BLAZING BEETLES: THE EFFECT OF TEMPERATURE ON THE LOCOMOTION OF A NAMIB DUNE BEETLE

Jonathan Philips, Anthony Cobos, & Timothy Higham
Department of Entymology

ABSTRACT

Deserts represent some of the harshest ecosystems for life to survive in. In order to thrive, species must find novel adaptations either through behavioral or physiological modulation. The Namib desert of Southern Africa is no exception. In the Namib, temperatures can swing from 10 to 30°C throughout the day. Previous studies have indicated that there is temperature dependence in muscle power output in ectotherms. With the Namib being an understudied ecosystem, the present study aimed to investigate if invertebrate muscle output is affected by wild temperature fluctuations. *Onmyacris plana*, a Tenebrionid beetle endemic to the dunes of the Namib, was chosen due to being dorsally flattened, which results in low heat storage capacity. Although the thermoregulatory strategies, running ability, size and metabolic needs of *O. plana* have been studied, there have been no studies on the effect of temperature on the running performance of *O. plana*. We collected 8 beetles from the dunes and kept them in a vivarium in the lab. Beetles were subjected to three temperatures, to represent temperatures commonly experienced throughout the day. They were then placed on a 1-meter trackway in the lab and we made them run while recording them with a high-speed camera. Using the Matlab DLTdv5 digitizing tool we quantified their speed throughout the trials and analyzed differences in speed for the three treatments. We found no significant differences between running ability for beetles running at daytime temperatures and had significant difficulty in getting the beetles to run at temperatures experienced early in the morning. Our findings suggest that the beetles have a threshold muscle temperature which, if met, is sufficient for running at maximal speeds.

KEYWORDS: *Locomotion; Power; Tenebrionidae; Temperature; Behavior*

Jonathan Philips

Department of Entomology

Jonathan Philips is a fourth year Biology student at UCR. Over the course of his time here, he has been involved with three research labs and just last year, found his passion for Entomology. Last August, Jonathan studied abroad in Namibia, Africa and was able to study a beetle species native to the Namib desert. In addition to this, he also volunteers in an Entomology lab. Jonathan plans on returning to UCR for graduate school in the field of Entomology.

FACULTY MENTOR

Timothy Higham, *Associate Professor in the Department of Evolution, Ecology, and Organismal Biology*

Dr. Higham is an Associate Professor in the Department of Evolution, Ecology, and Organismal Biology. He received his PhD from the University of California, Davis. His research focuses on the ecological and evolutionary biomechanics of lizards, snakes, and fishes. Current work involves the predator-prey interactions between rattlesnakes and kangaroo rats, the dynamics of adhesion in geckos, and evolution of fish prey capture. He conducts fieldwork in a number of countries, including Namibia, French Guiana, Trinidad & Tobago, and Canada. He is an Alexander von Humboldt fellow, and is the graduate advisor for admissions in the Department of Evolution, Ecology, and Organismal Biology.

BLAZING BEETLES: THE EFFECT OF TEMPERATURE ON THE LOCOMOTION OF A NAMIB DUNE BEETLE

INTRODUCTION

From whales to aphids, temperature has a wide range of effects on the biological systems of animals. For instance, muscle function is greatly affected by temperature. Desert dwelling ectotherms have been analyzed for differences in running performance at different temperatures. In vivo raceway experiments have been done with the southwestern American lizard, *Dipsosaurus dorsalis*. Limb cycling frequency was analyzed at three trial temperatures and stride frequency was found to increase with temperature (Swoap et al. 1993). In ectotherms, it has been found that increased temperatures are associated with increased muscle power output and increased stride frequency.

In the tobacco hawkmoth, *Manduca sexta*, it has been found that maximum muscle power output and the operating frequency for muscle power output were both influenced by temperature with muscle power output being the most temperature-sensitive variable. In addition, it has also been determined that overall temperature dependence of both these variables decreases as temperature increases (Stephenson and Josephson, 1990). This suggests invertebrates have a minimum muscle temperature necessary for maximum muscle power output.

