

Structure and thermal biology of subterranean army ant bivouacs in tropical montane forests

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Received: 1 March 2016 / Revised: 21 May 2016 / Accepted: 23 May 2016 / Published online: 4 June 2016
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Abstract Active brood-warming in army ant nests (bivouacs) is well documented for surface-dwelling *Eciton burchellii* and *E. hamatum* colonies in lowland tropical forests. However, little is known about thermoregulation by the below-ground bivouacking army ants that comprise all other species in subfamily Dorylinae. Here we report the first observations of subterranean *Labidus praedator* bivouacs in tropical montane and premontane conditions (Monteverde, Costa Rica), and present the first evidence for active nest warming in underground bivouacs. We measured bivouac temperatures at depth increments of 10 cm through the center of a 1565 m elevation bivouac and compared these to simultaneous measurements at the same soil depths 1 m outside the bivouac. The bivouac was actively heated to over 6 °C higher than the adjacent soil. Another bivouac showed warming of up to 3.7 °C above surface ambient. We measured critical thermal maxima (CT_{max}) and minima (CT_{min}) of *L. praedator* workers of a range of body sizes including callows, as well as thermal tolerances of inquiline millipedes from the bivouac. CT_{max} varied positively with worker body size. CT_{min} was lower for mature than for callow workers. Symbiotic millipedes had lower CT_{max} and higher CT_{min} than ant workers. Temperatures below the thermal tolerance ranges of symbiotic millipedes and near the bottom thermal tolerance range for callow workers were recorded in the bivouac

periphery and in adjacent soil, suggesting active bivouac warming protects some members of *L. praedator* bivouac communities from cold-limitation at high elevations in the tropics.

Keywords *Labidus praedator* · Dorylinae · Homeostasis · Thermoregulation · Nest architecture · Microclimate · Soil buffering · Myrmecophile

Introduction

The typically soft-bodied, altricial brood of many social insects are more sensitive to thermal variation than adult nest mates (Nalepa 2011). Social insect nests are often thermally homeostatic with temperature control achieved by passive and/or active thermoregulation (Seeley and Heinrich 1981; Jones and Oldroyd 2007). Passive thermoregulation involves behavioral responses to environmental thermal gradients (Jones and Oldroyd 2007). Examples of passive thermoregulation among social insects include foraging site and nest site selection, nest construction and orientation, and relocation of brood within nests (Coenen-Stass, Schaarschmidt and Lamprecht 1980; Frouz 2000; Penick and Tschinkel 2008; McGlynn et al. 2010; Jílková and Frouz 2014). Actively thermoregulating organisms use physiology to modify internal or ambient temperatures via metabolic heating or physical activity (Seeley and Heinrich 1981; Jones and Oldroyd 2007). Examples of active thermoregulation among social insects include worker clustering, flight muscle twitching to generate heat, and wing-fanning to promote evaporative cooling (Heinrich 1993; Anderson, Theraulaz and Deneubourg 2002; Weiner et al. 2010). Most ant species rely on passive thermoregulation to modify nest temperatures

Electronic supplementary material The online version of this article (doi:10.1007/s00040-016-0490-2) contains supplementary material, which is available to authorized users.

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because workers are wingless, preventing active thermoregulation via fanning or flight muscle shivering (Hölldobler and Wilson 1990). However, some Neotropical army ants in the subfamily Dorylinae create homeostatic thermal conditions within their temporary nests (bivouacs) via active thermoregulation (Jones and Oldroyd 2007). In two above-ground active species, *Eciton burchellii* and *E. hamatum*, bivouacs are composed of hundreds of thousands of clustering worker bodies that surround and insulate the brood and queen. These bivouacs are actively warmed via collective metabolic heat, and bivouac temperature variation is lower than ambient (Schneirla, Brown and Brown 1954; Jackson 1957; Coenen-Stass, Schaarschmidt and Lamprecht 1980; Franks 1985, 1989; Anderson, Theraulaz and Deneubourg 2002; Jones and Oldroyd 2007; Kadochová and Frouz 2014). All other army ant species bivouac below ground (Rettenmeyer 1963). Here, we present the first measurements of subterranean bivouac thermal physiology and brood developmental synchrony in the army ant *Labidus praedator* (Smith 1858).

