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Fight *versus* flight: physiological basis for temperature-dependent behavioral shifts in lizards

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Summary

Previous studies have demonstrated that a behavioral shift from flight to aggressive behavior occurs at low temperatures in some lizards. Our data for the agamid lizard *Trapelus pallida* demonstrate how the effect of temperature on whole organism performance traits such as sprint speed (much lower performance at lower temperature) and bite force (largely independent of temperature) may explain the shift from flight to fight behavior with decreasing temperature. Moreover, our data hint at the physiological basis for this effect as isolated muscle power output, twitch and tetanus time traits, relevant to sprinting, appear to be strongly temperature-dependent muscle properties. Maximal muscle force production, on the other hand, appears largely independent of temperature. Unexpectedly, differences in the physiological properties of jaw *versus* limb muscle were observed that enhance the ability of the jaw muscle to generate maximal force at all temperatures tested. Thus our data show how behavioral responses may be determined by the limitations set by temperature on physiological processes.

Key words: behavior, temperature, locomotion, biting, muscle physiology.

Introduction

The role of behavior in driving evolutionary changes in organisms over time is often debated. Although some authors suggest that behavior can act to trigger evolutionary changes in morphology, physiology and ecology (e.g. Mayr, 1963; Wyles et al., 1983), others suggest that regulatory behaviors may actually constrain evolutionary change by buffering the effect of environmental variation (see Huey et al., 2003). Using a null model approach, Huey and co-workers elegantly demonstrated how in the case of thermal physiology of lizards, behavior can effectively slow down the evolution of thermal sensitivity of ecologically relevant performance traits such as sprint speed. Specifically, changes in environmental temperature will cause behavioral changes that allow lizards to maintain a set range of body temperatures and thus will annul the need for evolution of thermal sensitivity of performance traits.

A classic example of a temperature-induced behavioral shift is the change from flight to aggressive behavior observed in some lizard species (Hertz et al., 1982; Crowley and Pietruszka, 1983; Mautz et al., 1992). In at least two species of agamid lizards (Huey et al., 1982), a xantusiid lizard (Mautz et al., 1992) and *Gambelia wislizennii* (Crowley and Pietruszka, 1983), animals change their behavior from trying to run away from a potential predator at higher temperatures to standing their ground and showing threatening displays and actual biting at lower temperatures. Although the change in behavior can be explained by the impairment of locomotor capacity at low temperatures (Bennett, 1980; Hertz et al., 1982; Bennett, 1990; Van Damme et al., 1990; Swoap et al., 1993), the actual shift towards biting and aggression is often considered a last resort alternative.

Aggressive behaviors and biting are likely also dependent on physiological processes that are themselves temperature dependent. For example, endurance capacity and maximal exertion, both traits likely important in the context of aggression and defensive behaviors, are strongly temperature dependent (Bennett, 1990). Biting, however, is one aspect of defensive behavior that is presumably less dependent on changes in temperature. Previous workers have shown that the force generation capacity of muscle has a low thermal dependence in most organisms studied, including lizards (Putnam and Bennett, 1982; Bennett, 1985; Marsh and Bennett, 1986). Thus, bite force generation, a trait relevant in aggressive interactions (Lailvaux et al., 2004; Huyghe et al., 2006) and likely also in a defensive context, may be less dependent on temperature than, for example, sprint speed. If so, then this may provide a physiological backdrop for the observed shift in

behavior. Moreover, this would suggest that the physiological properties of muscle (i.e. temperature dependence of force generation *vs* rate dependent processes) drive the observed shift in behavior.

Here, we test the hypothesis that a physiological drive causes changes in behavior by measuring the thermal dependence of sprint speed and bite force in *Trapelus pallida*, a lizard known to show a temperature-dependent shift in behavior (Hertz et al., 1982). Moreover, we examine the physiological basis for the observed differences in thermal dependence of sprint speed and bite force by investigating the effect of temperature on muscle contractile properties for limb and jaw muscle in the same species.