The mechanism by which muscle is affected by temperature is related to the definition of muscular power. Power is defined by the force multiplied by the contractile speed of the muscle. Calcium ion sequestration, which is vital for the crossbridge cycle and therefore contractile speed, is greatly influenced by temperature. Q10, a measurement of change in a system as a consequence of increasing temperature by 10 degrees, was analyzed in frog leg muscle. The Q10 of hind frog leg muscle for the crossbridge cycle and sarcoplasmic reticulum calcium ion sequestration was found to be 3.4 between 0-10 °C (Rall and Woledge, 1990). The rate of the mechanism behind contraction of muscle is restricted by the temperature of the muscle and any increase in temperature at low temperatures.

Figure 1. An example of a male *Onymacris plana*. Although there is no difference in the running ability of males and females, females are more rounded and curved around the edges of the elytron.

The Namib desert is home to an impressive array of ultra-psammophilous tenebrionid beetles. The shifting dune system is sparsely vegetated, with Bushman's grass (*Stipagrostis ciliata*) and !nara bush (*Acanthosicyos horridus*, ! is a click in the native language) being the most commonly found (Koch 1961). Tenebrionids can be seen running from shade source to shade source or found taking cover a few inches under the sand of a hummock created by plants. Observational studies on the genus *Onymacris* (Tribe: Adesminii) found that these beetles undergo behavioral thermoregulation. Thermoregulatory strategies include stilting to rapidly increase body temperature, squatting to decrease body temperature, climbing to escape the boundary layer of heat, and burrowing to both raise and lower their body temperatures (Henwood 1975). These beetles have been observed to be very active throughout most of the day, first emerging from the sand mid-morning when the sands heat up and retiring in the late afternoon. Since these beetles are highly active throughout the day, through a range of temperatures, it is possible that muscle power output is only loosely dependent on temperature.

All of the behavioral strategies discussed above have been observed in *O. plana*, a discoid dune beetle with exceptional locomotor abilities (Henwood 1975). Metabolic rates of *O. plana* have been tracked while running on a treadmill. While at speeds ranging from 0-13 cm/s, the volume of oxygen used by the beetle was found to linearly increase. However, from 13-22 cm/s, there was no change in the respiration needs of the beetles (Bartholomew et al. 1985). This suggests that there is a behavioral or physiological trait that enhances the running ability of *O. plana* at high speeds.

The literature value for average speed is 90 cm/s and no difference has been found between running speeds of either sex. Running speed and its effect on internal body temperature has been studied with *O. plana*. It was found that running, no matter the speed, had no effect on the body temperature of the beetle (Nicolson et al. 1984). *O. plana* has the highest muscle mass to weight ratio of the tenebrionids of the Namib desert (Nicolson et al 1984) which results in the incredible speeds of the species. The surface-area-to-volume ratio is high for *O. plana*, due to its saucer shape. *O. plana* have a body shape and size that is small enough that their heat storage capacity is minimal (Henwood 1975).

We hypothesized that external temperature would have an effect on the running performance of *O. plana*. We predicted that there would be a positive relationship between temperature and running speed.

METHODS

Animals

The investigation that is being reported here was conducted at Gobabeb Training and Research Institute, Namibia. We collected eight specimens by hand (Higham and Russell, 2010) from the dune 2.1 km southwest of Gobabeb. We collected all of our specimens between 9:00 am and 12:00 pm because *O. plana* are most commonly found feeding in this interval. As the sun comes out, *O. plana* will emerge from the sand and begin to stilt to gain heat. Once they have reached a sufficient temperature, they tend to run from bush to bush in search of food.

The specimens were kept in a vivarium with a lid and a hole drilled in the side for ventilation. We also provided them with sand to burrow into and *S. ciliata* to eat *ad libitum*. Of the beetles we collected, seven of them were male and only one of them was female. We continued to look for more females to observe any gait differences, but there were none to be found. It is unknown if females have different behavioral thermoregulatory strategies than males.

