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Research article

Thermal preference for fungus culturing and brood location by workers of the thatching grass-cutting ant *Acromyrmex heyeri*

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Summary. Thermal preferences for location of brood and fungus by workers of the thatching grass-cutting ant *Acromyrmex heyeri* were investigated in the laboratory, using a temperature gradient between 10 °C and 37 °C. Workers from a large laboratory colony were motivated to pick up exposed brood and fungus by removing a small part of the protective nest thatching. Single workers carrying pieces of fungus or brood items were then gently removed from the colony and placed at one end of the temperature gradient, either at 10 or 37 °C. They were allowed to relocate the fungus or brood following their temperature preference. Both the probability of relocating an item and the temperatures selected were recorded after 45 minutes. The probability of relocation largely depended on the starting temperature, being higher for workers starting at 37 °C than at 10 °C, for both fungus and brood-carrying workers. For those relocated items, the selected temperatures averaged 25 °C and 24 °C for fungus and brood, respectively, when workers started from the hot end of the gradient. When workers started from the cold end of the gradient, the selected values were slightly lower, 22 °C and 21 °C for fungus and brood, respectively. These values match closely the temperature range in which maximal growth of the isolated attine ant fungus is achieved, between 20 and 25 °C, as reported in the literature, indicating that the thermoregulatory behavioral responses of workers maximize fungal growth. Thermal preferences and the construction of thatch mounds are discussed as regulatory responses for the control of fungus growth in leaf-cutting ants inhabiting temperate regions.

Key words: Leaf-cutting ants, temperature, fungus culturing, brood, nest climate.

Introduction

In ant colonies, the control of adequate microclimatic conditions for brood development is achieved through both the construction of a nest and the behavioral tracking of the appropriate conditions inside the nest by nurse workers that carry the motionless brood (Seeley and Heinrich, 1981; Roces and Núñez, 1995). Although the nest architecture can help to stabilize the climatic fluctuations inside the nest, brood transport by nurse workers ultimately represents a fine-tuning response to cope with excessive deviations from the adequate values.

The New World fungus-growing ants, tribe Attini, live in symbiosis with a fungus they cultivate, which provides the colony members with food. Although workers cover only a portion of their energetic demands by feeding on the fungus staphyllae, the fungus represents the unique food source for the developing larvae (Quinlan and Cherrett, 1979; Bass and Cherrett, 1995). As a consequence, workers are expected to maximize the growth of their symbiotic fungus not only by selecting suitable vegetable substrate, but also by an active choice of proper locations for culturing, according to the prevailing microclimatic conditions inside the nest. For instance, workers of a laboratory colony of *Atta sexdens rubropilosa* were observed to relocate the fungus garden along a humidity gradient, choosing the environments with the highest humidity (Roces and Kleineidam, 2000). With regard to temperature, it is known that the isolated attine ant fungus has strict demands of temperature for proper growth in vitro (Quinlan and Cherrett, 1978; Powell and Stradling, 1986). But remarkably, there are no published studies dealing with thermal preferences of attine ant workers for fungus culturing, even though they are responsible for the relocation of the fungus gardens when temperature varies beyond suitable values.

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It has been suggested that field colonies of the genus *Atta* move the fungus to different subterranean chambers according to their temperature (Eidmann, 1935; Weber, 1972). This appears to be a long-term response, since leaf-cutting ant species building large and complex subterranean nests are expected to achieve a more or less natural control of temperature in deep chambers buffered by surrounding soil (Kleineidam and Roces, 2000). In contrast, leaf-cutting ant species constructing more simple nests closer to the surface, particularly those inhabiting the southernmost latitudes of the attine ant distribution range (e.g., *Acromyrmex lobicornis*, *A. ambiguus*, *A. lundi, A. heyeri*; Farji Brenner and Ruggiero, 1994), may be more exposed to marked changes in environmental temperature.

Colonies of the thatching grass-cutting ant *Acromyrmex (Moellerius) heyeri* occur in open habitats of Uruguay, Brazil and the Pampean grasslands of Argentina (Bonetto, 1959). They construct a thatch mound with dry grasses and soil that protects a central and single fungus garden usually located at the general soil level (Gonçalves, 1961). Mound-building in *Acromyrmex* is unique among fungus-growing ants, and seems to represent an adaptation to low temperatures, since the thatch mound insulates the fungus to some extent and prevents rapid cooling (Weber, 1972). On the other hand, mounds may be warmed more rapidly by the sun than subterranean nests. As a consequence, behavioral control of fungus temperature is expected to be particularly developed in mound-building *Acromyrmex* species inhabiting areas that undergo large temperature variations.