Previous studies of army ant thermoregulation have primarily focused on above-ground *E. burchellii* and *E. hamatum* bivouacs (Schneirla, Brown and Brown 1954; Jackson 1957; Rettenmeyer 1963). Lowland *E. burchellii* bivouacs thermoregulated their brood at relatively stable elevated temperatures of 28 ± 1 °C (average 2 °C higher than ambient, maximum 6 °C higher than nocturnal low temperatures) (Schneirla, Brown and Brown 1954; Franks 1989), while lowland bivouacs of *E. hamatum* were on average 1 °C higher and less thermally variable than ambient conditions of 22–29°C (Jackson 1957). Active bivouac thermoregulation and homeostasis is thought to be tightly linked to the synchronous brood development cycles that characterize the dichotomous stately (egg/pupal) and nomadic (larval) phases of *Eciton* colony activity (Schneirla, Brown and Brown 1954; Jackson 1957; Franks 1989). Whether active or passive bivouac thermoregulation and brood synchrony occur in other species of Dorylinae is unknown. *Eciton* species are not representative of Dorylinae, in part because they raid and frequently bivouac above ground (Schneirla 1933; Schneirla, Brown and Brown 1954; Rettenmeyer 1963; Gotwald Jr 1995). In contrast, most Neotropical army ant species are at least partly subterranean, bivouacking underground and raiding partly or entirely below ground (Rettenmeyer 1963; Gotwald Jr 1995). These differences in foraging and bivouacking soil microhabitat correspond to differences in sensory investment and thermal tolerance (Baudier et al. 2015; Bulova et al 2016). Due to the challenge of tracking underground mobile nests, the subterranean bivouacs of many common army ants remain undescribed (Rettenmeyer 1963; Berghoff et al. 2002; Dunn 2003). Above-ground bivouacking in *Eciton* is a derived state among army ants (Brady 2003;

Brady et al. 2014). Therefore, data on bivouacking behavior in other doryline genera can provide evidence of how bivouac thermoregulation evolved in the above-ground species.

Labidus praedator is an abundant subterranean-bivouacking army ant that raids both on the surface of the forest floor and underground (Rettenmeyer 1963; Kaspari and O'Donnell 2003; O'Donnell et al. 2007). *Labidus praedator* ranges from the southern United States (N30°50', W93°45') to Argentina and Southern Brazil (S30°2', W51°12'). *Labidus praedator* occurs in the lowlands near sea level, though this species is most abundant from 1000 to 1600 masl in Costa Rica (Schneirla 1949; Watkins 1976; Kaspari and O'Donnell 2003; Longino 2010; O'Donnell et al. 2011; Economo and Guénard 2016). Brood development in *L. praedator* is apparently synchronous with stately and nomadic colony phases observed in Neotropical lowland wet forests of Panama (N09°09', W79°51') as well as in the tropical dry forest of southern Mexico (N18°27', W96°12') (Rettenmeyer 1963; Sudd 1972). Possible evidence of seasonal asynchrony in brood development has been reported at the southernmost extent of this species' range in Paraguay (S 25°20', W57°32') during extended winter stately phases with air temperatures reaching below 12.5 °C at night (Fowler 1979). Fowler (1979) suggested temperatures within a Paraguay *L. praedator* bivouac were less variable than surface air temperatures but bivouac temperatures were not elevated. However, Fowler (1979) measured temperature at a single bivouac point and did not measure adjacent soil temperatures, making interpretation of the data problematic. We asked whether tropical *L. praedator* colonies warm and/or buffer temperatures within their bivouacs when exposed to relatively low ambient temperatures in montane and pre-montane forest.

Air temperatures are relatively low year-round in high-elevation tropical sites, which can select for localized low-temperature adaptations (Janzen 1967; Ghalambor et al. 2006). However, army ant colonies are nomadic. For example, colonies of the highly epigeic army ant *E. burchellii parvispinum* move nomadically across elevations in pre-montane and montane forests of Monteverde, potentially experiencing a variety of mean annual temperatures at different elevations (Soare et al. 2014). *Eciton burchellii* bivouacs are more likely to be located in sheltered refuges in montane forest than in lowland forest, suggesting army ant nest site selection is a behavioral mechanism for dealing with thermal challenges (Soare et al. 2011). Underground environments have reduced daily and seasonal thermal variation, but closely match the local annual mean air temperature (Harkness and Wehner 1977; Parton and Logan 1981; Tschinkel 1987). Subterranean bivouacking *L. praedator* therefore likely experiences less temporally

variable temperatures, but similar geographic (elevational) variation in mean temperature, compared to surface-bivouacking army ants. To date, there are no published records of bivouacking behavior or thermal biology for montane or premontane subterranean army ants. Here, we report observations of the structure and thermal properties of subterranean bivouacs of *L. praedator* from montane forests (1500–1565 masl) and premontane forest (950 masl) near the center of this species' latitudinal range in Costa Rica (approximately 10°N) (Watkins 1976). We compared bivouac temperature conditions with thermal tolerances of the ants and their symbionts, and addressed two questions regarding bivouac thermal properties: 1. do subterranean army ants maintain elevated bivouac temperatures? 2. Do surface or sub-surface conditions exceed thermal tolerance limits of army ant workers or symbionts at high elevations, and do bivouacs buffer against these conditions?

In tropical premontane wet forests of Monteverde, Costa Rica, mean annual temperature at 1460 masl is 18.8 °C (Nadkarni and Wheelwright 2000). We recorded average air temperatures of 15.9 °C while recording bivouac temperatures at 1565 masl in March of 2015; March is a relatively cool dry-season month in Monteverde (Nadkarni and Wheelwright 2000). This temperature is lower than optimum for brood development in most tropical ant species (Franks 1989; Abril, Oliveras and Gómez 2010; Kipyatkov and Lopatina 2015). We therefore predicted active metabolic warming would be used to elevate *L. praedator* bivouac temperatures at this high elevation site.

