ECOGRAPHY

Research

Plastic collective endothermy in a complex animal society (army ant bivouacs: *Eciton burchellii parvispinum*)

Kaitlin M. Baudier, Catherine L. D'Amelio, Elisabeth Sulger, Michael P. O'Connor and Sean O'Donnell

K. M. Baudier (http://orcid.org/0000-0001-8450-3788) (kmbaudier@gmail.com), C. L. D'Amelio, E. Sulger, M. P. O'Connor and S. O'Donnell, Dept of Biodiversity Earth and Environmental Science, Drexel Univ., Philadelphia, PA, USA. KMB also at: School of Life Sciences, Arizona State Univ., Tempe, AZ, USA. CLD, ES, MPO and SO also at: Dept of Biology, Drexel Univ., Philadelphia, PA, USA.

Endothermic animals do not always have a single adaptive internal temperature; some species exhibit plastic homeostasis, adaptively allowing body temperature to drop when thermoregulatory costs are high. Like large-bodied endotherms, some animal societies exhibit collective thermal homeostasis. We tested for plasticity of thermoregulation in the self-assembled temporary nests (bivouacs) of army ants. We measured core bivouac temperatures under a range of environmental conditions and at different colony developmental (larval vs pupal brood) stages. Contrary to previous assertions, bivouacs were not perfect thermoregulators in all developmental stages. Instead, bivouacs functioned as superorganismal facultative endotherms, using a combination of site choice and context-dependent metabolic heating to adjust core temperatures across an elevational cline in ambient temperature. When ambient temperature was low, the magnitude of metabolic heating was dependent on colony developmental stage: pupal bivouacs were warmer than larval bivouacs. At cooler high elevations, bivouacs functioned like some endothermic animals that intermittently lower their body temperatures to conserve energy. Bivouacs potentially conserved energy by investing less metabolic heating in larval brood because the high costs of impaired worker development may require more stringent thermoregulation of pupae. Our data also suggest that site choice played an important role in bivouac cooling under high ambient temperatures at low elevations. Climate warming may expand upper elevational range limits of *Eciton burchellii parvispinum*, while reducing the availability of cool and moist bivouac sites at lower elevations, potentially leading to future low-elevation range contraction.

Keywords: altitude, nest site choice, thermal regulation

Introduction

Endothermic animals do not necessarily have a single adaptive body temperature. Homeothermy is a strategy with costs and benefits, and plastic body temperature variation can be adaptive for endotherms in some environmental conditions (Bauwens et al. 1999, Ruf and Geiser 2015). Depending on the costs of thermal

homeostasis, body temperatures can covary with diel, seasonal, or geographic temperature differences, and with metabolic opportunities and demands (Angilletta Jr et al. 2002, Angilletta 2009).

Like endothermic animals, insect societies can exhibit group (collective) homeothermy, even when composed of poikilothermic workers (Jones and Oldroyd 2006). A major function of thermal homeostasis in eusocial insect nests is enabling growth and development of temperature-sensitive altricial brood (Franks and Sendova-Franks 1992, Jones et al. 2004, Penick and Tschinkel 2008, Becher et al. 2009). Adult social insects often engage in brood-specific thermoregulation behaviors such as fanning or direct incubation (Heinrich 1975, Franks and Sendova-Franks 1992, Cook et al. 2016). However, social insect nests are generally assumed to be tightly thermoregulated superorganisms with a narrow range of ideal internal temperatures (Jones and Oldroyd 2006). We propose that, like endothermic organisms, insect colonies may exhibit adaptive plasticity in thermoregulation. We predicted colony internal temperatures would vary in response to both environmental pressures (i.e. differing ambient temperatures) and to changes in internal colony needs.

