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Self-heating by large insect larvae?

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ABSTRACT

Do insect larvae ever self-heat significantly from their own metabolic activity and, if so, under what sets of environmental temperatures and across what ranges of body size? We examine these questions using larvae of the Japanese rhinoceros beetle (*Trypoxylus dichotomus*), chosen for their large size (> 20 g), simple body plan, and underground lifestyle. Using CO_2 respirometry, we measured larval metabolic rates then converted measured rates of gas exchange into rates of heat production and developed a mathematical model to predict how much steady state body temperatures of underground insects would increase above ambient depending on body size. Collectively, our results suggest that large, extant larvae (20–30 g body mass) can self-heat by at most 2 °C, and under many common conditions (shallow depths, moister soils) would self-heat by less than 1 °C. By extending the model to even larger (hypothetical) body sizes, we show that underground insects with masses > 1 kg could heat, in warm, dry soils, by 1.5–6 °C or more. Additional experiments showed that larval critical thermal maxima (CT_{max}) were in excess of 43.5 °C and that larvae could behaviorally thermoregulate on a thermal gradient bar. Together, these results suggest that large larvae living underground likely regulate their temperatures primarily using behavior; self-heating by metabolism likely contributes little to their heat budgets, at least in most common soil conditions.

1. Introduction

Like many ectotherms, insects face the problem of attaining reasonable body temperatures—temperatures that permit adequate performance, including feeding, growth, and reproduction—in environmental conditions that vary in space and time. In cool environments, insects may live far down on the left sides of their thermal performance curves (Deutsch et al., 2008). For insects in such a situation, there are exponential increases in performance to be gained by finding ways to achieve higher body temperatures. Insects often do so using behavioral thermoregulation (May, 1979; Heinrich, 1993), which involves moving through locally available mosaics of microsites and choosing sets of conditions that give higher body temperatures (Bakken, 1992). For individual insects, this is equivalent to choosing sites that increase inputs, and decrease outputs, to its heat budget—for example, by choosing microsites with relatively high air temperatures, high levels of incoming visible and infrared radiation, and relatively warm nearby objects (Woods et al., 2015) or that give low rates of heat loss by evaporation and convection (Gates, 1980). In hot environments, by contrast, the problem is to avoid body temperatures so high that the insect's performance is declining. In such environments, the insect needs to decrease inputs of heat and, if possible, increase outputs. In general, actively seeking out cooler conditions also involves behavioral thermoregulation.

Insects may also warm themselves up significantly using heat produced by their own metabolism. Whether they do so depends primarily on mass-specific intensity of metabolism, body size, and rates of heat loss, which are lower across well-insulated surfaces. Birds and mammals, most of which have high mass-specific metabolic rates and large body sizes (compared to insects), are the consummate endothermic homeotherms. Most produce enough heat to sustain constant body temperatures in the range of 35–40 °C. Insects of course are smaller and, with some significant exceptions (see below), have mass-specific metabolic rates that are an order of magnitude or more lower per unit body mass than those of birds and mammals (Robinson et al., 1983); in general, their body temperatures are much more closely coupled to ambient environmental conditions. Much of the time, they are ectothermic poikilotherms. Even so, it may be possible for insects to produce significant metabolic heat. The best examples are large flying adults, which can have extraordinarily high mass-specific metabolic rates (Bartholomew and Casey, 1978). Adults of several species of moths maintain thoracic temperatures above 38 °C when flying, which is possible because their thoracic muscles produce heat at such high rates (Heinrich, 1970; Casey, 1981). Indeed, some flying insects produce so much metabolic heat that they risk overheating (Heinrich, 1980, 1993); the difficulty then is to shed heat rapidly enough. This

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may explain why some large beetles and moths fly only at night.

Because holometabolous insect larvae do not power locomotion nearly as intensely as adults, their mass-specific metabolic rates while active generally are much lower. For example, a fifth-instar caterpillar of *Manduca sexta* consumes 30–50 μmol O_2 g^{-1} h^{-1} (Greenlee and Harrison, 2005) whereas an adult *M. sexta* in free flight consumes about 2 mmol g^{-1} h^{-1} (Heinrich, 1971a), which is 40 – 65× greater on a mass-specific basis. Nevertheless, several factors may allow larvae to self-heat. First, larvae generally have simple sac-like bodies, with relatively small ratios of surface area to volume and therefore relatively small surfaces across which to lose heat. Second, not all larvae have low mass-specific metabolic rates; for example, some herbivorous caterpillars feed and digest at very high rates, with that activity supported by high mass-specific rates of metabolism (Kingsolver and Woods, 1997). Third, larvae of some species can grow to very large absolute body sizes (always larger than the adults into which they metamorphose). Large insects generally have higher absolute metabolic rates (Chown et al., 2007) and thus produce more metabolic heat, possibly causing them to self-heat to greater extents. Fourth, larvae of many species live in confined spaces—underground, in rotting vegetation or wood, in constructed shelters—where there is little potential for evaporative or convective cooling (for example of bird eggs in such an environment, see Seymour and Bradford, 1992). In effect, those confined spaces can act as insulation.

