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Dung beetles use their dung ball as a mobile thermal refuge

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At midday, surface temperatures in the desert often exceed 60°C. To be active at this time, animals need extraordinary behavioural or physiological adaptations [1]. Desert ants, for instance, spend up to 75% of their foraging time cooling down on elevated thermal refuges such as grass stalks [2]. Ball-rolling dung beetles work under similar thermal conditions in South African savannahs. After landing at a fresh dung pile, a beetle quickly forms a dung ball and rolls it away in a straight line, head down, walking backwards [3]. Earlier studies have shown that some dung beetles maintain an elevated body temperature to gain a competitive advantage [4-6], and that heat shunting may prevent overheating during flight [7-8]. However, we know little about the behavioural strategies beetles might employ to mitigate heat stress while rolling their dung balls. Using infrared thermography and behavioural experiments, we show here that dung beetles use their dung ball as a mobile thermal refuge on which to climb and cool down, while rolling across hot soil. We further demonstrate that the moist ball functions not only as a portable platform, but also as a heat sink, which effectively cools the beetle as it rolls or climbs onto it.

To examine how dung beetles avoid heat stress on hot soil, we compared the behaviour of *Scarabaeus (Kheper) nigroaeneus* rolling their balls at midday from the centre of two sandy, circular arenas (ø 3m) in their natural South African habitat. One arena was shaded in the morning to keep it cool (median ground temperature 51.3°C), while the other one was exposed to full sunlight (median 57.2°C). At ground temperatures below 50°C, beetles exit the arena along a straight 1.5 m path without pausing. On hotter soil, however, they occasionally stop, climb onto their ball and perform a particular preening behaviour during which the front legs are repeatedly brought into contact with the beetles' mouth-parts (see Supplemental Video 1). Like desert ants, the beetles may regurgitate liquid onto their front legs during this contact to cool them down by evaporative cooling [9]. After preening, beetles usually perform an orientation dance [10] before continuing to roll.

Beetles perform this ball climbing behaviour progressively more often with increasing ground temperature (Figure 1A). Overall, they climbed onto their ball almost seven times as often in the hot arena as they did in the cool arena (3.1 ± 0.4 climbs/m vs. 0.45 ± 0.15 climbs/m; $W(52) = 1128$, $p < 0.001$). The average roll time between consecutive ball climbs dropped from 18.3 ± 0.98 s in the cool arena to 5.8 ± 0.34 s in the hot arena ($W(52) = 1258$, $p < 0.001$). As a result, beetles spent almost 70% of their time on the ball when the ground temperature exceeded 60°C.

What triggers this ball climbing behaviour? Infrared thermography (see Supplemental Video 2) shows that during each rolling phase, the surface temperature of the beetles' front legs (protibia) increases by as much as 10°C and then decreases again when the beetle is on the ball. At the same time, the thorax temperature changes only minimally (Figure 1B). The front legs of the beetles that climbed their balls most often in the hot arena cooled down by an average of about 7°C within 10 s on the ball (Figure 1C). To test if an increase in leg temperature during a rolling phase triggers ball climbing, we applied insulating "boots" made from dental silicone to the beetles' front legs (Figure 1D, inset) before testing them in a hot arena. With the boots on, the average rolling time between consecutive ball climbs almost doubled from 10.7 s to 20.8 s in the same individuals. Beetles with boots consequently climbed their ball 35% less often than without boots (Figure 1D, left bar group; $W(41) = 326$, $p < 0.01$), indicating that ball climbing is mediated by temperature changes in the front legs. This behaviour may have evolved to protect the sensory organs on the legs and head, as well as the beetles' brain, from temperature-related damage [9].