Army ant colony members may not be the sole beneficiaries of a climatically moderated bivouac. Army ant colonies are host to the most species-rich array of animal associates known to science (Rettenmeyer et al. 2011). Many of these nest associates are arthropod species that live within the bivouac (Rettenmeyer 1962a; Eickwort 1990; Beeren, Maruyama and Kronauer 2016; Parker 2016). Myrmecophiles that live within the nest of their hosts are referred to as inquilines (Rettenmeyer 1962a). Little is known about potential thermal benefits of inquilinism, but the thermal biology of army ant bivouacs is potentially relevant to the climate niche and responses of these ants and of associated symbionts to climate change. Brood, callow (newly eclosed) workers, and myrmecophilic inquilines are seldom seen outside bivouacs except during colony emigrations, suggesting bivouacs could buffer them from thermal extremes (Schneirla, Brown and Brown 1954; Rettenmeyer 1962a, 1963; Rettenmeyer et al. 2011). We tested whether surface or sub-surface conditions exceeded the thermal tolerance limits of inquiline *Calymmodesmus* sp. millipedes collected from a subject *L. praedator* bivouac.

Methods

Nest structure

Two *L. praedator* bivouacs were observed in montane forest in July 2014, March 2015, and June 2015 on the Pacific slope of the continental divide near Monteverde, Costa Rica. Another bivouac was observed in premontane forest in April 2016 in the Children's Eternal Rainforest near San Gerardo Research Station on the Atlantic slope. The taxon referred to here as *L. praedator* was morphologically consistent with 'matte-face' *Labidus* sp. Cac 1 as described by Barth, Moritz and Kraus (2015). External nest structure and ant activity were observed over multiple days for two active bivouacs (bivouacs A and B) as follows:

Bivouac A (N10°18.113' W84°48.109', 1500 masl) was observed active 11–22 July 2014. During this time we noted external nest structure, lightly probing the bivouac with a machete and spade to assess ant presence and activity in the vicinity of the surface. We encountered bivouac A on 11 July 2014, and checked the bivouac site four additional times on 20 July 2014, 22 July 2014, 27 July 2014, and nearly 1 year later on 18 June 2015 (after the ants had departed). The ants had emigrated sometime between 22 July and 27 July 2014, the last two observations being of the evacuated bivouac site. We did not excavate this bivouac to observe brood developmental stage. However, the absence of discarded pupal cases prior to and after the colony's emigration from this site suggests the colony's brood were larvae. Larval brood are associated with the nomadic phase in *Eciton* (Schneirla, Brown and Brown 1954; Jackson 1957).

Bivouac B (N10°17.816' W84°47.951', 1565 masl) was an active stary-phase bivouac observed 19–26 March 2015. Observations of surface nest structure, colony presence and surface activity were made during this time in the same manner as for bivouac A. Bivouac B was first seen on 19 March 2015. We checked the bivouac site seven additional times on 20, 21, 22, 24, 25 and 26 March 2015, and 18 June 2015. Our March field season ended before the emigration of this colony, but the ants were absent from this site on 18 June 2015. After 5 days of temperature data collection (below), bivouac B was excavated twice, followed by observations and photographing of internal structure. The two excavations of bivouac B took place 24 h apart (25 and 26 March 2015), and yielded consistent results, the ants having reformed the bivouac structure overnight.

Bivouac C (N10°22.375' W84°46.532', 950 masl) was an active stary-phase bivouac observed on 22 April 2016. Due to a mat of thick buttress roots associated with the bivouac, a full excavation could not be completed, but we performed a partial excavation and nest surface description.

Temperature and humidity measurements

Temperature and relative humidity of bivouac B were recorded using alternating iButton hygrochron (measuring humidity and temperature), and thermochron (measuring only temperature) data loggers placed alternately every 10 cm along a thermally inert (wooden) vertical probe from soil surface to 40 cm depth in the bivouac (iButton: Maxim IntegratedTM, San Jose, CA, USA) (Fig. 1). A reference probe with an identical configuration was placed in soil 1 m away from the bivouac (Fig. 1). Data were collected every 5 min for 5 days. Temperature accuracy of all iButtons was confirmed to be within ± 0.5 °C (the manufacturer-reported instrument error for thermochrons) via hot and cold water bath (42 and 0 °C respectively) using a certified glass thermometer. Relative humidity accuracy of hygrochrons was confirmed to be within ± 1 % at 0 and 100 % by using desiccants and suspension over enclosed water bath at 25 °C. Additional temperature readings were taken at various depths during excavation using a hand-held infrared (IR) thermometer (BAFX Products, Milwaukee, WI, USA). IR thermometer accuracy was confirmed using a calibrated thermocouple. Temperatures were recorded with the IR thermometer for the bivouac C surface, soil surface 1 m away from the bivouac, and in the upper portions of the underground bivouac cavities at 10 and 15 cm (within a gallery underneath one of the roots).

