent a species that uses more than one substrate type, we included *T. oreadicus*, a generalist species typical of Cerrados that moves amongst the rocks, fallen branches and bushes (Colli et al., 1992). We quantified the maximal sprinting capacity on rocky and sandy surfaces and the mean number of jumps produced for each of the three *Tropidurus* species. This latter variable was considered a behavioral indicator of propensity to jump in undisturbed animals and a physiological index of endurance in tests with uninterrupted stimulation. We also examined aerobic metabolic scopes as a whole-organism measurement of the ability to sustain activity aerobically. Additionally, to explore the underlying physiological basis of any interspecific variation in performance, we compared fiber type composition, power output and maximal activities of key metabolic enzymes (citrate synthase, pyruvate kinase and lactate dehydrogenase) of the iliofibularis muscle.

## Materials and methods

### Animals

Ten to 15 individuals of each *Tropidurus* species were collected by noose from different Brazilian localities: *T. itambere* Rodrigues 1987 (Atibaia, SP), *T. oreadicus* Rodrigues 1987 (Lageado, TO) and *T. psamonastes* Rodrigues 1988 (Ibiraba, BA). Animals were transported to the lab in cloth bags and maintained on a 14 h:10 h light:dark cycle at 27°C mean temperature in cages provided with 40 W lamps, allowing behavioral thermoregulation. Lizards were fed three times a week, always after performance experiments, and water was offered *ad libitum*. Individuals were maintained in captivity for one (*T. itambere*), two (*T. oreadicus*) or four (*T. psamonastes*) months. The general condition of each lizard was monitored every week by measurements of body mass. The animals used in the experiments maintained original body mass while in captivity.

### Locomotor performance tests

Sprint speed and jumping capacity were measured at 35°C in eight to 10 individuals of each species. One hour before the onset of experiments, animals were placed inside a climatic chamber set at the test temperature.

#### Sprint speed

Sprint speed was measured using a racetrack with five sets of photocells at intervals of 50 cm, connected to a computer using customized software. The substrate of this track could be changed to either rock or dry, white, fine-grained sand. Animals were stimulated to run by hand. Three trials on each substrate were conducted for each individual, and only the shortest time was used in computations. Races in which individuals ran against the walls of the racetrack or turned back before the end of the track were discarded and new trials were conducted an hour later. Both absolute and body length-independent sprint speeds were analyzed. Absolute sprint speed was considered indicative of the speed ecologically relevant for the lizards, since it indicates the time necessary to cross a specific distance whilst escaping from a predator.

Relative sprint speed (corrected by body length) is indicative of the speed physiologically relevant for the lizards, since it indicates how fast muscles can contract and impulse the body towards the movement, independently of the animal size. To verify whether or not performance had changed with captivity, the sprint speed of *T. oreadicus* and *T. psamonastes* was measured again prior to sacrifice, two and four months after the first trials; no changes were observed (*t*-test,  $P=0.735$ , d.f.=36).

#### Jumping capacity

Jumping capacity was tested on a jumprack (50 cm width; Fig. 2A) with barriers of different heights. Tests were conducted with one individual at a time. First, undisturbed animals inside the jumprack were filmed over 6 h, and jumping behavior and performance were quantified from the videotapes (Fig. 2A). In a second test series, individuals were stimulated to jump each barrier by tail tapping, starting with the lowest barrier, which was replaced by the next highest every time that the individual successfully jumped (Fig. 2B). The experiment was finished when the individual was exhausted and refused to move even when turned with the abdomen up. Jumps were classified as 'successful' when lizards crossed the barrier and as 'attempts' when lizards jumped but did not cross the barrier. Jumping success ratio for each species was calculated as the fraction of individuals that crossed the barriers in relation to the total number of individuals tested.

#### Respirometry

Metabolic scopes were calculated as the difference between activity metabolic rates (AMR), measured on a treadmill at horizontal level and inclined level, and resting metabolic rates (RMR). RMR and AMR were estimated from oxygen consumption in 9–10 individuals of *T. itambere*, *T. oreadicus* and *T. psamonastes* using open-flow respirometry, as described in detail by Withers (1977). Both experiments were conducted at 35°C using a PA-1 O<sub>2</sub> Analyzer (Sable Systems, Henderson, NV, USA) and a flow of 42.45 ml O<sub>2</sub> min<sup>-1</sup> for resting