In the present study, temperature preferences by workers of the thatching grass-cutting ant *Acromyrmex heyeri* for the location of both brood and fungus were investigated in the laboratory along a temperature gradient. The removal of brood and fungus from either 10 °C or 37 °C, and the temperatures selected to relocate them, were evaluated under otherwise constant conditions. This schedule allowed us to investigate the response of workers to low and high temperature values that are experienced by colonies in the field.

Materials and methods

Workers, brood and fungus used in the experiments were taken from a colony of *A. heyeri* collected in September 2000 in Joanico, Canelones, Uruguay (34°33′26″S, 56°15′59″W), and maintained in the laboratory at 25 °C under a LD cycle of 12:12 h. This temperature was chosen based on reports of highest growth rates of isolated attine ant fungus (Powell and Stradling, 1986). Experiments were performed in Würzburg in April 2001.

Temperature preference of *A. heyeri* workers to locate brood and fungus were analyzed in a controlled temperature gradient. It consisted of an acrylic box (30 cm long, 10 cm wide and 2 cm in height) placed on an aluminium base and located in a room at 22 °C. The temperatures of the two ends of the box floor could be independently regulated through Peltier-elements attached to a self-constructed thermostatic circuit. One end of the box floor was maintained at 10 °C (± 0.5 °C), and the other end at 37 °C (± 0.5 °C). Using this procedure, a roughly linear gradient of about 1 °C · cm^{-1} was achieved. Temperatures were measured directly at the box floor using thermistor-thermometers (Beckmann + Egle, Germany, Model MD 3150), at a resolution of 0.01 °C. Six thermometers were placed equidistantly along the box floor, thus allowing a long-term control of temperature. A relative humidity of 84% was maintained inside the box by placing a saturated solution of KCl (Solomon, 1951) in a narrow plastic container all along the box. This humidity was chosen, and not the most preferred one of 98% (Roces and Kleineidam, 2000), as it proved to be difficult to maintain a constant humidity at highest values because of the extreme temperature differences at the nest ends. Ants were prevented to contact the solution by covering the container with nylon gauze. Humidity inside the box was measured with a hygrometer at a resolution of 1% (measuring range at 25°C: between 2 and 98%).

Prior to each assay, thirty workers from the colony were placed inside the gradient-box and allowed to inspect the complete arrangement. This was essential to provide the box with colony odors, in order to avoid alarm behaviors by the workers during the assays. After at least three hours, workers were removed from the box and returned to the nest. Each assay was then initiated as follows. Workers from the large colony were motivated to pick up exposed brood and fungus by removing a small part of the nest thatching. As a reaction to this disturbance, single workers picked up either a fungus piece or a brood item, and searched for an appropriate place to relocate them. At this moment, five workers carrying fungus or a larvae, depending on the assays to be performed, were gently removed with forceps, put in a vial and placed together at one of the ends of the gradient-box, either 10 °C or 37 °C in independent assays. If workers showed signs of disturbance or alarm reactions, the complete group was removed and the procedure started again with the collection of five new ones. After 45 minutes, both the proportion of items moved from the end of the gradient-box and the temperatures selected to place the items were recorded. The items were usually piled and therefore occupied a small area of approximately 1 cm^2, so that we used the center of their distribution as the mean temperature chosen.

A total of 30 independent assays were performed with brood-carrying workers, each one using five new workers and items. Fifteen assays started with the workers being located at the cold end of the gradient, and other 15 were initiated from the hot end. Cold-end and hot-end assays were performed in random order. The same randomization schedule was used for the assays with fungus-carrying workers.

Results

Ants placed at the end of the temperature gradient with either fungus or brood quickly began to walk along the gradient searching for a suitable place to relocate the items. Usually after 15 minutes the items were either transported and placed at the selected temperature, or left at the starting point with no further removal. The observation time of 45 minutes assured that the behavioral responses completely stabilized, and that no transient responses were recorded.

The probability of relocating an item from the starting point largely depended on the starting temperature, being higher for workers moving from 37 °C than from 10 °C, for both fungus and brood-carrying workers (Fig. 1). Most of the fungus and the brood (81.3 and 97.4%, respectively) were relocated from 37 °C (Fig.1, grey bars; Chi-square goodness-of-fit to the ratio 1:0 for fungus: $\chi^2 = 66.13$, df = 1, $p < 0.001$; for brood: $\chi^2 = 94.74$, df = 1, $p < 0.001$). Worker responses from 10 °C differed for fungus and brood. Less than 20% of the fungus was relocated, and as a consequence, the final distribution of the fungus pieces could not be statistically distinguished from the initial. In contrast, ca. 40% of the brood was relocated from the cold end, so that their final distribution was significantly different from the initial (Fig. 1, white bars; Chi-square goodness-of-fit to the ratio