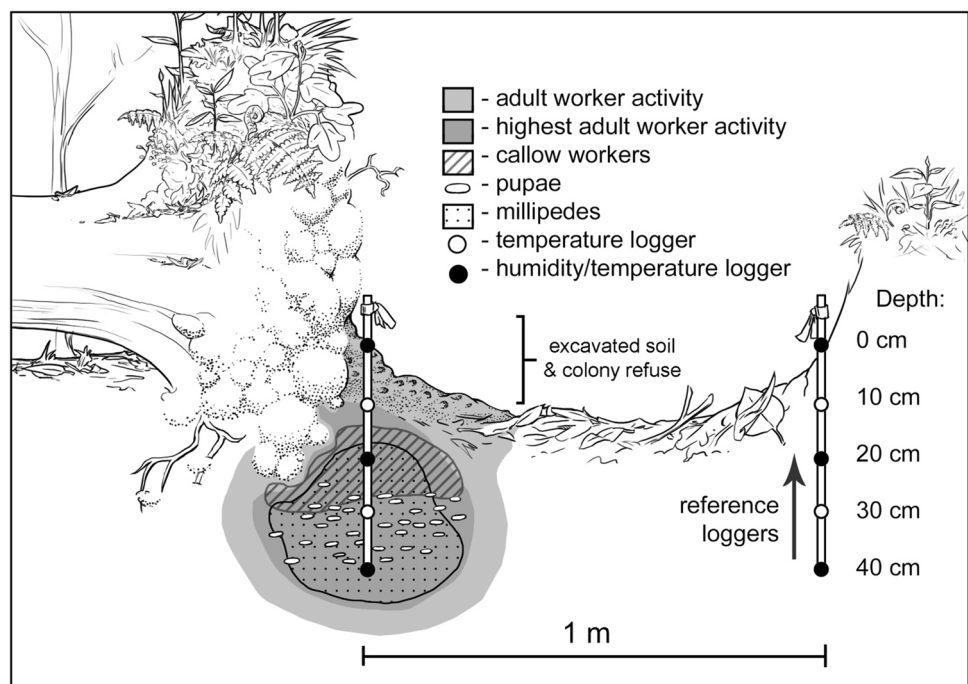
Thermal tolerance

Critical thermal maximum (CT_{max}) and minimum (CT_{min}) were measured for callow and non-callow workers of different body sizes, as well as for inquiline millipedes found within bivouac B. CT_{max} and CT_{min} were measured using standard dynamic methods (Lutterschmidt and Hutchison 1997; Diamond et al. 2012; Oberg, Toro and Pelini 2012) with thermal ramping at a rate of 1 °C every 10 min. Insects showing immobility for a duration of 10 s were considered to have surpassed their CT_{max} or CT_{min} respectively. Half of the ants were allowed to acclimate to lab conditions for 24 h prior to thermal assays (ranging from 19 to 26 °C over the course of the day), while half were run in thermal assays 30 min after collection. The thermal tolerances of these groups were compared to test for effects of acclimation.

Statistical analyses

All analyses were performed in R statistical software. We used standard linear multifactor analyses of variance to identify significant predictors of bivouac temperatures, environmental temperatures and thermal tolerances (Quinn and Keough 2002). We used the ANOVA function to test for significance of predictors by comparing the fit of linear models with and without the inclusion of each predictor variable. Starting with all measured predictors, Aikake Information Criteria (via the drop1 function) was used to

Fig. 1 Structure and probe placement for temperature and humidity measurements of bivouac B



select the order of predictor variable testing and elimination from the model. If the predictor had a significant effect on the model's fit, it was included in the full model for subsequent analyses. Mean, maximum and minimum daily temperatures and relative humidity were compared across depths and treatments (bivouac versus soil reference probes). Linear regressions of soil depth versus daily mean were also performed within each location. Predictors of CT_{max} and CT_{min} included head width (as a proxy for body size), whether the ant was from the acclimated or non-acclimated group, and whether ants were callow. Student's *t* tests were run to compare millipedes to all ant size classes for both CT_{max} and CT_{min} .

Results

Nest surface structure

Bivouac A was found active at the base of saplings and a small tree trunk at 1500 masl on 11 July 2014 (Fig. 2, Supplementary Fig. 1). Bivouac B was found alongside and below the root mass of a fallen tree on 19 March 2015 at 1565 masl (Fig. 1, Supplementary Fig. 1). Bivouac C was found between and beneath the intertwining roots of two live trees at the top of a ridge on 22 April 2016 at 950 masl (Supplementary Fig. 1). The surface structure of all three bivouacs consisted of low, wide mounds of loose excavated soil (in fine particles) intermixed with colony refuse. In all cases, the excavated soil was distinctive and easily visible against nearby leaf litter.