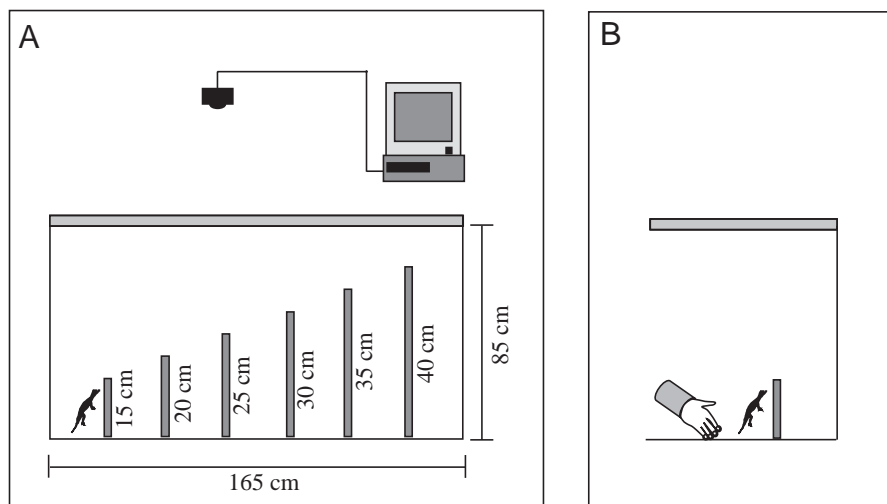


Fig. 2. Diagram of the jumprack used to verify motivation to jump (A, undisturbed animals) and jumping performance and endurance (B, by tapping lizards).

experiments and  $150.95 \text{ ml O}_2 \text{ min}^{-1}$  for activity experiments, with lizards in a post-absorptive state. Resting experiments were conducted with the lizard inside a metabolic chamber, and the oxygen consumption was recorded overnight (12 h). The lowest nadir of the signal generated by the gas analyzer (duration of at least 15 min) was used to calculate RMR. AMR were measured using a latex mask around the animal's head and stimulating the lizard to run on a treadmill set at  $1.2 \text{ m s}^{-1}$  at two inclinations ( $0^\circ$  and  $20^\circ$ ). AMR was calculated from the peak of oxygen consumption (usually three points, less than 1 min, since the animals don't run at a steady speed and present episodic ventilation). Three races were conducted at each incline, and the maximal value at each level of AMR was considered. All metabolic measurements were divided by body mass and corrected for standard conditions of pressure and temperature.

#### *Histochemistry*

Histochemistry was used to determine the proportion of different fiber types in the iliofibularis muscle of the three *Tropidurus* species. Four individuals of each species were weighed and then anesthetized and killed with an intraperitoneal injection of 0.5 ml of hypnol. The right legs were dissected and iliofibularis muscles removed without damaging the tissues. Muscles were dehydrated in talcum, frozen in liquid nitrogen and stored at  $-85^\circ\text{C}$ . Serial transverse sections ( $9 \mu\text{m}$  thickness) were obtained in a cryostat at  $-20^\circ\text{C}$  and stained with hematoxylin–eosin for morphological examination. Subsequent sections were also stained for NADH-TR (nicotinamide adenine dinucleotide tetrazolium reductase) and myofibrillar ATPase (alkaline preincubation pH 10.4 or acid preincubation pH 4.5) to evaluate oxidative activity and relative contraction speed (fast or slow), respectively (Bancroft and Stevens, 1982). Digital images from histological laminae were obtained from a light microscope connected to a computer by a video camera using the software program Stereo Investigator 2000 (MicroBrightField Inc., Colchester, VT, USA). Five to six fields of each cut were obtained using a  $10\times$  magnification. The frequency of fiber types was determined from digital images, and muscle fibers were classified as fast-glycolytic fibers (FG), slow-oxidative fibers (SO) or fast-oxidative and glycolytic fibers (FOG), according to Bancroft and Stevens (1982). We used only the NADH-TR results to perform statistical analyses because NADH-TR and ATPase reactions are complementary, which was confirmed by preliminary results. This complementarity is explained by the characteristic of oxidative fibers to sustain activity aerobically, contracting slower than glycolytic fibers, which contract fast but use mainly glycolytic pathways and fatigue quickly.

#### *Muscle mechanics*

Experiments of muscle mechanics were conducted to determine the ability of iliofibularis muscle both to generate power at different contraction velocities and to resist fatigue. Femur and tibia lengths were measured on 5–7 lizards of each

species. Body mass was measured to the nearest 0.01 g. Lizards were then killed using a guillotine. The right iliofibularis muscles were used for muscle mechanics and the left ones used for biochemical assays. Ilio-fibularis muscle was isolated at room temperature ( $25^\circ\text{C}$ ) in oxygenated (95%  $\text{O}_2$ ; 5%  $\text{CO}_2$ ) Ringer solution for reptiles ( $\text{NaCl}$   $145 \text{ mmol l}^{-1}$ ,  $\text{Na}_2\text{HPO}_4$   $2.15 \text{ mmol l}^{-1}$ ,  $\text{NaH}_2\text{PO}_4$   $0.85 \text{ mmol l}^{-1}$ ,  $\text{KCl}$   $4 \text{ mmol l}^{-1}$ , glucose  $10 \text{ mmol l}^{-1}$ ,  $\text{CaCl}_2$  solution  $2.5 \text{ mmol l}^{-1}$ , pH 7.60 at room temperature pre-oxygenation). Ilio-fibularis muscle is a flexor of the superior part of the leg and pulls the hind leg behind, pushing the body towards the movement (Romer, 1985). It is active during the swing phase of running or walking, when the femur is abducted and the knee is bent (Jayne et al., 1990). For each muscle, a small section of bone was left at the end of both proximal and distal tendons. Aluminum foil clips were wrapped around the tendons at either end of the muscle, leaving both sections of bone unwrapped to prevent tendon slippage in the clips.