Materials and methods

Animals and husbandry

Ten adult *Trapelus pallida* Reuss 1834 obtained through the commercial pet trade were used in the experiments. Animals were transported to the laboratory at the University of Antwerp and maintained on a 12 h:12 h light:dark cycle at a temperature of 28°C with basking spots at a higher temperature (50°C). Crickets dusted with calcium powder were provided twice weekly and water was always present.

In vivo performance traits

Animals were placed in cloth bags and transferred to an incubator set at the test temperature (20°C to 37.5°C, increasing in steps of 2.5°C) at least 1 h before the onset of testing. Sprint speed was measured using a custom designed 2 m long electronic race-track equipped with photo-cells placed 25 cm apart and connected to a portable computer. Lizards were induced to run at maximal speed across the track and three trials per individual were conducted. At least 1 h of rest was given between trials and at least 1 day between different test temperatures. Test temperatures were randomized for each performance trait. The fastest speed attained during any 25 cm interval was considered an animal's maximal sprint speed at any given temperature.

Bite forces were measured using a custom-designed bite force apparatus based on a piezo-electric Kistler force transducer connected to a handheld charge amplifier with maximum hold function (Herrel et al., 1999). Animals were induced to bite the apparatus five times at each temperature and the highest bite force recorded was considered an animal's maximal performance at that test temperature. As with the sprint speed trials, animals were placed in the incubator at least 1 h prior to testing, given at least 1 h in between trials and at least 1 day in between test temperatures. Temperatures for bite force trials were randomized and bite forces were measured 1 week after the termination of the sprint speed trials.

Animals were weighed before and after the performance trials to test for decreases in condition. Since none of the animals showed any significant differences in mass, data for all individuals were used in the analyses.

Muscle physiology

Six lizards were transported to the laboratory at Coventry University, UK, where they were maintained as described earlier. Animals were killed by concussion and destruction of the brain in accordance with the British Home Office Animals Scientific Procedures Act 1986, Schedule 1. Immediately after being killed, animals were immersed in a bath of lizard oxygenated Ringer solution (see Swoap et al., 1993) where the m. caudofemoralis and the m. adductor mandibulae externus superficialis posterior were dissected from the animal. For each muscle, a small section of bone was left intact at the origin and insertion. For the m. caudofemoralis, the entire row of vertebrae upon which the muscle originates was left intact. After dissection, muscles were placed in fresh Ringer solution bubbled with oxygen until used. The m. caudofemoralis (Fig. 1) was chosen for our experiments as it is the largest hindlimb muscle in lizards and retracts the femur during the stance phase in lizards (Nelson and Jayne, 2001). The m. adductor mandibulae externus superficialis posterior (Fig. 1) was selected for its ease of access and because it is active during the power phase of biting in agamid lizards (Herrel et al., 1997).

Isometric properties

Muscle preparations were attached *via* aluminum foil clips wrapped around the tendon or by clamping the bone directly. The muscles were attached to a calibrated load cell (UF1; Pioden Controls Ltd, Canterbury, UK) at one end and a motor arm (V201; Ling Dynamics Systems, Royston, UK) attached to a calibrated linear variable displacement transducer (DFG

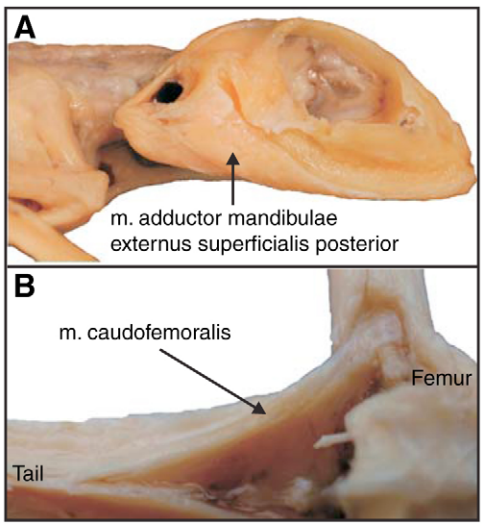

Fig. 1. (A) Lateral view of the head of a *Trapelus pallida* specimen with the skin, the jugal, the postorbital and the squamosal bones removed, thus exposing the m. adductor mandibulae externus superficialis posterior. The muscle originates tendinously at the dorsal aspect of the quadrate and inserts broadly along the lateral aspect of the dentary. (B) Ventral view of the right hindlimb and tail of *Trapelus* illustrating the insertion of the m. caudofemoralis onto the femur.