The surface mound of bivouac A was approximately circular and larger in area than the other two mounds



Fig. 2 Surface nest structure of bivouac A on 11 July 2014, showing the loose soil mound covered in fine bits of colony refuse; cleared bivouac surface appeared prominent and easily distinguished from surrounding leaf litter

(length: 124 cm, width: 141 cm). The surface mound of bivouac B was crescent-shaped around the base of the root mass of a fallen tree (length: 59 cm, width: 32 cm). Mid-elevation bivouac C was visible on the surface as a series of small mounds between root buttresses of two mature trees (entire area length: 81 cm, width: 76 cm). Unlike the other two observed bivouac mound surfaces, that of bivouac B was punctuated with craters approximately 1 cm in diameter. All three bivouacs were covered in a mixture of excavated soil and colony refuse. For bivouac B, this refuse consisted largely of discarded pupal cases, isopod tergites and other arthropod body parts (Supplementary Fig. 2). The surface of bivouac C was covered in what appeared to be fine bits of excavated decomposing wood in addition to excavated soil and colony refuse (cockroach tergites, isopod tergites, and discarded ant pupal cases).

The lack of discarded pupal cases on the bivouac surface of bivouac A suggests the colony was nomadic at the time. We also noted two possible abandoned bivouac sites within a 500 m radius of bivouac A with similar loose soil and broad circular shape, also located at the base of saplings. Bivouac B was in the late statary phase, with the majority of the pupae having eclosed by the time our observations in March 2015 were complete. Bivouac C was likely in the late statary phase as well, having large numbers of discarded army ant pupal cases among colony refuse.

In June of 2015, former bivouac sites of bivouac A and bivouac B were revisited and found to be void of any army ant activity. At this time, the soil on the surface of both abandoned nest mounds appeared to have sunken in the absence of *L. praedator* (27 cm subsidence for bivouac A, 17 cm subsidence for bivouac B) (Supplementary Fig. 3).

Internal nest structure and colony strata

The initial excavation of bivouac B was performed at 5 pm. At that time, some ant activity was observed on the soil surface. Excavating the top 10 cm of soil produced considerable defensive activity with the arrival of several hundred soldiers (also observed in bivouac A the previous year and bivouac C the following year). Upon excavation, bivouac B contained mature workers, callow workers of various size castes and hundreds of pupae, indicating synchronous brood development and a colony in the late statary phase; no army ant larvae were observed. The depth at which the first callow workers were observed was 17 cm, however, callow workers were at highest density from depths of 20–35 cm. These callow workers were interspersed with pupae found from 27 to 35 cm depth (Fig. 1).

Bivouac B did not appear to occupy a large central cavity, but rather consisted of many small tunnels (<1 cm) and chambers within loose soil. Soil adjacent to the bivouac was more compacted and less porous than soil within the

bivouac, particularly around roots of the fallen tree. Some tunnels were close to small (0–1 cm diameter) roots of this fallen tree, though the majority of the bivouac structure consisted of small interconnected chambers and tunnels independent of the tree's root system. On the second day of excavation, bivouac structure was similar, the bivouac having been reconstructed over night. There was one exception: fewer pupae were present than the previous day and callow workers were relatively more abundant. This is likely due to overnight eclosion of workers. We did not observe the emigration of bivouac B.

Excavating bivouac C within 10 cm of the surface of the bivouac revealed large galleries (approximately 2 cm in diameter) beneath some of the most superficial, large (>10 cm diameter) roots.

Nest-associated arthropods

Numerous white inquiline millipedes (genus *Calymmodesmus*) were found within bivouac B (Loomis 1959; Rettenmeyer 1962b). *Calymmodesmus* were in high density in the vicinity of the pupae and extended from 20 cm to 43 cm depth in the bivouac (Figs. 1, 3). Other inquilines included one other species of millipede, two morpho-species of Acari, two morpho-species of wingless phorid (*Ecitomyia* spp.) found roaming the mound surface, five sp. of staphylinid beetle (one individual limuloid beetle of genus *Vatesus* was collected in refuse atop the bivouac), and one species of Scydmaemidae beetle. Other notable associates encountered include one sp. winged Phoridae that arrived en masse when we excavated high ant-density portions of the nest, as well as two morphospecies of collembola (1sp. Entomobryidae, 1sp. Poduroomorpha). Only *Calymmodesmus* millipedes were used in thermal tolerance assays as they were the only myrmecophile collected in sufficient numbers to enable within-species replication of critical thermal measures. Photographs of

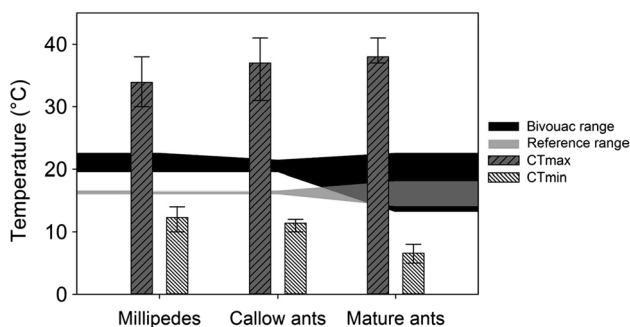


Fig. 3 Thermal tolerances of three types of assayed subjects extracted from the bivouac; *black background area* represents the bivouac temperatures recorded within the range of depths where each category was collected; *grey background area* represents reference probe temperatures at equivalent depths

each collected myrmecophile morphospecies are included in online supplementary materials.