Materials and Experimental design

In order to keep track of individuals, we painted numbers one through eight on the elytra of the abdomen. Our trial temperatures were 10°C, 20°C, and 35°C. We manipulated the temperature of the beetles by exposing them to a freezer and a heat lamp. While we were manipulating their body temperature, they were isolated in a separate plastic container. We used an infrared gun to measure the temperature of the beetles. The temperature was read every minute that they were in the freezer, which was kept at 0 °C. In the freezer, the external temperature of the beetles fell by 3°C/minute. We held the beetles 30 cm away from the heat lamp, which had a surface temperature of 80 °C, to reliably raise their temperature by 10 °C/minute.

The trials took place on a one-meter long raceway set up in laboratory conditions. We randomized all of the trials to prevent skewing of the data from the beetles acclimating to the lab. The running surface was lined with 60 grit sandpaper to provide grip for the pre-tarsus of the beetles. An Edgertronic camera was set up to record high-speed video at 500 frames per second. A computer was hooked up to the camera to provide playback. The camera was 1 meter away from the raceway.

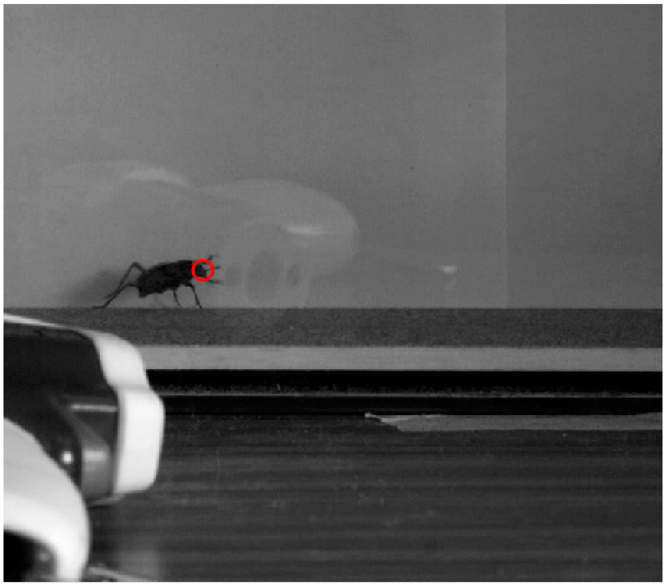

Figure 2. An example of the digitization of a trial recorded with high-speed video equipment.

In order to properly measure the distance that the beetle ran, we calibrated the camera by placing a ruler in the middle of the raceway and recording a short video of the ruler before beginning trials. Every time we moved the camera, we recalibrated in this manner. We then brought the beetle to the requisite temperature and placed it on the raceway, ensuring that it is always oriented in the correct direction. We used a thin 30 cm metal rod as a motivating stimulus to get the beetles to run. After each attempt at getting the beetle to run, we ensured that the beetles ran at least halfway down the track. (Note: the Edgertronic camera is always recording, we press "trigger" to save a specific interval of seconds before we pressed "trigger".) Our recording interval was six seconds. As the beetles do not tend to run in straight lines, we only recorded the trial if it ran the length of the raceway without hitting the sides. Each trial was replicated three times per individual at a given temperature; every individual was put through the same three temperature treatments.

Data analysis

To find the maximum instantaneous velocity of the trials, we used the Matlab DLTdv5 digitizing tool (Hedrick 2008). A single point was placed on the tip of the head, the mouthparts.

The software tracked the point through time; and sometimes it was necessary to manually track the point through the frames if the contrast was not high enough for the digitizing tool to track automatically. Each video had a point digitized from the time the beetle began to run, until it came to rest. We recorded the frame number that we began digitizing on and the last frame that was digitized. We chose to digitize the longest sprint in each trail. Once the trials were digitized, we exported the data to Excel and used the two-dimensional distance formula to find how many pixels the beetle advanced frame-by-frame. We then divided the number of pixels traveled by the frame rate to find pixels/second. Next, we used Excel to find the maximum velocity for each trial. After converting from pixels/second to cm/second, we picked the fastest trial per individual for each treatment for statistical analysis.