#### *Isometric studies*

The muscle preparation was attached *via* the foil clips to a load cell (UF1; Pioden Controls Ltd, Canterbury, UK; calibrated to  $77.6 \text{ mN V}^{-1}$ ) at one end and a motor arm (V201; Ling Dynamics Systems, Royston, UK) attached to an LVDT (linear variable displacement transformer; DFG 5.0; Solartron Metrology, Bognor Regis, UK; calibrated to  $1.35 \text{ mm V}^{-1}$ ) at the other. The muscle was then maintained at  $35\pm 0.5^\circ\text{C}$  in circulating oxygenated Ringer solution for reptiles. The preparation was stimulated *via* parallel platinum electrodes while held at constant length to generate a series of twitches (stimulus amplitude, pulse width and muscle length were adjusted to determine the stimulation parameters and muscle length corresponding to maximal isometric twitch force production). A tetanic force response was then elicited by subjecting the muscle to a train of stimulation (250 ms). After 5 min, a twitch response was then initiated to confirm that there had been no slippage of the tendons in the foil clips. In the rare cases where twitch force had dropped, the muscle was re-clipped and twitch force was again maximized before tetanic stimulation was resumed. Stimulation frequency (180–240 Hz) was then altered to determine maximal tetanic force. A rest period of 5 min was allowed between each tetanic response.

#### *Work loop studies*

After optimization of stimulation parameters, the muscle was then subjected to cycles of three sinusoidal length changes (work loops; Josephson, 1985). Muscle stimulation and length changes were controlled *via* a D/A board and an in-house program produced using Testpoint software (CEC, Bedford, NH, USA). Data were collected at a rate of 1500 points per work loop cycle. For each work loop cycle, muscle force was plotted against muscle length to generate a work loop, the area of which equated to the net work produced by the muscle during the cycle of length change (Josephson, 1985). The net work produced was multiplied by frequency of length change cycles to calculate net power output.

The total strain of length change cycles was maintained at 0.12 (i.e.  $\pm 6\%$  of resting muscle length), as in Swoap et al. (1993). The cycle frequency of length change was altered at random from 4 Hz to 14 Hz to ensure that a power output cycle frequency curve could be generated to encompass the range of lizard limb cycle frequencies measured in previous studies (Swoap et al., 1993; Nelson and Jayne, 2001). During these length changes the muscle was usually subjected to phasic stimulation (active work loop cycle) but sometimes these length changes were performed without stimulation (passive work loop cycle) to monitor the net work done on the muscle during the length change cycle. For passive work loop cycles, the net passive power (net passive work multiplied by cycle frequency of length change) was used to indicate the power input required (the power absorbed) to move the unstimulated muscle through length change cycles. The passive power input values were also used to indicate the relative stiffness of the muscle, as increased passive power input was accompanied by an increase in muscle stiffness (indicated by an alteration in the shape of the work loop).

Every 5 min, the muscle was subjected to a further set of three work loop cycles with stimulation parameters being altered until maximum net work was achieved. Before the fatigue run, a set of control sinusoidal length change and stimulation parameters were imposed on the muscle every 3–4 sets of work loops to monitor variation in the muscles ability to produce power/force. Any variation in power was found to be due to a matching change in ability to produce force. Therefore, the power produced by each preparation, prior to the fatigue run, was corrected to the control run that yielded the highest power output, assuming that alterations in power-generating ability were linear over time. All muscles still produced over 70% of maximal control run power by the end of each experiment, i.e. prior to the fatigue run.

Iliofibularis muscles were then subjected to a fatigue run consisting of 32 work loop cycles. Recovery from fatigue was monitored by regularly subjecting the muscle to a set of three control active work loop cycles.

#### *Muscle dimension measurements and calculations*

At the end of muscle mechanics experiments, the aluminum foil clips, bone and tendons were removed from iliofibularis muscles and each muscle was blotted on absorbent paper to remove excess Ringer solution. Wet muscle mass was determined to the nearest 0.0001 g using an electronic balance (FA 1604; Shangping Inc., Shanghai, Jiangsu, China). Mean muscle cross-sectional area was calculated from muscle length and mass assuming a density of  $1060 \text{ kg m}^{-3}$  (Mendez and Keys, 1960). Maximum isometric muscle stress was then calculated as maximum tetanic force divided by mean cross-sectional area ( $\text{kN m}^{-2}$ ). Normalized muscle power output was calculated as power output divided by muscle mass ( $\text{W kg}^{-1}$ ).

