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The thermal environment of the nest affects body and cell size in the solitary red mason bee (*Osmia bicornis* L.)

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ABSTRACT

Many ectotherms grow larger at lower temperatures than at higher temperatures. This pattern, known as the temperature-size rule, is often accompanied by plastic changes in cell size, which can mechanistically explain the thermal dependence of body size. However, the theory predicts that thermal plasticity in cell size has adaptive value for ectotherms because there are different optimal cell-membrane-to-cell-volume ratios at different temperatures. At high temperatures, the demand for oxygen is high; therefore, a large membrane surface of small cells is beneficial because it allows high rates of oxygen transport into the cell. The metabolic costs of maintaining membranes become more important at low temperatures than at high temperatures, which favours large cells. In a field experiment, we manipulated the thermal conditions inside nests of the red mason bee, a solitary bee that does not regulate the temperature in its nests and whose larvae develop under ambient conditions. We assessed the effect of temperature on body mass and ommatidia size (our proxy of cell size). The body and cell sizes decreased in response to a higher mean temperature and greater temperature fluctuations. This finding is in accordance with predictions of the temperature-size rule and optimal cell size theory and suggests that both the mean temperature and the magnitude of temperature fluctuations are important for determining body and cell sizes. Additionally, we observed that males of the red mason bee tend to have larger ommatidia in relation to their body mass than females, which might play an important role during mating flight.

1. Introduction

In 1847, Carl Bergmann reported that the body size of endotherms is positively correlated with geographic latitude. Bergmann proposed the role of increased heat conservation capacity in larger animals associated with their relatively small body surface area as an adaptive mechanism (Voorhies, 1996). Similar latitudinal clines in body size were recently discovered in ectotherms, indicating that the heat-conservation hypothesis cannot be universally applied to all types of organisms. Moreover, many ectotherms respond plastically to thermal developmental conditions by emerging at larger adult sizes in colder conditions than in warmer ones, in a pattern called the temperature-size rule (Atkinson, 1994). Limited evidence suggests that ectotherms respond to different developmental temperatures by changing not only body size but also cell size (Blanckenhorn and Llaurens, 2005; De Moed et al., 1997; French et al., 1998; Van Voorhies, 1996). According to the theory of optimal cell size (TOCS), the thermal sensitivity of cell size has fitness consequences (Atkinson et al., 2006; Czarnoleski et al., 2013, 2015; Kozłowski et al., 2003; Szarski, 1983). Czarnoleski et al. (2013) argued that because oxygen permeates more readily through lipids in membranes than through the aqueous environment in the cytosol (Subczynski et al., 1989), a tissue built of small cells provides a more extensive network of cellular membranes for oxygen distribution than does a tissue built of large cells. Consistent with this interpretation, fruit flies develop small cells under poor oxygen conditions (Heinrich et al., 2011; Zhou et al., 2007). Similarly, a high demand for oxygen in warm environments favours small cells, which increase the rate of oxygen delivery to mitochondria, even under normoxia. However, a tissue that consists of numerous small cells should be more costly than one consisting of fewer, large cells because a large part of the cellular energetic budget is devoted to the maintenance of the physiological functionality of cell membranes (Kozłowski et al., 2003). According to Czarnoleski et al. (2013), the low demand for oxygen in cold environments does not justify costly small cells.

Experiments on the thermal sensitivity of cells and body size have been typically performed under controlled laboratory conditions (e.g., Czarnoleski et al., 2013, 2015; Partridge et al., 1994), and complementary evidence from thermal experiments set in semi-natural

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conditions is lacking. Here, we performed a field experiment with the solitary red mason bee (*Osmia bicornis*, Linnaeus) to assess the role of the thermal environment inside nests in influencing cell size and body size among emerging bees. In contrast to social bees such as the honeybee (Bonoan et al., 2014) and bumblebees (O'Donnell and Foster, 2001; Weidenmüller et al., 2002), solitary bees do not regulate the temperature inside their nests. Because access to nesting sites is limited (Potts et al., 2005; Steffan-Dewenter and Schiele, 2008), solitary bees appear to typically develop over a wide range of thermal conditions, suggesting that experiencing thermal variations is a part of their biology. We manipulated the thermal environment of developing larvae by restricting female establishment of nests to the following three types of sites: two naturally occurring microhabitats, one constantly exposed to full sun (sun site) and one shaded (shade site); and a warm and temperature-constant environment, which was artificially created by heating the nests to the desired temperature (heated site). Using this design, we created a range of thermal conditions, with site differences in mean temperature (sun and heated vs. shade) and thermal variance (heated vs. sun). Based on the temperature-size rule and the TOCS, we expected that warm nests and thermally fluctuating nests would result in smaller bees that consisted of smaller cells.