Here we present a test of thermoregulation plasticity in social insect nests. We tested whether internal nest temperatures were invariant across ambient temperature variation (strict thermoregulation), as opposed to varying linearly with ambient temperature (thermoconformation). We measured social thermoregulation over a geographic (elevational) temperature cline, and across developmental changes in demand for thermoregulation, using the living nests (bivouacs) of the army ant *Eciton burchellii parvispinum*. Army ant bivouacs are composed entirely of interlocking worker bodies that cradle the brood and queen, warming the interior with the workers' collective metabolic heat (Schneirla et al. 1954, Jackson 1957, Franks 1989, Jones and Oldroyd 2006). *Eciton burchellii* bivouacs were previously asserted to be prime examples of near-perfect social thermoregulation, with stable internal temperatures of 28.5 ± 1°C (Schneirla et al. 1954, Franks 1989, Jones and Oldroyd 2006). However, previous studies of bivouac thermoregulation were conducted only in warm lowland tropical forests, where mean ambient temperature is within 2°C of mean internal bivouac temperature (Fig. 1; Schneirla et al. 1954, Jackson 1957, Teles da Silva 1977, Franks 1989). We took advantage of two features of *E. b. parvispinum* ecology and colony development. First, the geographic range of *E. b. parvispinum* spans over 1500 m in elevation in Costa Rica (Watkins 1976, O'Donnell et al. 2011). Bivouacs experience a range of thermal conditions across this altitudinal expanse (MacArthur 1972, Lazaridis 2011, Baudier et al. 2018a). Second, army ants have synchronous brood, meaning that larval and pupal stages are separated temporally: bivouacs typically house only larvae, or only pupae and eggs, at a given point in time (Supplementary material Appendix 1 Fig. A1, Rettenmeyer 1963, Gotwald Jr 1995).
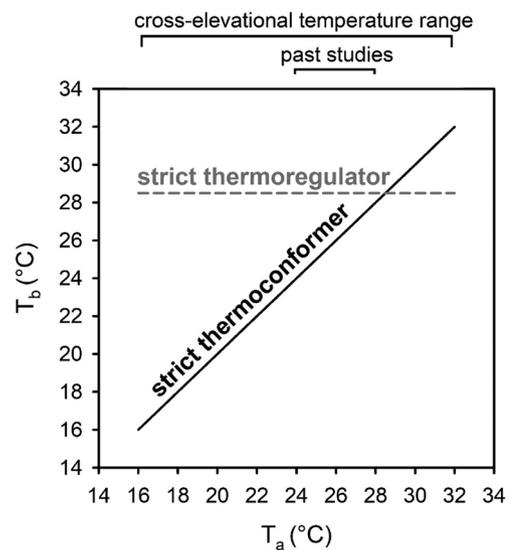
Figure 1. Adapted from Angilletta (2009), The predicted relationship between ambient temperature (T_a) and expected internal bivouac temperature (T_b) for strict bivouac thermoregulation (slope = 0), and strict bivouac thermoconformation (slope = 1). The range of ambient temperatures where above-ground bivouac thermoregulation has been previously studied in lowland tropical forests (Schneirla et al. 1954, Jackson 1957, Franks 1989), and the full range of cross-elevational temperatures of *Eciton burchellii parvispinum* in Costa Rica measured as part of this study are depicted above the graph.

The thermal needs of juvenile developmental stages (e.g. larvae versus pupae) often differ in Hymenoptera (Jay 1963, Liu et al. 2005, Li et al. 2016). Pupae are often more tightly thermoregulated than larvae (Kronenberg and Heller 1982, Roces and Núñez 1989, Ishay and Barenholz-Paniry 1995). Larvae are relatively robust to temperature variation, while pupae suffer lower eclosion rates or developmental anomalies when exposed to sub-optimal temperatures in many species (Luczynski et al. 2007, Bennett et al. 2015, but see Penick et al. 2017). This pattern may be due in part to temperature-sensitive neurological development occurring during the pupal stage (Groh et al. 2004, Jones et al. 2005, Luczynski et al. 2007, Becher et al. 2009). We predicted that pupal bivouacs would be more tightly thermoregulated than larval bivouacs in environments where it is energetically costly or difficult to maintain high bivouac temperatures, such as in relatively cool environments. Mean ambient temperature covaries reliably with elevation (MacArthur 1972, Lazaridis 2011), and bivouacs at high elevations are exposed to consistently lower temperatures than their lowland counterparts (Soare et al. 2011, Baudier and O'Donnell 2016). We predicted that cooler bivouac temperatures at high elevations would be common for larval bivouacs because larvae are more developmentally robust to thermal deviation.

To evaluate endothermy within bivouacs, the passive thermoregulatory effects of bivouac site selection should

be considered. Army ants are nomadic, frequently moving among bivouac sites. We predicted *E. b. parvispinum* would select cooler bivouac sites than ambient at their low elevation range limits, and conversely, that ants would select relatively warmer bivouac sites at their high elevation range limits. We also asked whether bivouac site choice (site exposure or below-ground location) differed across the elevation range of *E. b. parvispinum*. By accounting for the effects of site selection we were able to estimate the amount of thermoregulation performed exclusively by the living structure of the bivouac itself.

Temperature homeostasis within army ant bivouacs has implications for water balance. We predicted bivouacs would maintain relative humidities greater than 76%, given that adult *E. burchellii* workers show distress below this level (Schneirla et al. 1954). However, immature social insects often have higher desiccation risk than adult nestmates (Lindauer 1955, Human et al. 2006, Ellis et al. 2008). We asked whether and to what degree homeostasis of relative humidity is maintained within bivouacs, and whether ambient relative humidity or bivouac site selection affected bivouac relative humidity.