#### *Biochemistry*

Measurements of the maximal activities of pyruvate kinase (PK) and lactate dehydrogenase (LDH), from the glycolytic

pathway, and citrate synthase (CS), from the TCA cycle, were made using spectrophotometric techniques based on absorbance changes of cofactors after substrate addition. The differences between absorbance changes of control (without substrate) and after substrate addition were used to estimate maximal enzyme activity. Iliofibularis muscle samples from the left leg of *T. psamonastes*, *T. itambere* and *T. oreadicus* were collected when animals were killed for the muscle mechanics experiments. Muscle samples were immediately frozen in liquid nitrogen and stored at  $-85^\circ\text{C}$ . As the iliofibularis muscle from these species is so small, it is difficult to quickly separate the regions corresponding to red and white fibers prior to freezing. For this reason, a transversal section of the muscle, with both regions, was used in enzymatic assays.

Muscle samples were homogenized using a Marconi homogenator (Marconi Ltd, Piracicaba, São Paulo, Brazil) in ice-cold  $20 \text{ mmol l}^{-1}$  imidazol (pH 7.4) buffer with  $2 \text{ mmol l}^{-1}$  EDTA,  $20 \text{ mmol l}^{-1}$  NaF,  $1 \text{ mmol l}^{-1}$  phenylmethylsulfonyl fluoride (PMSF) and 0.1% Triton X-100. The homogenates were then submitted to sonication using a U-200S control unit (IKA-Labor Technik, Staufen, Germany) for three 10 s intervals and directly used in the assays. Measurements were performed at  $35^\circ\text{C}$  with a Beckman DU-70 spectrophotometer, following the changes in the absorbance of NADH at 340 nm or 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) at 412 nm, under substrate saturation and in the absence of inhibitory conditions. All reactions were performed at a final volume of 1 ml in duplicate. Results were expressed in  $\mu\text{mol}$  of substrate converted to product per min per g wet mass. Enzyme protocols followed Bergmeyer (1983), with minor modifications as follow: PK (E.C. 2.7.1.40):  $100 \text{ mmol l}^{-1}$  imidazol (pH 7.0),  $10 \text{ mmol l}^{-1}$   $\text{MgCl}_2$ ;  $100 \text{ mmol l}^{-1}$  KCl;  $2.5 \text{ mmol l}^{-1}$  ADP;  $0.02 \text{ mmol l}^{-1}$  fructose-1,6-biphosphate;  $0.15 \text{ mmol l}^{-1}$  NADH;  $12 \text{ U ml}^{-1}$  LDH,  $5 \mu\text{l}$  of muscle sample homogenate (diluted 1:10) and  $3.6 \text{ mmol l}^{-1}$  phospho(enol)pyruvate (omitted for control). LDH (E.C. 1.1.1.27):  $100 \text{ mmol l}^{-1}$  imidazol (pH 7.0);  $5 \text{ mmol l}^{-1}$  DTT;  $0.15 \text{ mmol l}^{-1}$  NADH,  $5 \mu\text{l}$  of muscle sample homogenate (diluted 1:30) and  $1 \text{ mmol l}^{-1}$  pyruvate (omitted for control). CS (E.C. 4.1.3.7):  $50 \text{ mmol l}^{-1}$  Tris (pH 8.0);  $0.1 \text{ mmol l}^{-1}$  DTNB;  $0.2 \text{ mmol l}^{-1}$  acetyl-CoA,  $28 \mu\text{l}$  of muscle sample homogenate (diluted 1:10) and  $0.9 \text{ mmol l}^{-1}$  oxalacetate (omitted for control).

#### *Statistical analysis*

Body mass and length and iliofibularis muscle mass and length were compared by conventional analysis of variance (ANOVA) between the three species. An arcsine transformation was conducted on fiber proportions (Zar, 1996). One-way ANOVA or Kruskal–Wallis (according to normality) were used to analyze sprint speed, mean total amount of jumps (with and without stimulus), metabolic scope, activities of CS, LDH and PK, fiber type proportion (FG, SO and FOG) and power output at each cycle frequency (4, 6, 8, 10, 12 and 14 Hz). From muscle fatigue experiments, power output at

10th, 20th and 30th loops was calculated as percentage of power output of 1st loop. Frequencies were arcsine transformed and compared in a two-way ANOVA. Passive power input was analyzed using one-way ANOVA.