We examine the potential for larval self-heating using the Japanese rhinoceros beetle (*Trypoxylus dichotomus*), which occurs in Japan, Taiwan, Korea and eastern China. On Honshu island, Japan, where these animals have been best studied, adult males defend wounds on the sides of *Fraxinus* sp. and other trees, which serve as feeding sites (Hongo, 2007a, 2007b; McCullough, 2012). Females visit these territories to feed and mate with males, leaving later to lay eggs in the soil near decaying humus (Karino et al., 2004). Eggs are laid between July and September, and larvae feed below ground until June – July of the following year (Plaistow et al., 2005). As they feed, larvae orient towards high concentrations of CO_2 (Kojima, 2015), which appears to lead them towards high quality, or especially fermented, humus. This also can lead to large aggregations of larvae (Kojima et al., 2012, 2014; Kojima, 2015), at depths down to 25 cm in the soil. At the end of their final (3rd) larval instar, animals construct brittle pupal cells, which are vulnerable to damage by other burrowing larvae.

Because of their extreme size, and the fact that they develop underground in decomposing—hot—organic matter, *Trypoxylus* larvae may be prone to overheating. First, larvae can reach over 30 g (D. Emlen, personal observation). If any larvae self-heat as a result of high metabolic rates and large body size, these are likely candidates. Second, their soil microhabitats provide little potential for evaporative or convective cooling and may insulate larvae from rapid heat loss by conduction. Using flow-through and thermolimit respirometry (Lighton and Turner, 2004), we measured larval metabolic rates and values of CT_{max}. In addition, we examined the ability of larvae to behaviorally thermoregulate on a thermal gradient bar. Lastly, we developed and analyzed a mathematical model of larval heat balance that predicts increases in body temperature from self-heating. We used the model to evaluate self-heating in soils of different temperatures and across a set of realistic body sizes. Finally, we extended the model to ask how much very large insect larvae (larger than extant) would self-heat.

2. Materials and methods

2.1. Animals

Larvae of the Japanese rhinoceros beetle were purchased from a commercial insect distributor (Yasaka Kabuto Kuwagata World, Hamada City, Japan) and reared to adulthood in the laboratory. Individuals were placed in plastic jars (1 L) containing substrate made from a 1:1 mixture of organic hardwood compost (Hiroki Gotoh, personal communication) and quick-fermented hardwood sawdust (Emlen et al., 2012). Additionally, eggs were collected from a local laboratory colony and allowed to grow to the third instar. 110 larvae were collected and placed in plastic jars (1 L) containing the previously described substrate mixture. We kept approximately 50 larvae at room temperature (22 °C) and another 60 in a temperature-controlled growth chamber at 10 °C to postpone pupation. Larvae were pulled from the growth chamber as needed and held at room temperature for 24 h before being used in experiments.

2.2. Larval metabolic rates

We estimated larval metabolic rates from rates of carbon dioxide emission, using flow-through respirometry. CO_2 levels were measured by an infrared gas analyzer (LI-7000, LI-COR, Lincoln, Nebraska) set up in differential mode. In this mode, dry, CO_2-free air from a cylinder of compressed breathing air (Norco, Boise, ID, USA) was first passed through the instrument's reference side, then past the larva and returned to the instrument's measurement side. The gas analyzer was calibrated using pure N_2 and 2000 parts per million (p.p.m.) CO_2 in N_2 (NorLab, Boise, ID, USA). Flow rates of gas were 200 mL min^{-1} (STP) and were regulated by a mass flow controller (Unit UFC-1100, 500 mL min^{-1} maximum flow rate, Yorba Linda, CA), which was controlled by a separate set of electronics (MFC-4, Sable Systems). The flow controller was recently calibrated at the factory, and we checked its flow at 200 mL min^{-1} against a bubble flow meter. Analog signals from the LI-7000 were sent to an A/D converter (UI2, Sable Systems) and then recorded using the ExpeData software (Sable Systems). Rates of CO_2 emission were converted to L min^{-1} O_2 consumption assuming a respiratory exchange ratio of 0.70 (Chown et al., 2007) and then transforming to μW of heat output using the conversion of 19.7 kJ L^{-1} of O_2 consumed (Schmidt-Nielsen, 1997) $\times 60$ s $min^{-1} \times 10^9$ μJ kJ^{-1}. Errors in estimates of the heat output per unit O_2 consumed will lead to an error of at most 7% in rate of heat production.

Carbon dioxide emission was measured for ten rhinoceros beetle larvae at room temperature (22 °C). Each larva was taken from its rearing jar (no prior period of food deprivation) and placed into a horizontal 110-mL cylindrical glass chamber (length 160 cm, diameter 3 cm) sealed by a Teflon end cap with two built-in O–rings. Air entered through a hole in the end cap and exited at the other end through a drawn out taper in the glass that was connected to Bev-a-line tubing, so that the chamber was flushed approximately twice per minute. Larvae were weighed and placed in the chamber 5–10 min prior to measurement to allow them to settle down. Sexes were unknown. Each larva's CO_2 output was measured for 15 min, during which time it was free to move. Movement had only slight effects on CO_2 emission, probably because movements themselves are very slow. CO_2 measurements were reported as the average p.p.m. for the last 7 min of each experiment. Because the larvae were not post-absorptive and we included periods during which they were moving, the metabolic rates derived from these measurements are neither resting nor standard; rather, they reflect digestion, absorption, and moderate activity. These conditions are the most appropriate for estimating rates of heat output during normal activity in the field.

2.3. Critical thermal maxima

The CT_{max} was determined for six larvae using thermal ramping. Because these beetle larvae are some of the largest insects ever measured, some in excess of 25 g, we developed a protocol based on work by Terblanche et al. (2007) and using the thermolimit analysis proposed by Lighton and Turner (2004) (see below). The primary considerations were to ramp slowly enough that larval body temperatures were in thermal steady-state with the temperature of the air in the