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

A. Herrel\(^1\)*, R. S. James\(^2\) and R. Van Damme\(^1\)

\(^1\)Laboratory for Functional Morphology, Department of Biology, University of Antwerp, Universiteitsplein 1, B-2610 Antwerpen, Belgium and \(^2\)Department of Biomolecular and Sport Sciences, Coventry University, James Starley Building, Priory Street, Coventry, CV1 5FB, UK

*Author for correspondence (e-mail: anthony.herrel@ua.ac.be)

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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.

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**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 (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.
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.
Muscle strain (10%) and cycle frequency (8 Hz) were 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

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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.
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 (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; $F_{1,8}=3.85; P=0.085$).

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].

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

<table>
<thead>
<tr>
<th>Muscle</th>
<th>N</th>
<th>TPB80</th>
<th>$T_{opt}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sprint speed (m s$^{-1}$)</td>
<td>10</td>
<td>9.86±2.54</td>
<td>35.63±1.49</td>
</tr>
<tr>
<td>Bite force (N)</td>
<td>11</td>
<td>16.88±2.56</td>
<td>31.33±1.68</td>
</tr>
<tr>
<td>Latency time (ms)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td>6</td>
<td>7.92±2.86</td>
<td>37.51±3.08</td>
</tr>
<tr>
<td>MAMESP</td>
<td>5</td>
<td>6.34±0.38</td>
<td>39.41±0.13</td>
</tr>
<tr>
<td>Time to peak twitch force (ms)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td>6</td>
<td>8.60±1.55</td>
<td>37.55±2.56</td>
</tr>
<tr>
<td>MAMESP</td>
<td>5</td>
<td>6.50±1.40</td>
<td>39.49±0.26</td>
</tr>
<tr>
<td>Time to 50% peak tetanic force (ms)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td>6</td>
<td>7.33±1.93</td>
<td>37.93±2.04</td>
</tr>
<tr>
<td>MAMESP</td>
<td>5</td>
<td>11.5±5.41</td>
<td>36.89±2.06</td>
</tr>
<tr>
<td>Time from peak force to half twitch relaxation (ms)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td>6</td>
<td>6.25±1.94*</td>
<td>36.60±3.51</td>
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<tr>
<td>MAMESP</td>
<td>5</td>
<td>5.73±0.26*</td>
<td>39.60±0.00</td>
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<tr>
<td>Peak twitch force (N)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>6</td>
<td>14.85±7.56</td>
<td>34.08±2.69</td>
</tr>
<tr>
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<td>21.33±0.32</td>
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<tr>
<td>Peak tetanic force (N)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CF</td>
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<td>17.67±4.69</td>
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<tr>
<td>MAMESP</td>
<td>5</td>
<td>21.54±0.75</td>
<td>32.20±2.24</td>
</tr>
</tbody>
</table>

CF, m. caudofemoralis; MAMESP, m. adductor mandibulæ 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.

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**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 mandibulæ 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).
whether their absolute size is too small to engage in fighting, is more strongly dependent on temperature in predator at lower temperatures (Rand, 1964). Whether bite force initiates an escape response sooner when confronted with a lineatopus from flight to fight at lower temperatures. Indeed, of bite force. However, not all animals show the behavioral shift allows an animal to take advantage of the thermal insensitivity strategy to a strategy involving biting might be a good one as it

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; Mauz 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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