## Results

### Locomotor performance tests

The results obtained for locomotor performance, metabolic scopes, fiber type proportion and enzymatic activities are presented in Table 1. All three *Tropidurus* species ran faster on rock than on sand, but no interspecific differences were detected in relative sprint speed on sand or rock (Table 1). *Tropidurus psamonastes* was faster in absolute sprint speed on sand than *T. itambere* (Table 1), with absolute speeds almost 50% higher than the sister-species specialized on rocky habitats. However, *T. psamonastes* was much less prone to jump than *T. itambere* or *T. oreadicus*, either undisturbed or when stimulated constantly (Table 1). Individuals of *T. psamonastes* did not cross any barrier on tests without stimulus, whereas at least 50% of the individuals for *T. itambere* and *T. oreadicus* crossed barriers of 15 cm and 20 cm high. On tests with stimulus, the jumping success ratios of *T. itambere* and *T. psamonastes* were similarly low (both crossed only the first barrier), while at least 50% of individuals of *T. oreadicus* also crossed the barrier of 20 cm in height. Snout–vent length of *T. psamonastes* was greater than that of *T. itambere*, while *T. oreadicus* had an intermediate value (ANOVA,  $F_{2,3}=5.146$ ,  $P=0.012$  after Bonferroni correction).

### Physiological parameters

Fiber type proportion and CS activity differed among the three species: whereas *T. psamonastes* exhibited almost 20% more FG fibers than *T. itambere* (Table 1), *T. itambere*

exhibited a marginally higher proportion of SO fibers than *T. psamonastes* (Table 1). *Tropidurus oreadicus* presented intermediate values of fiber type proportion and CS activity (Table 1). The proportion of FOG fibers did not significantly differ among the species (Table 1). Variation in muscle composition was mainly related to proportion, and not number, of fiber types, since the total amount of fibers did not differ among species (ANOVA,  $F_{2,3}=0.35$ ,  $P=0.714$ ). In relation to enzymatic activities, the three species possessed similar values for PK and LDH, but *T. psamonastes* exhibited lower activity of CS than *T. oreadicus* and *T. itambere* (Table 1).

Maximal isometric stress did not differ among the species (Table 1) and was within the range (85–180 kN m<sup>-2</sup>) previously reported for the iliofibularis of other lizard species at this temperature (Putnam and Bennett, 1982). The relationship between peak power output and cycle frequency did not vary between species (ANOVA,  $P>0.50$ , G.L.=2, Fig. 3A). The fatigue resistance of the iliofibularis was also similar between species (ANOVA,  $P>0.05$ , G.L.=2; Fig. 3B). The iliofibularis muscle of *T. psamonastes* was longer (ANOVA,  $F_{2,3}=11.73$ ,  $P<0.001$ , after Bonferroni correction) and thinner (ANOVA,  $F_{2,3}=4.39$ ,  $P=0.029$ , after Bonferroni correction) in comparison with *T. itambere* and *T. oreadicus*, resulting in a lower calculated cross-sectional area. The net passive power inputs differed between the three species at the cycle frequency of 14 Hz (ANOVA,  $F_{2,3}=6.83$ ,  $P=0.008$ ; Fig. 3C), which suggests differences in muscle stiffness.

The three species studied did not differ significantly in the metabolic scopes attained at either horizontal or inclined races (Table 1) and exhibited values close to 0.2 ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup> in all situations.

Table 1. Mean  $\pm$  S.E.M. for performance traits, fiber-type proportion, metabolic scopes and enzyme activities of *T. itambere*, *T. psamonastes* and *T. oreadicus*