The dung ball can serve thermoregulation in three ways: as an elevated platform (i), as a heat sink (ii), and by cooling the sand (iii). (i) With an average diameter of 3-4 cm, the ball provides the beetles with a portable *platform* to escape the hot sand, similar to the function grass stalks fulfil for desert ants [2,11]. (ii) Due to evaporative cooling, the moist dung ball has a temperature of 31.8°C ± 0.92 S.D. ($n = 12$). This is substantially cooler than the beetle and soil, and the ball may therefore act as a *heat sink*, both during rolling and while the beetle is on the ball. If true, warmer balls should be less efficient heat sinks, and beetles should climb onto them more often. To test this, we let beetles roll a cold ball (15.8°C ± 4.5 S.D.) and a hot ball (40.1°C ± 2.3 S.D.) from the centre of the hot arena, and found that the beetles climbed a hot ball 73% more often than a cold ball (Figure 1D, right bar group; $W(50) = 397.5$, $p < 0.001$). This indicates that a cool dung ball indeed works as an efficient heat sink. (iii) Because beetles roll their ball rather than drag it, the ball – preceding the beetle – *cools down the sand* the beetle is about to step on. The temperature of the soil just after ball and beetle crossed it is 1.5°C lower than it was before ($n = 6$ beetles, ball temperature 33.6°C ± 1.9 S.D.).

We conclude that dung beetles are unique among insects in transporting a mobile thermal refuge – their dung ball. The ball functions as both a platform and a heat sink, and additionally cools down the sand that the beetle is about to step on. Beetles climb onto the ball when their front leg temperature, and presumably their head temperature, increases. Transporting a mobile thermal refuge means that dung beetles do not have to deviate from their chosen path to seek shelter, can cross featureless terrain devoid of any refuges, and are constantly in touch with their ball should the need arise to defend it against conspecifics. This unique set of thermoregulatory behaviours allows dung beetles to operate in conditions when heat stress prevents most other arthropods from being active.

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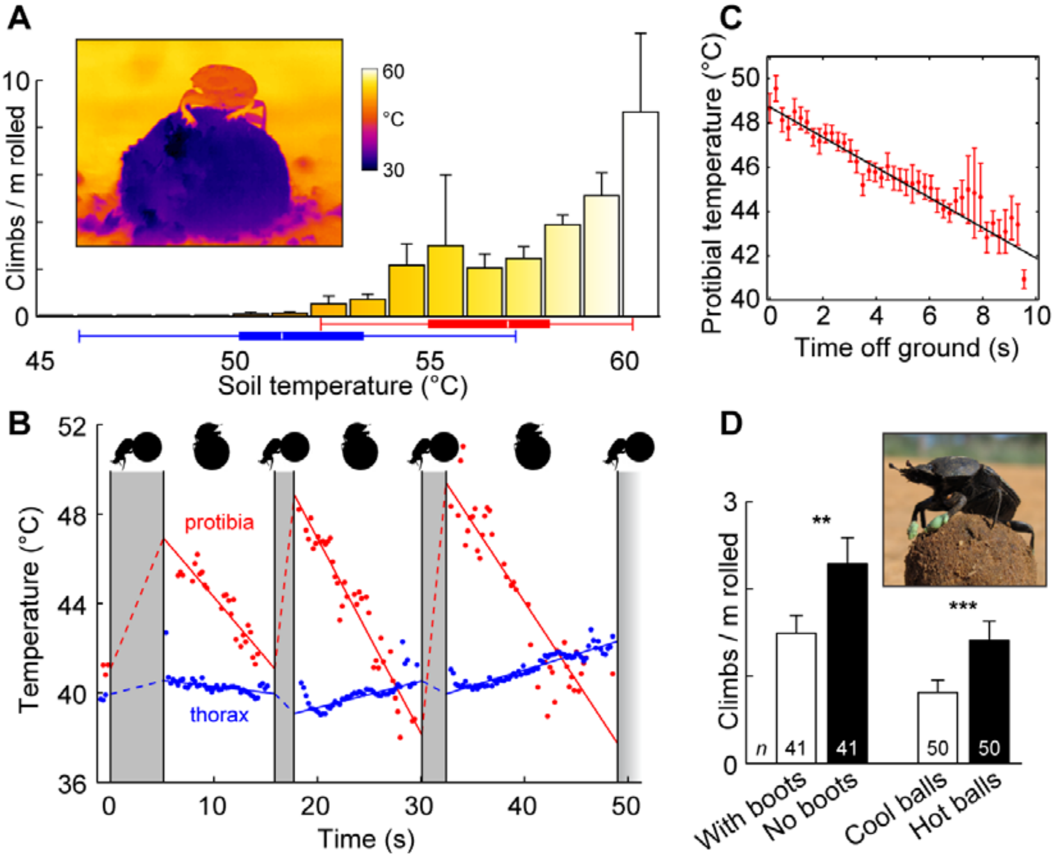

Figure 1. The dung ball as a mobile thermal refuge.