Temperature and humidity

Temperatures at bivouac B as measured by IR thermometer during excavation were 14–15 °C on the surface of the soil 1 m away from the bivouac, with 23.1 °C measured at the brood center of the bivouac during excavation. This was slightly higher than the maximum temperature of 22.6 °C recorded by the 40 cm depth iButton probe in the bivouac. Worker ants may have moved among bivouac depths in response to variation in surface temperature. At 7:15 am on a cold morning (13.6 °C measured by iButton on bivouac surface), adult worker ants were not observed at depths less than 13 cm, while on a warm day (surface iButton measured 19.1 °C at 9:49 am) ant activity was observed within 1 cm of the surface of the bivouac.

Mean, maximum and minimum daily temperatures (recorded by iButtons) were always equal or higher in the bivouac than at depth-matched reference points in the soil nearby (Figs. 4, 5; mean $F_{1,46} = 81.75$ $p < 0.001$; maximum $F_{1,46} = 166.55$ $p < 0.001$; minimum $F_{1,46} = 59.09$ $p < 0.001$). Mean daily temperature increased with depth from the surface within the bivouac ($R^2 = 0.91$,

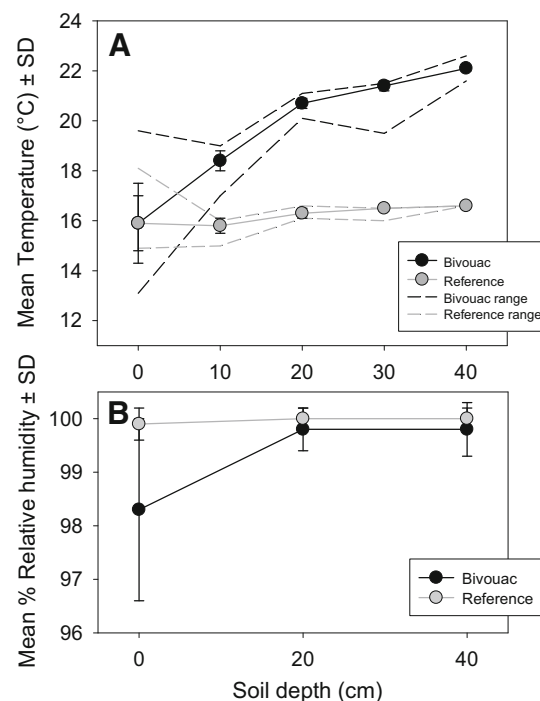


Fig. 4 a Bivouac and uninhabited (reference) soil temperatures at varying depths from the surface \pm standard deviation; perforated lines represent maximum and minimum recorded temperatures across all 5 days; **b** bivouac and reference percent relative humidity at varying depths from the surface \pm standard deviation

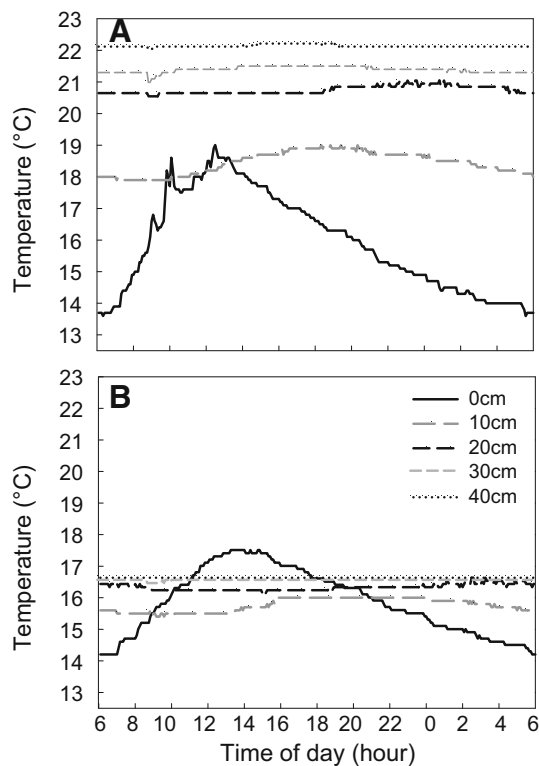


Fig. 5 Bivouac (a), and reference (b) temperatures across times of day, recorded every 5-min and averaged across 5 days of recording; different lines denote probe depth in soil