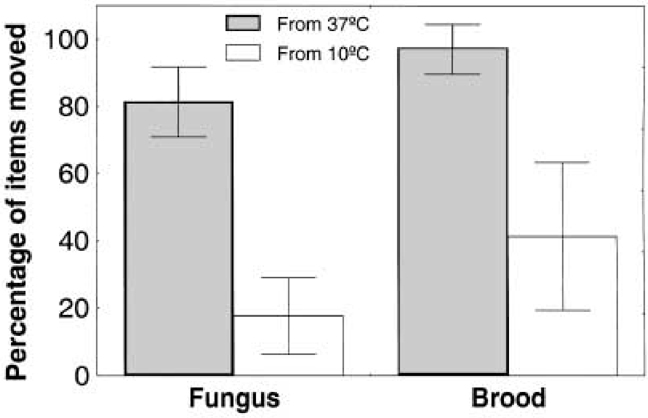

Figure 1. Proportion of items, fungus pieces or brood, transported along the thermal gradient by workers of *A. heyeri* (mean ± SD, n = 15). Workers started either from 37 °C or from 10 °C

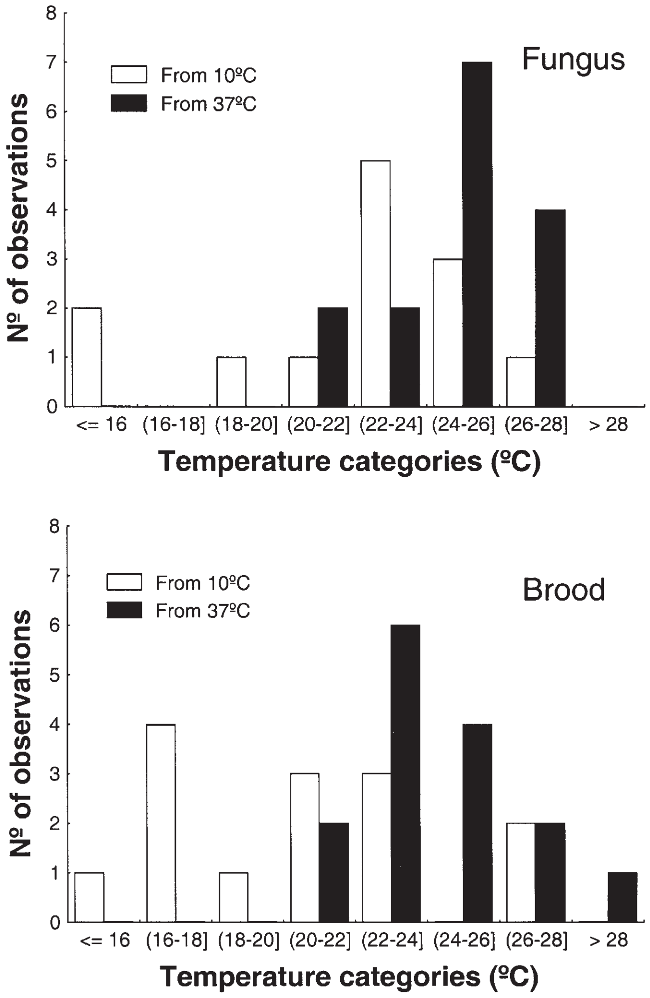

Figure 2. Temperature selected by *A. heyeri* workers to locate the fungus (a) or brood (b). Workers started either from 37 °C or from 10 °C, which represent the temperatures of the two ends of the gradient. In each assay, the relocated items were grouped by the workers at the selected temperature, so that each observation represents the response of five workers. For graphic representation of the frequency distribution, observations were grouped in intervals of 2 °C

1:0 for fungus: $\chi^2 = 3.13$, df = 1, p = 0.07, NS; for brood; $\chi^2 = 17.08$, df = 1, p < 0.001).

For the relocated items, the selected temperatures for both fungus and brood were similar when workers moved from 37 °C, i.e., 24.96 °C ± 1.80 for fungus and 24.35 °C ± 2.05 for brood (Fig. 2; mean ± SD; t-test for independent samples, t = 0.88, df = 28, p = 0.385, NS). The selected temperatures were also similar for both fungus and brood for workers starting at 10 °C: 22.11 °C ± 3.70 for fungus and 20.82 °C ± 3.90 for brood (Fig. 2, t = 0.87, df = 25, p = 0.389, NS). However, the temperatures selected for the brood depended on the starting point, being higher when workers started at 37 °C (Fig. 2, t = 3.07, df = 27, p = 0.005), whereas temperature selection for the fungus was slightly different, but statistically independent of the starting point (Fig. 2, t = 2.65, df = 26, p = 0.013).