5.0; Solartron Metrology, Bognor Regis, UK). The muscle was immersed in circulating oxygenated Ringer solution and brought to the desired test temperature (20°C to 40°C, increasing in steps of 5°C). The preparation was stimulated *via* parallel platinum electrodes while held at constant length to generate twitch responses. Stimulus amplitude, pulse width and muscle length were adjusted to obtain maximal isometric twitch force. Time from stimulus to twitch force increase (latency), time to peak twitch force, time from peak force to half relaxation and peak twitch force magnitude were measured. Each tetanic response was elicited by subjecting the muscle to a train of stimuli (250 ms duration), after which the muscle was given 5 min rest and then another twitch response to test for muscle slippage. Stimulation frequency was then adjusted to obtain maximal tetanic force with at least 5 min of rest between subsequent tetani. Time to 50% peak tetanic force and peak tetanic force magnitude were measured. After all relevant parameters were measured (for caudofemoralis, see power output measurements section), the temperature of the bath was adjusted to the next test temperature with at least 30 min in between successive testing of the muscle after temperature equilibration.

Power output

After optimization of stimulation parameters, the m. caudofemoralis was subjected to cycles of sinusoidal length changes (work loops) (Josephson, 1985). Muscle stimulation and length change were controlled *via* a D/A board and an in-house program using Testpoint software (CEC, Bedford, NH, USA). Data were collected at 1500 points/cycle. For each cycle muscle force was plotted against muscle length to generate a work loop, the area of which is the net work produced by the muscle during the cycle of length change. Net work was then multiplied by frequency to obtain net power. Muscle strain (10%) and cycle frequency (8 Hz) were determined based on high-speed recordings (Red Lake Motion Pro, set at 500 Hz) of *Trapelus pallida* running on a race track. Passive cycles (i.e. without muscle stimulation) were run at the end of each work loop experiment to determine net passive work and power. Stimulation parameters (stimulation onset and duration) were adjusted until maximal net work was obtained. At the end of a temperature series (35°C, 20°C and 40°C), the muscle was returned to its initial test temperature (35°C) to quantify the decrease in power output due to changes in force production capacity over time. All muscles were still able to produce over 80% of maximal control power at the end of each experiment.

At the end of each experiment, foil clips, bone and tendon were removed and the muscles blotted dry on filter paper to remove excess ringer solution. Wet muscle mass was determined to the nearest 0.1 mg using an electronic balance (BP211D; Sartorius, Goettingen, Germany). Muscle cross sectional area was calculated from muscle length and mass assuming a density of 1060 kg m^{-3} (Méndez and Keys, 1960). Maximum isometric muscle stress was then calculated as maximum tetanic force divided by mean cross sectional area (kN m^{-2}). Normalized muscle power was then calculated as power output divided by muscle mass (W kg^{-1}).

Analyses

Thermal dependence of performance and muscle physiology was investigated using the minimum convex polygon technique (Van Berkum, 1986). Based on the polygons, the thermal performance breadth at which 80% of maximal capacity could still be generated (TPB80) and the optimal performance temperature (T_{opt}) were calculated. Differences in TPB80 and T_{opt} between performance traits and muscles were tested using paired *t*-tests.