$F_{1,23} = 245$, $p < 0.001$); the mean temperature at 40 cm depth was 6.2 °C higher than the depth-matched reference point in soil (Fig. 4). Mean temperatures (\pm standard deviation) experienced by pupae at depths of 30–40 cm were 21.8 ± 0.4 °C. Daily mean belowground temperatures were only slightly higher than surface (0.8 °C at 40 cm depth) in soil with no ants ($R^2 = 0.75$ $F_{1,23} = 72$, $p < 0.001$) (Fig. 4). Time of day was a significant predictor of temperature ($F_{1,286} = 212.46$, $p < 0.001$), accounting for much of surface thermal, 10 cm, and 20 cm variability in both the bivouac and depth-matched reference points (Fig. 5). Daily fluctuation in temperature decreased similarly with soil depth in both the reference and bivouac samples (Fig. 5). Standard deviation of bivouac temperatures at each depth was equal to or slightly greater than those in the nearby soil (Fig. 4). The bivouac therefore experienced reduced variation in temperature relative to surface air temperature, but did not experience reduced variation in temperature compared to nearby soil. Relative humidity remained near 100 % as recorded across all surface and sub-surface probes for both bivouac and soil reference transects (Fig. 4). Daily maximum humidity was 100 % for all depths regardless of ant presence. Minimum relative humidity across 5 days was 88.1 % on the surface with a minimum bivouac humidity of 96.7 %.

Ambient and bivouac surface temperatures at premontane bivouac C were higher (as measured using IR thermometer) than corresponding temperatures at montane bivouac B. At 13:52 on 22 April 2016, surface soil temperatures 1 m from bivouac C ranged from 21.6 to 22.0 °C, while the surface of the bivouac was 22.0–23.6 °C. 10 cm below the bivouac surface ranged in temperature from 24.0 to 24.8 °C, and within a gallery beneath a root approximately 15 cm below the surface, temperatures ranged from 24.5 to 25.3 °C.

Thermal tolerances

CT_{max} ($F_{1,10} = 0.001$, $p = 0.919$) and CT_{min} ($F_{1,11} = 0.011$, $p = 0.973$) did not differ with 24 h of acclimation. Acclimation treatments were therefore pooled in subsequent analyses. Larger ants had higher CT_{max} ($F = 12.53$, $df = 14$, $p = 0.004$), but CT_{min} did not covary with body size ($F_{1,14} = 2.540$, $p = 0.135$). Callow ants had higher CT_{min} than non-callow ants ($F_{1,13} = 106.66$ $p < 0.001$), while CT_{max} did not differ significantly between callow and non-callow ants ($F_{1,12} = 0.489$ $p = 0.498$) (Fig. 4). CT_{max} was higher for mature worker ants than inquiline millipedes ($F = 19.119$, $p < 0.001$). *Labidus praedator* workers also had lower CT_{min} than millipedes ($F = 28.619$, $p < 0.001$). Low temperatures were not below CT_{min} for any of the assayed individuals at the bivouac depths where they occurred, (Fig. 3). However, minimum recorded surface temperatures were colder than tolerable by the most sensitive millipedes, and were only 1.1 °C warmer than tolerated by the most sensitive callow workers (Fig. 3; Supplementary Table 1).

Discussion

Bivouac site selection

All observed active and apparently abandoned montane and premontane *L. praedator* bivouac sites occurred at the base of live or dead trees (Supplementary Fig. 1), suggesting (together with previous accounts) that *L. praedator* selects bivouac sites in loose soil or cavities created by live tree roots, fallen trees, or other similar structures (Sumichrast and Norton 1868; Rettenmeyer 1963; Sudd 1972; Monteiro, Sujii and Morais 2008). However, both high volumes of loose soil on the surface and soil subsidence after ant departure indicate excavation by the ants (Supplemental Fig. 2). Bivouac B was able to rebuild overnight suggesting rapid excavation of several liters of soil.

Bivouac thermal and humidity conditions

Above-ground bivouacking army ants achieve nest homeostasis via active metabolic warming and active thermal buffering using the interlocked bodies of the ants themselves (Schneirla, Brown and Brown 1954; Franks 1989; Jones and Oldroyd 2007). Our data show that two high-elevation *L. praedator* colonies at a tropical latitude (N10°18') actively warmed their bivouacs via collective metabolic heating, while possibly relying on passive thermal buffering effects of soil to reduce diel thermal fluctuations in temperature. Nest heating via combined metabolic activity of ant workers in close spatial proximity is an active thermoregulation mechanism (Jones and Oldroyd 2007), while bivouac moderation of widely varying air temperatures could not be distinguished from passive soil buffering. The *L. praedator* placement of immobile pupae and callow workers within bivouac B along the thermal gradient, and adult worker movements in response to solar warming, are passive thermoregulatory mechanisms common among many ant species (Jones and Oldroyd 2007; Penick and Tschinkel 2008).

The warmest regions of the stately *L. praedator* bivouac B were the lower level strata containing the brood (pupae) and millipedes. Thermal probes showed these regions were on average 21.8 ± 0.4 °C, with infrared recordings of up to 23.1 °C at the brood center. This is not as high as previously suggested thermoregulatory target windows of *E. burchellii* (28 ± 1 °C) and *E. hamatum* (26.6 ± 1.1 °C) in the lowlands (Jackson 1957; Franks 1989). At 40 cm depth from the surface, the *L. praedator* bivouac sustained mean daily temperatures 6.2 °C higher than at the same depths in surrounding soil. This is a greater and longer-sustained warming effect than previously reported for any army ant bivouac (Jackson 1957; Franks 1989).