|   | <i>T. itambere</i>           | <i>T. psamonastes</i>          | <i>T. oreadicus</i>           | Statistical test                 | d.f. | <i>P</i> |
|---|------------------------------|--------------------------------|-------------------------------|----------------------------------|------|----------|
| Absolute sprint speed on sand (cm s <sup>-1</sup> )                               | 171.6 $\pm$ 10.53            | 230.0 $\pm$ 20.72 <sup>b</sup> | 211.4 $\pm$ 14.22             | Kruskal–Wallis (Dunn correction) | 2    | 0.020    |
| Absolute sprint speed on rock (cm s <sup>-1</sup> )                               | 211.2 $\pm$ 21.02            | 270.5 $\pm$ 13.75              | 257.9 $\pm$ 17.42             | ANOVA                            | 2    | 0.069    |
| Relative sprint speed on sand (body length s <sup>-1</sup> )                      | 19.3 $\pm$ 1.24              | 24.2 $\pm$ 2.19                | 23.8 $\pm$ 1.58               | Kruskal–Wallis (Dunn correction) | 2    | 0.091    |
| Relative sprint speed on rock (body length s <sup>-1</sup> )                      | 23.8 $\pm$ 2.37              | 28.5 $\pm$ 1.44                | 29.1 $\pm$ 2.00               | ANOVA                            | 2    | 0.136    |
| Total amount of jumps with stimulus   | 76.1 $\pm$ 9.24 <sup>a</sup> | 13.9 $\pm$ 3.18 <sup>a</sup>   | 44.9 $\pm$ 10.41 <sup>a</sup> | ANOVA (Bonferroni correction)    | 2    | 0.001    |
| Total amount of jumps without stimulus  | 57.2 $\pm$ 12.53             | 0.5 $\pm$ 1.58 <sup>c</sup>    | 14.5 $\pm$ 6.50               | Kruskal–Wallis (Dunn correction) | 2    | 0.001    |
| Metabolic scope – horizontal (ml O <sub>2</sub> g <sup>-1</sup> h <sup>-1</sup> ) | 0.2 $\pm$ 0.02               | 0.2 $\pm$ 0.01                 | 0.2 $\pm$ 0.02                | Kruskal–Wallis                   | 2    | 0.614    |
| Metabolic scope – inclined (ml O <sub>2</sub> g <sup>-1</sup> h <sup>-1</sup> )   | 0.2 $\pm$ 0.01               | 0.2 $\pm$ 0.01                 | 0.2 $\pm$ 0.01                | ANOVA                            | 2    | 0.389    |
| % Fast-glycolytic fibers (FG)   | 37.0 $\pm$ 1.91              | 56.2 $\pm$ 7.14 <sup>b</sup>   | 47.3 $\pm$ 4.55               | ANOVA (Bonferroni correction)    | 2    | 0.028    |
| % Slow-oxidative fibers (SO)  | 28.8 $\pm$ 2.96              | 16.6 $\pm$ 3.32                | 17.9 $\pm$ 2.05               | ANOVA                            | 2    | 0.052    |
| % Fast-oxidative-glycolytic fibers (FOG)  | 34.2 $\pm$ 2.40              | 27.2 $\pm$ 4.27                | 34.8 $\pm$ 3.12               | ANOVA                            | 2    | 0.346    |
| Activity of PK ( $\mu$ mol min g <sup>-1</sup> tissue)                            | 288.6 $\pm$ 21.26            | 248.3 $\pm$ 20.33              | 359.4 $\pm$ 23.36             | ANOVA                            | 2    | 0.133    |
| Activity of LDH ( $\mu$ mol min g <sup>-1</sup> tissue)                           | 709.7 $\pm$ 50.21            | 675.1 $\pm$ 45.12              | 702.3 $\pm$ 60.01             | ANOVA                            | 2    | 0.866    |
| Activity of CS ( $\mu$ mol min g <sup>-1</sup> tissue)                            | 8.0 $\pm$ 0.83               | 3.6 $\pm$ 0.23 <sup>c</sup>    | 9.6 $\pm$ 0.62                | Kruskal–Wallis (Dunn correction) | 2    | 0.002    |
| Maximal isometric muscle stress (kN m <sup>-2</sup> )                             | 165.8 $\pm$ 8.67             | 156.2 $\pm$ 11.22              | 144.6 $\pm$ 10.67             | Kruskal–Wallis                   | 2    | 0.457    |

Letters indicate statistical significant differences as follows: <sup>a</sup>three species different; <sup>b</sup>*T. psamonastes* different from *T. itambere*; <sup>c</sup>*T. psamonastes* different from *T. itambere* and *T. oreadicus*.

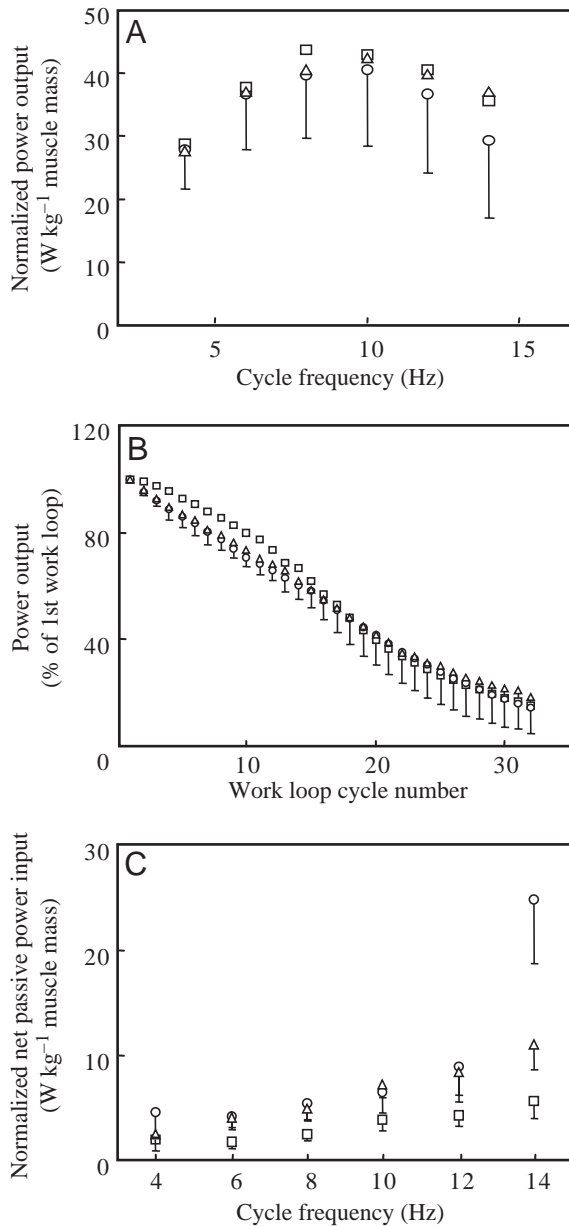


Fig. 3. Mean net normalized power output at different cycle frequencies (A), fatigue curves (B; given by percentage of power output produced on the first loop) and mean net passive power input measured at different cycle frequencies (C) of iliofibularis of *T. itambere* (triangles), *T. psamonastes* (squares) and *T. oreadicus* (circles). Bars correspond to standard errors, which are presented only for *T. oreadicus* in A and only for *T. itambere* in B because they are similar for the three species. In C, standard errors are presented for all species.