Discussion

For both fungus- and brood-carrying workers, the probability to relocate items was strongly dependent of the temperature initially experienced by the workers. All items were quickly removed from 37 °C, and the values selected to place them were less variable than those chosen when workers removed items from 10 °C. These results clearly indicate the importance of avoiding high temperatures that are expected to be harmful for both fungus and brood. In fact, temperatures above 30 °C are lethal for the isolated attine ant fungus (Powell and Stradling, 1986), and it has been observed that a slight temperature increase causes workers of a laboratory colony of *Acromyrmex octospinosus* to move the fungus to a lower value (Quinlan and Cherrett, 1978).

On the other hand, brief exposure to low temperatures seems not to compromise the growth and development of fungus. Powell and Stradling (1986) reported that the growth of the isolated fungus can be stopped at low temperatures, but it continues when the temperature is increased again. In addition, even small colonies of *Acromyrmex lundi* with brood kept at 5–8 °C for 5 days recover and behave normally when temperature increases again (Weber, 1972). As for the brood transport, ants in our experiments responded to both starting temperatures, even though the probability of relocating brood was markedly lower when workers moved from 10 °C. This is unexpected if we consider that in insects, the developmental rate of the brood positively depends on temperature (Gilbert and Raworth, 1996), and workers are expected to try to maximize the growth rate of the brood. But on the other side, field colonies are sometimes exposed to even lower values for short periods (pers. obs.), so that immediate responses may not be as critical as it is the case for high temperatures. Chilling is unlikely to be responsible for the lower percentage of items moved in our experiments, as workers were not observed to be chilled or to move particularly slow at 10 °C, and field observations indicate that they are active even at lower temperatures.

The selected temperatures averaged 25 °C and 24 °C for fungus and brood when workers started from the hot end of the gradient, and were slightly lower, 22 °C and 21 °C, when they started from the cold end. These values match very well the temperature range in which maximal growth of the isolated attine ant fungus is achieved, as reported in the literature. Powell and Stradling (1986), working with the symbiont fungus of three attine ant species, found highest growth rates between 20 and 25 °C. The *A. octospinosus* isolated fungus grew best at 24.5 °C, and hardly at all at 10 °C and 37 °C (Quinlan and Cherrett, 1978). Similar results were obtained by Cazin et al. (1989) working with *Atta cephalotes* isolated fungus, which grew better at 25 °C than 30 °C, and not at all at 37 °C. Therefore, our results show for the first time in leaf-cutting ants that the thermoregulatory behavioral responses of workers indeed maximize fungal growth. The behavioral preferences are also congruent with reports about fungus temperatures recorded in field colonies: 25–28 °C for *Atta sexdens* (Stahel and Geijskes, 1940; Eidmann, 1935, but 20.2 °C were reported by Parra et al., 1974), 27.5 °C for *Atta vollenweideri* (Kleineidam and Roces, 2000), 25.6 °C for *Acromyrmex coronatus* (Parra et al., 1974), and around 27 °C for *A. heyeri* (Zolessi and Abenante, 1998).

What is the ecological relevance of the thermoregulatory responses by workers of *A. heyeri*? This species has a distribution area that includes the temperate Neotropical zone (Fowler et al., 1986), so that extreme air temperatures are usual and they are expected to largely influence nest temperature. For instance, mean temperatures of 10 and 28 °C were measured in the fungus garden of the mound-building leaf-cutting ant species *Acromyrmex lobicornis* in winter and summer, with soil surface temperatures of 4 and 31 °C, respectively (Quiran and Pilati, 1998). Our results suggest that in winter, workers may first try to move the brood to more protected areas inside the nest, and then the fungus. Or they may even relocate the fungus on an hourly-basis following the daily changes of temperature, as suggested by Zolessi and Abenante (1998), or seasonally, as reported for other fungus-growing ant species (Weber, 1957; Lapointe et al., 1998; Navarro and Jaffé, 1985). In this context, it is unknown whether worker thermal preferences vary following a daily pattern, as reported for the ant *Camponotus mus* (Roces and Núñez, 1995). Measurements of temperature preferences at different times of the day are already under way and will be reported elsewhere together with long-term field measurements of fungus temperatures.

It is important to note that the particular architecture of the *A. heyeri* nest, with a singly fungus garden located at the ground level and covered with thatch, may on the one hand limit the possibility of fungus relocation following temperature changes. But on the other hand, mound construction may favor fungal growth during cold periods by allowing the colony to be rapidly warmed by the sun (working as a solar collector as discussed for other ant species; Steiner, 1929), and by providing insulation. The question whether workers, in addition to their relocation behavior, also respond to long-term changes in climatic conditions by modifying the mound architecture, for instance by altering the tunnels that permeate the mound, remains to be experimentally investigated.

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