2. Methods

2.1. Study animals

The red mason bee (*O. bicornis*) nests in pre-existing cavities, such as reed stems or cracks in wood. A nest consists of linearly located cells, each containing an egg that is provisioned with pollen (Raw, 1972). Similar to many solitary species, the red mason bee is gregarious; however, females do not cooperate and provide food only to their own offspring. After food provisioning and egg laying, each cell is sealed, and larvae develop without contacting their parents. In temperate regions of Europe, the development of *O. bicornis* is completed by September (Wasielewski et al., 2011), and bees overwinter as adults, emerging in the spring.

2.2. Experimental design

At the onset of the experiment, nine artificial trap nests were constructed from bundles of reed straws. Thermal loggers (Thermochron iButton DS1921G, Maxim/Dallas iButton Products, accuracy ± 1 °C, measurements to the nearest 0.5 °C) were placed in the central of the trap nests and set to record the temperature every 120 min. On April 17, 2012, three trap nests per treatment condition were placed outdoors near the Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland. The treatments consisted of (i) exposure to direct sun (sun) or (ii) shade (shade) and (iii) controlled exposure to nest temperatures above 31 °C in a shaded area (heated). Each trap nest in treatment (iii) was wrapped in a wire aquarium heater connected to a thermostat. The following day, 80 cocoons originating from a bee culture that overwintered at ca. 4 °C were placed near each trap nest to allow the emerging females to establish nesting aggregations in our trap nests (Moroń et al., 2014; Steffan-Dewenter, 2002). The females were able to freely choose among all of the experimental trap nests; however, their choices were likely biased toward the nearest ones. The thermoregulation system in treatment (iii) was activated on 7th May after the mother bees had started nesting; therefore, the elevated temperatures established in the trap nests did not affect the females' initial choices of nesting site. Based on the timing of development in the red mason bee (Giejdasz and Wilkaniec, 2002; Wasielewski et al., 2011), we estimated that the developing larvae would have likely completed development by August 23. The temperatures recorded inside each trap nest between May 7 and August 23 were assumed to characterise the thermal conditions experienced during development (Table 1). Consistently, the thermoregulation system in treatment (iii) was active only to August 23. After overwintering, the nests were opened, and we conducted body size and cell size measurements of the bees.

Table 1
Thermal conditions in experimental trap nests and sample sizes in the experiment. M – males, F - females.

Trap nest	Treatment	Mean temperature (°C)	Standard deviation of temperature (°C)	Comments	Sample size
1	heated	31.40	3.06		3 F
2	heated	22.59	7.63	heater failure	8 M, 14 F
3	heated	30.17	2.89		4 M, 25 F
4	sun	20.17	7.86		3 M, 2 F
5	sun	20.33	8.05		6 M, 2 F
6	sun	20.61	8.91	no nests founded by bees	0
7	shade	19.94	5.29		14 M, 16 F
8	shade	20.35	5.92		10 M, 18 F
9	shade	20.71	5.90		10 M, 20 F

2.3. Measurements

After opening the nests, the bees were removed from their cocoons, weighed to the nearest 0.001 g and preserved by freezing for further analyses. Where the number of bees exceeded 30 per trap nest, we randomly chose 30 bees. Following earlier studies, we assessed the cell size of bees by measuring their ommatidia (Blanckenhorn and Llaurens, 2005; Stevenson et al., 1995). We measured the ommatidia from nail polish eye replicas according to methods of Schramm et al. (2015). An eye of a pinned bee was covered with a layer of transparent nail polish. After 4 h, each replica was removed with forceps and spread onto a histological slide. The slides were photographed under a Nikon Eclipse 80i light microscope (Japan) with a 10x objective using an AxioCam MRc5 camera and Zen 2011 software (Zeiss, Germany). The area with the largest ommatidia in the eye was visually selected and measured, and the ommatidia within this area were counted. The mean ommatidium area was calculated for each bee. Measurements and counts were made by using ImageJ (Schneider et al., 2012).

2.4. Statistical analysis

All analyses were performed in R ver. 3.2.3. (R Core Team, 2015) using the packages *lmer* (Bates et al., 2015), *lmtest* (Zeileis and Hothorn, 2002), *MASS* (Venables and Ripley, 2002), *car* (Fox and Weisberg, 2011) and *lmerTest* (Kuznetsova et al., 2016). Our primary hypothesis concerned the effects of thermal conditions experienced during development on ommatidia size (our proxy of cell size) and body mass. We used general linear mixed modelling (GLMM) with treatment (i-iii) and sex as fixed effects and trap nest nested within treatment as a random effect. The interaction between treatment and sex was also included in the models to investigate potential different responses of males and females to developmental conditions. As a change in cell size can be coupled with a change in body size, we analysed ommatidia size with an additional GLMM, which included body mass as a numerical covariate and the interactions of the covariate with treatment and sex. One sun trap nest with no established nests and one heated trap nest with a malfunctioning thermoregulation system were excluded from the analysis.

3. Results

Overall, bees established more nests in the shade than in the sun.