### Discussion

The *Tropidurus* species considered in this study contrasted in terms of locomotor performance and behavior. The sand specialist *T. psamonastes* jumps rarely but has high absolute sprint speed on sandy substrates, and the rock specialist *T. itambere* exhibits lower absolute sprint speed on sand but jumps more frequently. The more distantly related *T. oreadicus*

exhibits intermediate values for both number of jumps (on tests with constant stimulation or on undisturbed lizards) and absolute sprint speed. Although all three species exhibited comparable speeds on rocky substrates (both absolute and relative; see Table 1), the absolute speed of *T. psamonastes* is higher in its typical substrate, sand, thus favoring Arnold's paradigm (Arnold, 1983). Absolute speeds are of particular ecological relevance because they define how fast a lizard can flee from a potential predator. Differences in absolute sprint speed may be partially explained by the larger body length of *T. psamonastes* in comparison to *T. itambere*, allowing greater speeds due to the increased limb length while still maintaining the contractile properties of the muscle. However, the interspecific variation in locomotor performance could also be related to differences in stride frequency (Biewener and Gillis, 1999; Irschick and Jayne, 1999; Jayne and Irschick, 1999) and/or differences in parameters of muscle-tendon unit design, such as tendon dimensions (Biewener, 1998; Alexander, 2002). Additionally, subtle differences in foot morphology in sand species may elicit alterations in absolute sprint performance (Carothers, 1986; Arnold, 1995; Melville and Swain, 2000). Although only one hind limb muscle was examined, which limits our ability to evaluate the extent to which muscle physiology explains differences in locomotor performance, it is possible to suggest that physiological traits are involved as well, because *T. psamonastes* possesses a higher proportion of FG fibers in the iliofibularis (see below).

The three species studied exhibited the same rank (*T. itambere* > *T. oreadicus* > *T. psamonastes*) in both types of jumping test (i.e. with undisturbed and constantly stimulated individuals). The number of jumps for undisturbed animals was considered to be a measurement of an individual's propensity or motivation to jump, while the number of jumps for lizards stimulated until exhaustion was considered to be an index of jumping endurance. The interspecific differences observed suggest a contrast in jumping endurance that might have an underlying basis in muscle and metabolic physiology. The tendency towards a higher proportion of SO fibers in the iliofibularis of *T. itambere* (high jump endurance species) supports this prediction, although this trend is not statistically significant and not supported by either enzymatic or whole-animal metabolic analyses. The magnitude of differentiation in jumping performance among the three species does not parallel their taxonomic distances. The differences between the sister-species *T. itambere* and *T. psamonastes* are greater than those between them and the congeneric *T. oreadicus*, the more distantly related species (Frost et al., 2001). The strong divergence in jumping performance between the two sister-species suggests that *Tropidurus* species exhibit significant phenotypic plasticity of behavioral, physiological and locomotor traits and that this lability might rapidly lead to enhanced performance and fitness in their specific habitats.

The fiber type and enzyme profile of *Tropidurus* are probably related to ecological demands. *Tropidurus psamonastes* exhibits high absolute sprint speeds on sand and a high proportion of FG fibers in comparison with *T. oreadicus*

and *T. itambere*. The latter frequently locomotes on vertical surfaces and exhibits a comparatively high proportion of oxidative fibers, a trait that may be required for the increased work against gravity. Overall, our results support the relationship between fiber type proportion and locomotor performance that has been reported in other lizard genera (Putnam et al., 1980; Gleeson and Harrison, 1988; Mutungi, 1989, 1992; Young et al., 1990; Mirwald and Perry, 1991). These studies suggest high proportions of FG fibers in fast species (Putnam et al., 1980; Gleeson and Harrison, 1988; Bonine et al., 2001), while slower and more aerobic species exhibit high proportions of SO fibers (Mutungi, 1989; Bonine et al., 2001). Because of spatial restrictions, and given that an increase in number of fibers implies an increment in metabolic costs, an increase in proportion of one fiber type necessarily implies a decrease in the amount of the other fiber types (Alexander, 2000). SO fibers generally consume less ATP per unit force generated and use mainly oxidative pathways such as the TCA cycle, while FG fibers use mainly energy derived from oxygen-independent pathways (Hochachka and Somero, 1984; Hochachka, 1994). Dominant fiber-type might also be correlated with typical limb cycle frequency during locomotion because slow fibers are designed to produce power at slow speeds whereas fast fibers work optimally at the frequencies used during fast speeds (James et al., 1995).