Results

Temperature dependence of in vivo performance traits

As has been demonstrated previously for agamid lizards (Hertz et al., 1982), maximal sprint speed is strongly temperature dependent in *T. pallida*, with a fairly narrow TPB80 (10°C) and an optimum at 36°C (Fig. 2, Table 1). Bite force, on the other hand, was maintained at about 80% of its maximal capacity over nearly the entire range of temperatures tested resulting in a broad TPB80 (17°C) and a lower T_{opt} (31°C). Both the optimal temperature and TPB80 were significantly different between sprinting and biting ($P<0.05$).

Effects of temperature on isometric properties

Rate-dependent muscle properties such as latency, the time from peak twitch to half relaxation, and the time to peak force development, showed strong temperature effects and a fairly narrow TPB80 (Fig. 3, Table 1). Interestingly, for nearly all

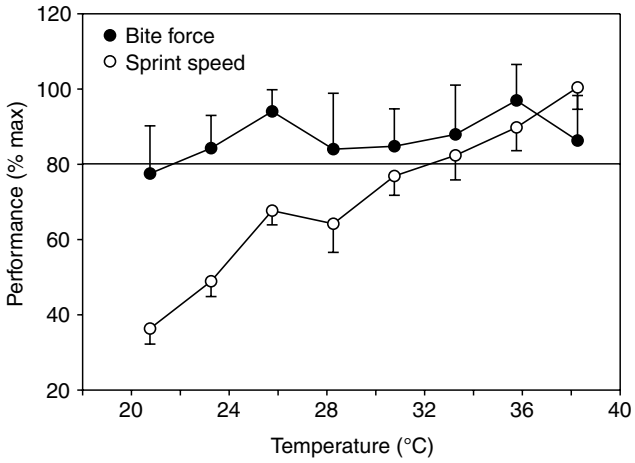

Fig. 2. Temperature dependence of *in vivo* whole organism performance traits in *Trapelus pallida*. Values are means ± s.e.m. (N=10). Note that bite force (filled circles) remains at almost 80% of its maximal capacity for nearly the entire range of temperatures tested. Sprint speed (open circles) on the other hand is strongly dependent on temperature and drops below 80% of its maximal capacity at a temperature of about 30°C.

Table 1. *Thermal dependence of performance traits and muscle physiology*

	Muscle	*N*	TPB80	T_{opt}
Sprint speed (m s^{-1})		10	**9.86±2.54**	**35.63±1.49**
Bite force (N)		11	**16.88±2.56**	**31.33±1.68**
Latency time (ms)	CF	6	7.92±2.86	**37.51±3.08**
	MAMESP	5	6.34±0.38	**39.41±0.13**
Time to peak twitch force (ms)	CF	6	**8.60±1.55**	**37.55±2.56**
	MAMESP	5	**6.50±1.40**	**39.49±0.26**
Time to 50% peak tetanic force (ms)	CF	6	**7.33±1.93**	37.93±2.04
	MAMESP	5	**11.54±5.41**	36.89±2.06
Time from peak force to half twitch relaxation (ms)	CF	6	6.25±1.94*	**36.60±3.51**
	MAMESP	5	5.73±0.26*	**39.60±0.00**
Peak twitch force (N)	CF	6	14.85±7.56	**34.08±2.69**
	MAMESP	5	11.62±3.26	**21.33±0.32**
Peak tetanic force (N)	CF	6	17.67±4.69	32.73±1.24
	MAMESP	5	21.54±0.75	32.20±2.24

CF, m. caudofemoralis; MAMESP, m. adductor mandibulae externus superficialis posterior; T_{opt}, optimal performance temperature; TPB80, 80% performance interval.

Values are means ± s.d. Values in bold are significantly different for the CF *vs* MAMESP or sprint speed *vs* bite force comparison. *Differences with *P*-values between 0.1 and 0.05.

Raw data are available from the authors upon request.

of these properties, with the exception of the time to 50% peak tetanic stress, the jaw closer muscle showed a narrower TPB80 and a higher T_{opt} than the femur retractor muscle (see Table 1). Force generation capacity, on the other hand, showed a much broader TPB80 and lower T_{opt} than that for rate-dependent processes. Although the TPB80 for the two muscles are not significantly different, the jaw closer muscle generates a higher proportion of its maximal capacity at 20°C (89 *vs* 79% of maximal stress respectively; $F_{1,8}$=3.85; *P*=0.085).