Statistics

We ran a repeated measures ANOVA using R studio (R studio team 2018). A repeated measures ANOVA was utilized because our trials were taking multiple measurements of temperatures effect on the same test subjects in order to see if there is a correlation between temperature and running ability. Our independent variable being tested was external temperature. Our dependent variable was speed of the beetles. Covariables include leg length and total mass of the beetles.

RESULTS

We had considerable difficulty getting the beetles to run at 10 °C. Of the eight individuals, five of them did not run at all at 10 °C. This suggests that at this body temperature, muscle output is too low for the beetles to run. Due to the surface area of the discoid beetle, heat is easily gained and shed by the beetle. When removed from the freezer, it was imperative that we place them on the raceway immediately. If left on the raceway for longer than a minute, the beetle would warm up beyond 1°C of 10°C. The same is true for 20°C and similarly, the beetles would decrease in temperature at a rate of roughly 4°C/minute when at 35°C. Although, the beetles always ran along the raceway when at all trail temperatures higher than 10°C. Since we were unable to get several of the beetles to run at 10°C, we only ran the repeated measures ANOVA for 20°C and 35°C trials. Our result ($p<0.2973$) for temperature indicates that, at least at higher temperatures, running speed is independent of temperature. We also found that total mass ($p<0.7504$) was an insignificant indicator of running speed. We measured the right hind leg length and used a repeated measures ANOVA to analyze the effect of leg length on running ability. The leg length was found to be an insignificant indicator of running performance ($p<0.6523$).

The average speed of the beetles did increase with each increase in trial temperature, but the standard error was very high for all temperatures, therefore this result was insignificant. The average speed for 10°C is the average for the three individuals that ran at 10°C.

The instantaneous velocity of *O. plana* is erratic and the resulting graph of speed over time can have very sharp peaks and troughs. To smooth the data, we used Excel to find the average speed of every three points. We then graphed the resulting data. Our data does not support our hypothesis. The fact that the beetles did not perform well at 10°C and ran at similar speeds for the other two trial temperatures suggests that at and above a certain temperature, running performance is constant. This is consistent with Stephenson and Josephson's findings that temperature dependence of muscle power output decreases as temperature decreases.

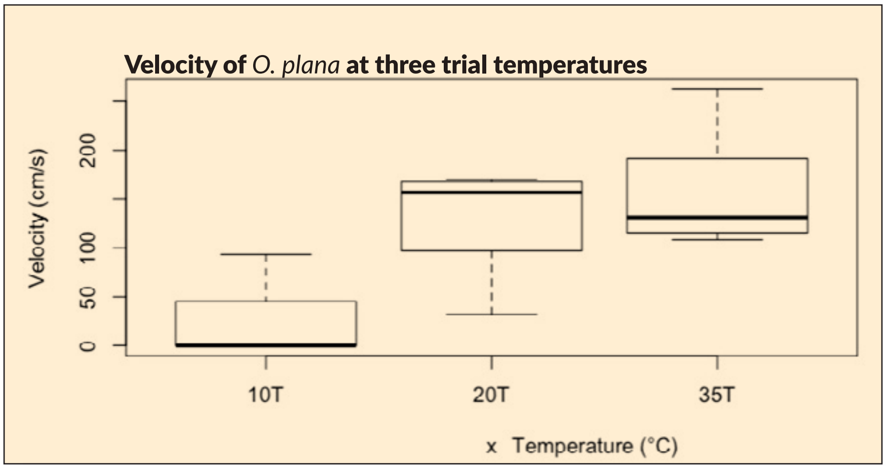

Figure 3. The box plot above shows the difficulty in getting *O. plana* to run at 10°C and the similarity in running capability at 20°C and 35°C.

DISCUSSION

When the beetles ran at lower speeds, they pitched, rolled, and yawed due to the movement of the legs. The femur was lifted higher and swung forward at a smaller angle than it did at higher speeds. At maximum velocity, the beetles lifted their body, so the femur was more parallel with the ground and swung their leg forward at a larger angle, resulting in a very level running stride.