(A) With rising soil temperature, beetles climb onto their dung balls more frequently while rolling (*n* = 104). Red and blue box plots show temperature ranges in the hot and cold arena, respectively. Thermal image (inset) illustrates the elevated front leg (protibia) temperature just after the beetle climbed onto the ball. (B) Temperature of the right front leg (red) and thorax (blue) of a beetle during its first three ball climbs (soil temperature 55.8°C). Periods of rolling are shaded grey. Solid lines are regression lines, dashed lines interpolations. (C) Front leg (protibial) temperature profile (regression line in black) averaged over 84 ball climbs from 7 beetles (soil temperature 54.3°C – 62.8°C). (D) With silicone “boots” on their legs (inset), beetles perform fewer ball climbs. Similarly, beetles climb onto cool balls (15.8°C ± 4.5 S.D.) less often than hot balls (40.1°C ± 2.3 S.D.).

Numbers in text and figure show mean ± S.E.M. unless otherwise indicated; all statistical comparisons were performed using Wilcoxon signed-rank tests.

Supplemental Videos

Supplemental videos can be found at xxxxxxxxx.

Supplemental Video S1: Dung beetle rolling its ball on hot ground.

On hotter soil, beetles occasionally stop, climb onto their ball and perform a particular preening behaviour during which the front legs are repeatedly brought into contact with the beetles' mouth-parts.

Supplemental Video S2: Thermal video of a dung beetle rolling on hot soil.

Infrared thermography shows that during each rolling phase, the surface temperature of the beetles' front legs (protibia) increases by as much as 10°C and then decreases again when the beetle is on the ball.

Supplemental Experimental Procedures

Animals and experimental setup

All experiments were performed with *Scarabaeus (Kheper) nigroaeneus* in its natural habitat on the game farm "Stonehenge" in South Africa (24.3°E, 26.4°S) in February 2011 and 2012. The dung beetles were captured using pit-fall traps and kept in plastic boxes (30x22x22 cm) in the shade, where they were provided with soil and fresh cow dung. To ensure that all beetles were under similar heat stress at the start of an experiment, they were placed onto an area of loose (and therefore hot) sand until they exhibited their characteristic climbing and preening behaviour indicating heat stress. The beetles were then placed onto a dung ball in the centre of a circular experimental arena of 3 m diameter, from where they immediately rolled the ball away in a straight line. The beetles' 1.5 m paths out of the arena were filmed from beside the arena with a camcorder (Sony HDR-HC5E or Samsung VP-HMX20C) at 25 frames per second, or with a thermal camera (FLIR ThermaCam A325) at 4.3 frames per second.

Video analysis & Statistics

From the videos, we measured how often each beetle climbed its ball, as well as the timing and duration of each "ball climb" and "rolling" period. A "ball climb" was defined as any period during which the beetle stopped rolling and retreated onto the ball, without contact between the front legs and the substrate. A ball climb starts on the frame after the last ball movement, and ends on the frame before the first ball movement of the next rolling period. Ball climb duration is then the time difference from ball climb start to ball climb end. Roll duration is the time difference from ball climb end to the next ball climb start. The last roll period, which ended when the beetle crossed the arena perimeter, was ignored except in those trials where the beetle never climbed its ball.

Neither the number of ball-climbs nor the ball-climb/roll durations are normally distributed. We therefore used Wilcoxon signed-rank tests (the non-parametric equivalent of a paired t-test) for all statistical tests. To test for an effect of the order of conditions, we calculated generalised linear mixed models (GLMM, Poisson family, using beetle identity as a random term) in R (v2.11.0).