We also recorded higher bivouac surface temperatures relative to soil surface temperatures 1 m away, and increasing temperatures with bivouac depth, within the superficial portions of mid-elevation bivouac C (950 masl). Both suggest that bivouac metabolic warming is not a montane bivouac phenomenon. However, the fact that even superficial portions of mid-elevation bivouac C were warmer than the warmest portions of montane bivouac B (1550 masl), suggests that internal bivouac temperature is not uniform across elevations, and that high elevation bivouacs may struggle to raise bivouac temperatures to those optimum for brood. Temperatures in above-ground bivouacs at high elevations have yet to be measured, but will likely shed further light on this interplay between microhabitat and elevational thermal effects on bivouac warming.

Ants in general, and particularly soft-bodied ant larvae, are susceptible to desiccation in a wide variety of environments (Hölldobler and Wilson 1990). For bivouac B,

although our humidity and temperature measurements were taken during the dry season, sub-surface and surface relative humidities near 100 % were recorded regardless of ant presence (Fig. 4). This suggests *L. praedator* bivouacs are not limited by moisture availability in the lower montane wet forest life zone. Future studies of how *L. praedator* bivouacs respond to dry season conditions in seasonally dry forests (such as in Guanacaste) may shed light on adaptations for dealing with low humidity.

The presence of discarded tergites in the refuse of *L. praedator* confirms previous observations that this species feeds largely on isopods in the premontane life zones of Monteverde (Supplementary Fig. 2) (Longino, J. T. pers. comm.) (Longino 2010), with evidence of small cockroaches and other insects consumed as well. We observed *L. praedator* feeding on terrestrial amphipods at a raid fronts in Monteverde and San Gerardo in June 2015 and April 2016 respectively. Although we observed refuse atop all montane and premontane bivouacs, there are no accounts of either loose-dirt mound construction or refuse-topping in low elevation *L. praedator* bivouacs (Sumichrast and Norton 1868; Rettenmeyer 1963; Fowler 1979). Whether this refuse topping serves as an adaptive thermal warming function remains to be tested.

Thermal sensitivities of bivouac occupants

Inquiline millipedes (*Calymmodesmus* sp.) were more thermally sensitive to both heat and cold than mature or callow ants, while callow worker ants differed from mature workers in their ability to function at low temperatures. The higher CT_{min} of callow workers may be related to differences in cuticular thermal resistance (Galushko et al. 2005). The impact of cold on callow movement may, at least in part, explain the common observation of callow workers being carried in emigrations immediately following the stately phase (Rettenmeyer 1962b, 1963). In the case of *Calymmodesmus* millipedes, bivouac surface temperatures were within 1.1 °C of the mean CT_{min} for all millipedes, and were lower than what could be tolerated by the most sensitive individuals. Choice of location within the bivouac corresponded to these sensitivities, with *Calymmodesmus* being found deep within the nest where temperatures were farthest from their CT_{min}. These findings show millipede thermal specialization to bivouac homeostatic conditions, suggesting an obligate relationship between host and inquiline. This more thermally stable region of the bivouac was also where the pupae were housed, indicating a narrow thermal tolerance range for *L. praedator* pupae. Callow workers were less sensitive than millipedes and correspondingly were encountered at shallower depths than inquiline millipedes or pupae, where temperatures are more variable over the course of the day. The widest distribution

of activity within the bivouac was seen by the mature workers, inhabiting bivouac depths from 0 cm down beyond 45 cm in low densities. The cold sensitivity of callow adult workers combined with low temperature ambient conditions suggests that active warming in *L. praedator* bivouacs is an adaptive response to cold limitation at this high elevation site.

Acknowledgments We thank anonymous reviewers who provided useful feedback. Students at the Monteverde Friends School, Nicole Arcilla, Johnathan Ogle, Heather Gosse, Rumaan Malhotra, Catherine D'Amelio, and Elisabeth Sulger provided field assistance. Historic Monteverde, Martha Campbell, Lucy, Wilford and Benito Guindon permitted access to private lands. The Monteverde Conservation League permitted access to the Children's Eternal Rainforest for work in San Gerardo. We thank John T. Longino and Susan Bulova for project feedback. Christoph von Beeren, Jon Gelhaus, and Jason Weintraub aided in myrmecophile identification and imaging. Michael O'Connor, Steven Pearson, Tom Radzio and Dane Ward assisted with thermal equipment. Research was conducted under research and collection permits issued by the Costa Rican government (MINAET). Funding provided by start-up funds and NSF grant IOS-1207079 to S. O'D, and by the Organization for Tropical Studies Tyson Research Fellowship as well as the Academy of Natural Sciences of Drexel University McLean Fellowship to K. M. B.

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