Material and methods

Two main field sites were chosen at the elevational extremes of *E. b. parvispinum* in Costa Rica: low-elevation Guanacaste (Santa Rosa National Park 10°53′N, 85°46′W bivouacs sampled at 268–312 m a.s.l.; Maritza Biological Station 10°57′N, 85°29′W bivouacs sampled at 402–607 m a.s.l.) and high-elevation Monteverde (10°18′N, 84°49′W, bivouacs sampled at 1160–1602 m a.s.l.). Within each site we located *E. b. parvispinum* colonies by trail-walking (Kumar and O'Donnell 2009, Soare et al. 2011). Army ant raid columns were followed in the direction of carried food items to reach bivouacs. Bivouacs were checked nightly for signs of emigration and colonies were followed to the next bivouac site to identify successive bivouacs of the same colony whenever possible. Geographic coordinates and elevation were measured using handheld GPS units (Garmin GPSMAP® 62S, Garmin International, Olathe, KS, USA). Bivouacs encountered more than 300 linear meters apart within 24 h of each other were assumed to be different colonies, as this exceeds maximum recorded length of a single overnight emigration (Teles da Silva 1982). Data were collected in the wet season of 2013 (24–27 July Monteverde n = 1 bivouac) and 2015 (10 June–11 July Monteverde n = 36 bivouacs; 12 July–13 August Santa Rosa n = 32 bivouacs), and in the transition from dry season to wet season of 2016 (5 April–1 May Monteverde n = 19 bivouacs; 2 May–16 May and 19 May–2 June Santa Rosa n = 10 bivouacs; 16 May–19 May Maritza n = 6 bivouacs).

Temperature and relative humidity were measured using Ibutton Thermochron and Hygrochron data loggers (Maxim Integrated™, San Jose, CA, USA). Ambient control probes measured conditions on the forest floor approximately 1 m away from each bivouac site. Ambient probes were protected from direct insolation via plastic housings. Bivouac probes consisted of four loggers placed 5 cm apart along a plastic dowel starting from the apical tip. When relative humidity was measured for the bivouac, a Hygrochron logger was placed at the apical position. Prior to probe insertion, a small observation hole was made in the bivouac and brood depth measured to the nearest centimeter using a ruled dowel. The depth of the most superficial brood was estimated using the depth of the single larva or pupa closest to the most exposed surface of the bivouac.

Logger probes were positioned within the bivouac so that brood surrounded the apical tip of the probe. Following the bivouac reforming around the probe, the ensuing 30 min of temperature logs were not counted to allow for instrument equilibration. Loggers ran 24 h a day collecting temperature and/or relative humidity logs every 5 min. Changes in bivouac volume and relative probe position were monitored using time-lapse PlotWatcher Pro game cameras (Day 6 Outdoors, Columbus, GA, USA). Thermal conditions of each bivouac were recorded for up to five days, shorter for bivouacs which emigrated before the end of 5 d. Most nomadic bivouacs were measured for 1 to 2 d due to frequent emigration. Mean temperature and relative humidity in the bivouac and ambient, and within-colony standard deviations were calculated across days and times of day at each bivouac site. Because bivouac structure breaks down during emigration, temperature and humidity logs recorded during bivouac emigrations were excluded from all analyses.

Brood temperatures were a subset of the bivouac thermal probe measurements that were within the immediate vicinity of the brood. After equilibration time following probe insertion, temperature logs for one hour (n = 12 logs) across the depths at which brood had been observed within the bivouac (brood core) were considered representative of the brood conditions. Brood type was recorded and generally corresponded with colony statary/nomadic phase, but some early nomadic and late statary bivouacs housed both pupae and larvae. We analyzed the relationship of brood versus ambient temperature for these transitional bivouacs separately.

We measured empty bivouac site temperature and humidity immediately following colony emigration to evaluate the effects of site choice. Bivouac site conditions without ants were monitored by leaving all probes in-place after each colony had emigrated. Control ambient temperatures were simultaneously collected from a reference probe on the forest floor 1 m from the abandoned bivouac site as before. Empty bivouac site temperature data were recorded for a minimum of 8 h, maximum 24 h after each bivouac had emigrated.

We conducted a bivouac site-type survey to assess whether ambient temperature affected site choice. Bivouacs were scored for relative exposure and for above- versus below-ground location in Monteverde and Guanacaste for a total of two months at each site. Fifty-five bivouacs were observed in Monteverde in June of 2015 and April of 2016, and

48 bivouacs were observed in Guanacaste in July 2015 and May 2016. A bivouac was considered 'exposed' if ≥ 50% of the bivouac surface was visible with the naked eye, a bivouac was deemed 'sheltered' if some portion of the bivouac surface less than 50% was visible, and a bivouac was considered 'enclosed' if no portion of the bivouac surface was observable, as in previous indices (Schneirla et al. 1954, Teles da Silva 1977, Soare et al. 2011). Whether bivouac sites were above-ground or below-ground was also recorded. Chi-squared tests of independence were used to test for the effects of elevation (Monteverde vs Guanacaste) and colony phase (nomadic vs statary) on bivouac exposure (exposed, sheltered, enclosed) and tendency to be below-ground (subterranean vs above-ground).