O. plana does not need to increase its metabolic rate while traveling at higher speeds. This is representative of a species that has adaptations that allow it to run faster without higher metabolic needs. It has been hypothesized that the saucer shape of the beetle promotes lift when the beetle is at high speeds, thereby reducing the amount

	N	p-value
Temperature	8	0.2973
Leg length	8	0.6523
Mass	8	0.7504

Figure 4. p-values of the three variates.

Temperature (°C)	Average speed (cm/s)	Standard error
10°C	61.34	(+/-) 21.319
20°C	130.57	(+/-) 18.286
35°C	155.96	(+/-) 20.983

Figure 5. Temperature, average speed and standard error of the trials.

of weight that the legs have to push (Bartholomew et al 1984). Our high-speed trials indicate that *O. plana* is level throughout its stride when running at high speed.

Muscle power output was found to be decreasingly associated with temperature as the temperature was raised (Stephenson and Josephson 1990). These findings help elucidate our results. We suggested that there was not much variance in the speeds of *O. plana* at 20°C and 35°C because these beetles have full locomotor function if at or above a threshold temperature. This phenomenon has been observed in other beetles. The effect of temperature on the running ability of other beetles has been studied. Tiger beetles (*Cicindela hybrida*) were found to also have an increased average speed as temperature increased, but the variance was high, and temperature's effect was found to be insignificant much like *O. plana* (Dreisig, 1981). This threshold temperature would be the temperature that would allow the muscle to sufficiently shuttle calcium ions in and out of muscle cells while retracting and contracting. Their ideal locomotor metabolic needs are met at a low enough temperature that the beetles can run at full speeds during most of the day. This would be expected of a beetle that lives in an environment with wildly changing temperatures. In the winter, temperatures in the Namib can reach highs of 30°C and nighttime lows of 10°C.

CONCLUSION

The locomotor ability of *O. plana* was determined by running the beetles along a raceway at three trial temperatures. We found that there was no significant difference in the velocity of beetles at 20°C or 35°C. We also noted that *O. plana* has a unique locomotor ability; when flipped upside down, the beetle can right itself with use of its legs.

There were notable effects on the willingness to run and right themselves at 10°C, indicating that temperature does have an effect on the locomotor ability of *O. plana* at lower temperatures. It is unknown if this is a behavioral adaptation due to the individual recognizing its decreased running ability, or if it is a physical inability to move. Further investigations are needed. The muscle fiber in