Experiment 1: Rolling on hot and cold soil

To test for an effect of soil temperature, two arenas were created by digging down to a depth of 10 cm until harder soil was reached. Both arenas were then flattened and covered with an even layer of approximately 1 cm deep, loose sand. Prior to each experimental day, one "hot arena" was chosen. This arena was covered with a black plastic tarpaulin overnight to reduce temperature loss and then exposed to the sun during the morning. Conversely, the second arena ("cold arena") was kept cool by leaving it uncovered overnight and shading it with the tarpaulin in the morning. Each arena alternated as hot or cold on consecutive days.

52 beetles were then individually placed into the centre of one arena, and filmed while they rolled their balls away. Once they exited the arena, they were placed into the other arena and the procedure repeated. Half the beetles experienced the hot arena first; the other half rolled their balls in the cold arena first. Which temperature was presented first was alternated between beetles, although this had no significant effect on the number of ball climbs (GLMM; no effect of order, $\chi^2 = 0.055$, $p = 0.81$; no order-condition interaction, $\chi^2 = 0.56$, $p = 0.46$).

The temperature of the soil in the two arenas was measured as an average from a ring around the beetle's position just before it commenced rolling. The total range of soil temperatures observed was 45.0 to 60.3°C, with median temperatures of 51.3°C and 57.2°C in the cold and hot arena, respectively. One outlier at 62.8°C followed the same trend as the rest of the data in Figure 1A (7.3 climbs / m), but was removed from the figure for clarity of display.

Experiment 2: Rolling with boots

To test whether an increase in leg temperature triggers ball climbing behaviour, we covered the beetles' front legs (protibia) with insulating "boots" made from fast-drying dental silicone (Coltène/Whaledent President light body polyvinylsiloxane). 41 beetles were then individually placed into the centre of a hot arena, and filmed while they rolled their balls away. Half the beetles started with boots on; the other half started without boots. Once the beetles exited the arena, boots were removed or new ones applied. Beetles were placed back into the centre of the arena and the procedure repeated. Due to the time required to fit and remove silicone boots, the second condition was performed 9.0 ± 6.4 min (mean ± S.D.) later when boots were applied and 3.3 ± 3.9 min later when they simply had to be removed.

Once again, the order of presentation had no significant effect on the number of ball climbs beetles performed (GLMM; no effect of order, $\chi^2 = 0.99$, $p = 0.32$; no order-condition interaction, $\chi^2 = 1.01$, $p = 0.60$). Both the average time spent on the ball and the time between two successive ball climbs almost doubled when wearing boots (from 10.9 s to 19.9 s and from 10.7 s to 20.8 s, respectively), indicating that the boots slow both the heating and cooling of the legs equally, and therefore fulfil a purely insulating function.

The total range of soil temperatures in this experiment (measured as for experiment 1) was 51.7 to 60.1°C.

Experiment 3: Rolling a hot or a cold ball

To test whether dung balls function as a heat sink, beetles were provided with balls that were either cooled (by placing them in a freezer) or heated (by leaving them in the sun for at least 30 minutes). In both conditions, balls were wrapped in aluminium foil to prevent desiccation. 50 beetles were individually placed into the centre of a hot arena, and filmed while they rolled their balls away. Half the beetles were tested with a cold ball first and then immediately placed back into the centre of the arena on a hot ball. For the other half of the beetles, the order was reversed. Which ball temperature was tested first was alternated between beetles. Again, the order of presentation had no significant effect on the number of ball climbs beetles performed (GLMM; no effect of order, χ^2 = 0.33, p = 0.56; no order-condition interaction, χ^2 = 0.69, p = 0.41).

Soil temperatures were measured as for experiment 1. Ball temperatures were averaged over the ball surface visible in the thermal camera just before the beetle started rolling. The range of soil temperatures observed was 53.0°C to 59.4°C, the range of temperatures recorded across all individual balls was 8.4°C to 44.8°C, with mean temperatures of 15.9°C and 40.1°C for the cold and hot balls, respectively.

Despite all care being taken to prevent dehydration of the ball, it is possible that, by the time the beetle exits the arena, the balls are not equally wet anymore. However, since each roll only took an average of 43 and 53 seconds (with a cold and hot ball, respectively), differences between the balls should be negligible.