In addition to a high proportion of FG fibers, *T. psamonastes* exhibits low values for CS activity in comparison with the other two congeneric species and with the same muscle in other lizard species (Guppy and Davison, 1982; John-Alder, 1984; Garland and Else, 1987; Gleeson and Harrison, 1988). The values of CS in *T. psamonastes* are comparable to the red and white muscles of sluggish species of fishes (Hochachka and Somero, 1984; Suarez et al., 1986; Moon and Mommsen, 1987; Moyes et al., 1992) and to the leg muscles of amphibians (Taigen et al., 1985). It is possible that there were no strong selective pressures for increasing the oxidative capacity of the iliofibularis muscle in *T. psamonastes*, since this species relies predominantly on short bursts of running to escape from predators or explore the environment. By contrast, *T. oreadicus* and *T. itambere* may have evolved higher oxidative capacities in their muscles associated to frequent use of jumps and vertical locomotion to explore higher portions in the habitat.

The exposed habitat of sand dunes, with greater distances between refuges, might favor lizards that exhibit high sprint speeds when escaping from predators, and sand species would probably need increased muscle power output at higher limb cycle frequencies to achieve higher sprint speeds. By contrast, the species from rocky environments, *T. itambere* and *T. oreadicus*, should possess greater muscle fatigue resistance correlating with their increased jumping endurance. However, both iliofibularis maximum muscle power output at different cycle frequencies and fatigue resistance are comparable among the three *Tropidurus* species, despite differences in locomotor performance and muscle morphology. Peak power output was between 40.5 and 44.0 W kg<sup>-1</sup> for each species, which is somewhat lower than the values for the iliofibularis of

*Dipsosaurus dorsalis* (Swoap et al., 1993). Despite the similarity in muscle contractile properties between the *Tropidurus* species studied, it is still possible that the *in vivo* performance of the iliofibularis differs among species, as performance of skeletal muscle fibers during movement is affected by length trajectory, amplitude and frequency of stimulation, initial fiber length and velocity of contraction (Marsh, 1999; Nelson and Jayne, 2001). Our study also found that species differed in the power required to lengthen the iliofibularis, given by the higher passive power input observed in *T. itambere* and *T. oreadicus* (Fig. 3C), which indicates greater muscle stiffness. This increased stiffness could feasibly occur as a result of more frequent jumping, which involves a lot of eccentric muscle activity (i.e. the muscle is active while it is being stretched), leading to muscle damage. Muscle damage of this type tends to lead to changes in collagen type or increased collagen content (Williams et al., 1988) acting as a protective mechanism to increase the forces subsequently required to stretch the muscle. The high jumping success ratio of *T. oreadicus* is possibly linked to this high net power input observed at high cycle frequencies.

The lack of differences in power output and fatigue resistance of *Tropidurus* iliofibularis muscles could also be supplemented by changes in other features not analyzed, such as tendon or limb bone compliance (not measured in *in vitro* work loops with isolated muscles), which may amplify *in vivo* muscle power output (Biewener and Roberts, 2000; Blob and Biewener, 2001). Alternatively, it is also possible that the iliofibularis is not the primary muscle limiting *in vivo* performance (Nelson and Jayne, 2001) or that *in vitro* measurements of power output may not reflect interspecific differences in *in vivo* conditions (Marsh, 1999). Animals may recruit different muscle groups or change activation patterns to vary contractile function (Biewener and Gillis, 1999) in response to shifts in environmental parameters (e.g. changes in substrate inclination) or activity mode (e.g. shift from running to jumping). In addition, kinematic adjustments may also play an important role in differences observed in jumping performance (Toro et al., 2003) and absolute sprint speed (Irschick and Jayne, 1999; Jayne and Irschick, 1999). It has been argued that natural selection and learning by experience may favor changes in kinematic parameters, such as step length, stance duration and limb cycle frequency, which maximize speed or minimize energetic costs (Alexander, 2000).

In conclusion, the present study shows remarkable divergence in absolute sprint speed on sand between closely related *Tropidurus* species that is correlated with physiological differentiation in the proportion of fiber types in the iliofibularis muscle. Additionally, propensity and endurance for jumping activity vary among *Tropidurus* species, with greater performance observed for those species exploiting more complex structural habitats. Differences in absolute performance on sand appear to be explained by some organismal traits, including body size, but are better predicted by proportion of glycolytic fibers in the iliofibularis than by



any other physiological parameter measured. The biomechanical and biochemical profiles of the iliofibularis muscle appear to be poor predictors of performance, but this trend may not apply to other muscles underlying locomotor performance. Evolutionary changes in behavior seem to be equally important for locomotor differentiation and may undergo more rapid divergence than morphology or muscle physiology (Blomberg et al., 2003). Further investigation of other physiological parameters that may constrain performance and/or the endocrinal basis for motivation may prove productive.

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