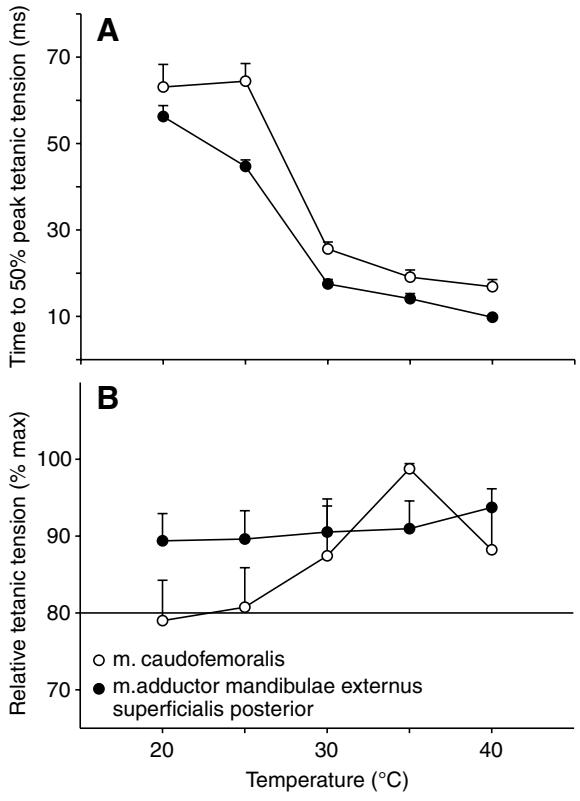

Fig. 3. The effect of temperature is different for the two muscles tested *in vitro*. Values are means ± s.e.m. (*N*=6). (A) Time (ms) needed to reach peak force in a tetanic stimulation increases significantly (over sixfold) as temperature decreased from 40°C to 20°C. Note also how the jaw closer muscle (m. adductor mandibulae externus superficialis posterior) is markedly faster than the femur retractor (m. caudofemoralis) at all temperatures. (B) Relative tetanic force as a function of temperature. Note how the peak force delivered by the jaw muscle is nearly independent of temperature (filled circles) whereas a notable optimum in force production appears to be present for the femur retractor (35°C; open circles).

Effects of temperature on muscle power output

As was previously demonstrated for the iguanid lizard *Dipsosaurus dorsalis* (Swoap et al., 1993), muscle power output was also strongly temperature dependent in *T. pallida*. The caudofemoralis muscle in *T. pallida* at 20°C was able to generate only 55% of the peak active power produced at 35°C (Fig. 4). The shape of the work loops in *T. pallida* at different temperatures is also remarkably similar to that observed for *D. dorsalis* [compare fig. 6 in Swoap et al. (Swoap et al., 1993) with Fig. 4B].