Experiment 4: The cooling effect of dung balls

To determine how much a dung ball cools the sand, six beetles were filmed while rolling their balls over hot sand with the thermal camera positioned above them. The temperature profile of each point of the beetle's track was analysed (excluding the points where the beetles climbed onto the ball) before and after beetle and ball crossed it. The before-temperature T_b was defined as the mean temperature of frames -10 to -5 before t_0 (the time when the centre of the ball rested on that particular patch of sand). The after-temperature T_s was measured 1.5 seconds after t_0.

Infrared thermography

All temperatures were measured using thermal videos taken with a FLIR ThermaCam A325 at a resolution of 320x240 pixels, recorded at 4.3 frames per second. Using ThermaCAM Researcher Pro v2.10 (FLIR Systems Ltd.), thermal videos were converted into files readable by custom-written software in MATLAB (The Mathworks Inc.). With this software, the temperature of the front legs was measured at the centre of the tibia whenever it was visible while a beetle was on top of its ball (to prevent reflections from the ground). The temperature of the thorax was determined in an analogous manner.

To obtain accurate temperature readings in thermography, two parameters have to be known: the infrared emissivity ε of the material to be measured, and the apparent temperature of the background T_{refl} that is reflected on the object. The infrared emissivity of beetles, sand and dung was determined using a direct reflection method [S1]. This method basically involves measuring the object's reflectivity (which is 1 - ε) by comparing the reflections of a hot and a cold reference object from its surface. In detail, we followed this procedure:

1. Samples were placed on a table in front of a hot reference (a plastic container filled with hot water). The apparent temperatures (using $\varepsilon_{container} = 1$) of the hot reference and of its reflection from the sample were determined.
2. Following this, the procedure was repeated with a cold reference (a styrofoam sheet at room temperature).
3. Using the videos of these two calibration sequences, we then manually adjusted the sample emissivity until both measurements showed the same sample temperature.
4. In this way, we determined the emissivity of the beetle cuticle ($\varepsilon_{cuticle} = 0.951 \pm 0.038$ S.D., $n = 30$), the sand ($\varepsilon_{sand} = 0.975 \pm 0.014$ S.D.; $n = 4$), and the dung ($\varepsilon_{dung} = 0.957$, $n = 1$).

Considering how close these values are to the default setting used in most thermal cameras ($\varepsilon_{default} = 0.95$), this default was used for all our measurements. An error of 0.02 in this value translates into an approximate error of 1.2°C in absolute temperature estimates (calculated for a 40°C object), and 0.24°C in temperature differences (calculated for a 40°C and a 60°C object).

To create a constant background temperature T_{refl} for all field measurements, we took care to only measure temperatures when we could be sure that the object was reflecting the clear sky. Since a direct measurement of sky temperature was prevented by the limited range of our camera (-20°C to 120°C), we determined it using the following procedure:

1. A mirror was filmed at an angle where it reflected a plastic reference sheet. Knowing the mirror's emissivity ($\varepsilon_{mirror} = 0.796$, measured as described above) and the apparent temperature of the plastic sheet, this allowed us to calculate the mirror's temperature.
2. We then recorded a reflection of the sky in the mirror, and manually adjusted T_{refl} in our analysis program until the resulting temperature equalled the mirror's known temperature.
3. In this way, we obtained a mean apparent sky temperature of -44°C ± 18 S.D. ($n = 9$).

In our experiments, we used a rounded value of -40°C for T_{refl}. An error of 20°C in this value translates into an approximate error of 0.4°C in absolute temperature estimates (calculated for a 40°C object), and 0.05°C in temperature differences (calculated for a 40°C and a 60°C object).

Our estimates of beetle cuticle emissivity are slightly higher than those reported in a recent thermographic study on two other *Scarabaeus* species (0.79 for *S. sacer* and 0.81 for *S. cicatricosus*; [5]). These different values could reflect species differences or could simply be a consequence of different measurement techniques. Using these lower measurements for beetle cuticle emissivity, our estimates of both absolute beetle temperatures and temperature differences would be several degrees higher than reported.

Supplemental References

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