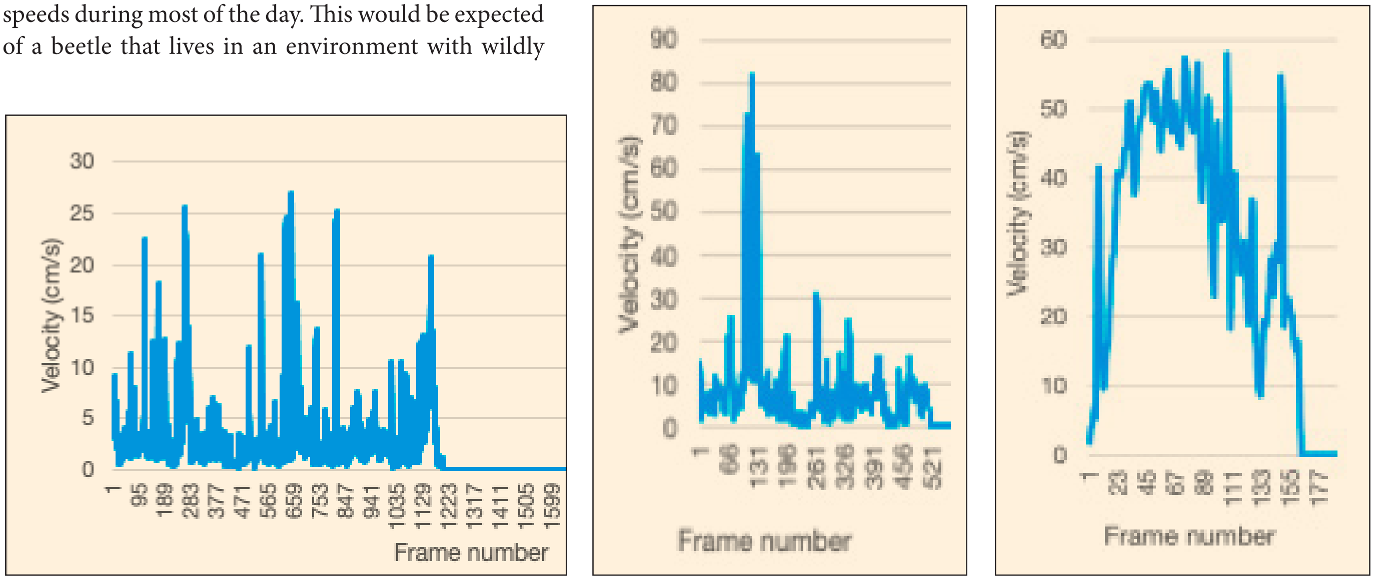

Figure 6. A smoothed rendering of velocity at every frame of the video at 10°C (left), 20°C (middle), and 35°C (right). These three representative trials were all by the same individual.

the leg should be directly studied for power output at different temperatures to remove the behavioral component of running speed. We suggest that the beetles are able to run at comparable speeds at the two higher trial temperatures due to the threshold temperature for maximum muscle contractile speed being met. It has been hypothesized that the *O. plana* does not have to increase its use of oxygen at high speeds due to a change in gait and the aerodynamic nature of the beetle's body. Qualitatively, we saw a change in gait and overall body posture when transitioning from a slower to a faster speed. The gait change at higher speeds results in the body traveling levelly and steadily forward.

REFERENCES

Bartholomew, G. A., Lighton, J. R. B. and Louw, G. N. (1985). Energetics of locomotion and patterns of respiration in tenebrionid beetles from the Namib Desert. *Journal of Comparative Physiology B* 155, 155–162

Dreisig, H. (1981). The Rate of Predation and Its Temperature Dependence in a Tiger Beetle, Cicindela Hybrida. *Oikos* 36, 196

Hedrick, T. L. (2008). Software techniques for two- and three-dimensional kinematic measurements of biological and biomimetic systems. *Bioinspiration & Biomimetics* 3, 034001

Higham, Timothy E. , Russell, Anthony P.,(2010) Divergence in locomotor performance, ecology, and morphology between two sympatric sister species of desert-dwelling gecko, *Biological Journal of the Linnean Society*, Volume 101, Issue 4, Pages 860–869

Koch, C. (1961) Some aspects of abundant life in the vegetationless sand at the Namib Desert dunes. *Scientific Papers of the Namib Desert Research Station* 9-92

Morgan, K. Temperature regulation, energy metabolism and mate-searching in rain beetles (Plecoma spp.), winter-active; endothermic scarabs (Coleoptera). *Journal of Experimental Biology* 1987 128: 107-122

Nicolson, S., Bartholomew, G. and Seely, M. (1984). Ecological correlates of locomotion speed, morphometries and body temperature in three namib Desert tenebrionid beetles. *South African Journal of Zoology* 19, 131–134

Stephenson, R. D. and Josephson, R. K. (1990). Effects of operating frequency and temperature on mechanical power output from moth flight muscle. *Journal of Experimental Biology* 149, 61–78

Swoap, S. J., Johnson, T. P., Josephson, R. K. and Bennett, A. F. (1993). Temperature, muscle power output and limitations on burst locomotor performance of the lizard Dipsosaurus dorsalis. *Journal of Experimental Biology* 174, 185–197

RStudio Team (2018). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA

Rall, J.A. and Woledge, R. C. Influence of temperature on mechanics and energetics of muscle contraction. *American Journal of Physiology* 259, 197-203