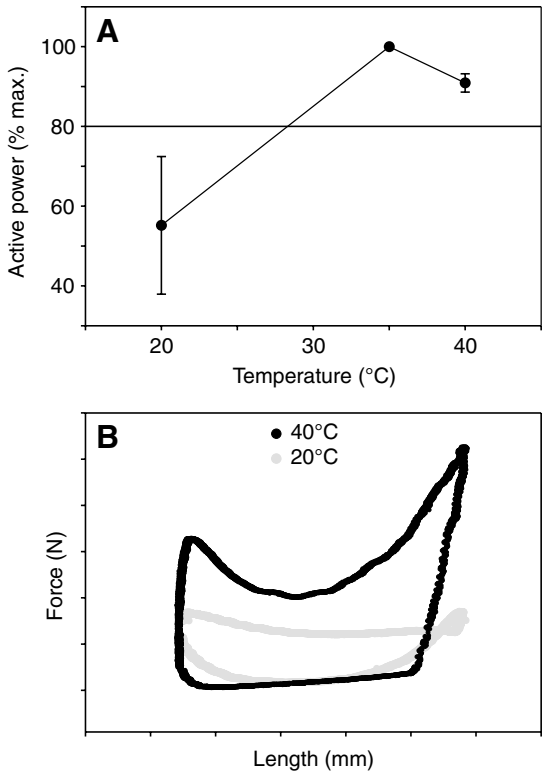

Fig. 4. The effect of temperature on peak active power of the m. caudofemoralis in *Trapelus pallida*. Values are means ± s.e.m. (*N*=6). (A) A clear decrease in muscle power output is notable between 35°C and 20°C. (B) Examples of work loops at 40°C and 20°C, respectively. Note how the shape and size of the work loops differ markedly at the two temperatures.

Discussion

Our data on the thermal dependence of sprint speed in *T. pallida* are in accordance with previous studies on lizards (e.g. Bennett, 1980; Bennett, 1990; Huey and Kingsolver, 1989; Van Damme et al., 1990; Swoap et al., 1993) that show that sprint speed is optimized across a relatively narrow range of temperatures (note, however, that the optimal performance range for sprints speed may be broader than that of other physiological processes). Bite force, on the other hand, appears largely independent of temperature and *Trapelus* lizards can maintain 80% of their maximal performance across a very broad range in temperatures. Only at the lowest test temperatures (20°C) does any decrease in bite force capacity become apparent (Fig. 2). These data suggest that the shift from an escape strategy to a strategy involving biting might be a good one as it allows an animal to take advantage of the thermal insensitivity of bite force. However, not all animals show the behavioral shift from flight to fight at lower temperatures. Indeed, *Anolis lineatopus*, for example, does not switch from flight to fight behavior at lower temperature, but rather becomes warier and initiates an escape response sooner when confronted with a predator at lower temperatures (Rand, 1964). Whether bite force is more strongly dependent on temperature in *Anolis* lizards, or whether their absolute size is too small to engage in fighting, remains unclear at this point but could be tested by measuring the thermal dependence of bite force in these animals.

The strong thermal dependence of rate-dependent processes involved in muscle performance in *T. pallida* is also in accordance with previous lizard data (Putnam and Bennett, 1982; Bennett, 1985; Marsh and Bennett, 1986; Swoap et al., 1993). Consequently, muscle power output and thus also sprint speed are strongly reduced at lower temperatures (see also Swoap et al., 1993), and may explain why lizards start to run earlier (Rand, 1964) or switch to alternative behavioral strategies (Hertz et al., 1982; Crowley and Pietruzka, 1983; Mautz et al., 1992) at lower temperatures. Our data also support previous studies that have shown that muscle force is largely independent of temperature and only decreases significantly at temperatures below 25°C (Putnam and Bennett, 1982; Marsh and Bennett, 1986). Unexpectedly, the drop in force at low temperatures was minimal for the jaw adductor muscle (Fig. 3) and force remained at nearly 90% of its maximal capacity for the entire range of temperatures tested. This, in turn, supports the observation that bite force in lizards also remains at roughly 80% of its maximal capacity across the entire range of test temperatures. The thermal dependence of rate-dependent processes, on the other hand, was greater for the jaw muscle than for the femur retractor (Table 1). Thus both muscles tested appear tuned towards their functional roles by being least dependent in the property that appears most relevant to their function task (force generation for the jaw muscle; power output, activation and relaxation times for the femur retractor).

Our data thus suggest that the shift from flight to aggressive behavior at low temperatures in lizards such as *T. pallida* is driven by the differential effect of temperature on rate dependent muscle performance *versus* peak muscle force generating ability. Thus, muscle physiology appears to permit behavioral responses in ectothermic organisms such as lizards. Clearly further comparative studies focusing on a set of closely related species that differ in their behavioral response to a change in temperature, such as agamid lizards of the genus *Trapelus*, would be needed to test the evolutionary significance of our finding.

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