Statistical analyses

All statistical analyses were performed in R (ver. 3.1.2; R Core Development Team). We first compared ambient temperature and relative humidity across elevations to confirm that adequate altitudinal sampling had occurred to generate significant thermal and humidity variation. This was done via two separate linear regressions of either ambient temperature or ambient relative humidity as a function of elevation.

To test the predictions of bivouac thermoregulation versus thermal conformation, we examined internal bivouac temperature as a function of ambient temperature. Linear regressions were used to test for significant deviance of slopes from 0 (the prediction for strict thermoregulation). If the slope was significantly greater than 0, then 95% confidence intervals of the fitted model slopes were checked. Slopes significantly greater than 0 and with 95% confidence intervals including 1 were considered evidence of strict thermal conformation. Any slope value significantly greater than 0 and significantly less than 1 using the same criteria was considered evidence of imperfect or intermediate thermoregulation. The same approach was used to analyze relative humidity data. All relative humidity data were arcsine-transformed to normalize distributions.

An ANCOVA was used to test for differences between larval and pupal bivouacs in the relationship between mean ambient temperature and mean brood temperature. A separate ANCOVA was used to test for differences between brood stages in the relationship between ambient relative humidity and bivouac relative humidity (arcsine transformed).

To test for effects of ambient temperature on empty bivouac site temperature, a linear regression between mean empty site temperature and mean ambient temperature was performed. Ambient relative humidity and empty bivouac site relative humidity were similarly regressed in a separate analysis.

Net metabolic heating of ants and brood was estimated as mean bivouac temperature while ants were present minus the mean bivouac site temperature after ants had emigrated. To test whether active bivouac warming differed across mean ambient temperatures, estimated metabolic heating was regressed against mean ambient temperature.

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.vk13np4> (Baudier et al. 2018b).

Results

Bivouac temperature

Ambient temperature decreased as elevation increased across the geographic range sampled ($F = 410.4$, $df = 35$, $p < 0.001$, $R^2 = 0.92$), confirming that elevations were sampled sufficiently for subsequent analyses. Pupal bivouac temperatures varied significantly less with ambient temperature than did larval bivouac temperatures (Fig. 2A; ANCOVA $F_{1,17} = 6.84$, $p = 0.019$). As ambient temperature decreased at high elevations, mean brood temperature within larval bivouacs decreased ($F = 20.7$, $df = 10$, $p = 0.001$, $R^2 = 0.64$, $T_{larvae} = 10.26 + 0.65 \times T_a$). For larval bivouacs, a slope of 1 was outside of the 95% confidence interval of the observed slope (0.65 ± 0.28), suggesting a thermoregulatory strategy which was intermediate between strict thermoconformation and strict thermoregulation (Fig. 2A). In contrast, across a similar elevation and temperature range, the slope of pupal bivouac temperatures was not significantly different from 0 ($F = 5.22$, $df = 6$, $p = 0.063$, $R^2 = 0.38$), indicating strict thermoregulation of pupal bivouacs (Fig. 2A). Mixed groups of larvae and pupae in transitional bivouacs were thermoregulated similarly to pupae: the slope of the relationship between ambient and mixed brood core temperature was not significantly different from 0 ($F = 3.74$, $df = 7$, $p = 0.094$, $R^2 = 0.26$), suggesting strict thermoregulation of mixed broods when pupae were present.

Bivouac humidity

Ambient relative humidity was significantly higher at high elevations than in the lowland seasonal dry forests of Guanacaste ($F = 50.76$, $df = 19$, $p < 0.001$, $R^2 = 0.71$). Unlike for temperature, both larval and pupal brood cores were both always humid regardless of ambient variation in humidity. Larval and pupal bivouacs did not differ in hygroregulation strategy (ANCOVA, $F_{1,12} < 0.01$, $p = 0.019$) or mean humidity ($F = 1.06$, $df = 1$, $p = 0.321$). The slope between ambient and brood relative humidity was not significantly different from 0 ($F = 0.54$, $df = 13$, $p = 0.474$), suggesting strict hygroregulation. This was also the case in general throughout all regions of the bivouac (Fig. 2C; $F = 1.95$, $df = 18$, $p = 0.18$). Together, this suggests that bivouacs are always strictly humidity-regulated, not only when a particular brood-stage is present, and not only in areas of the bivouac where brood are present. Mean relative humidity in bivouac brood cores ± standard deviation was 93.34
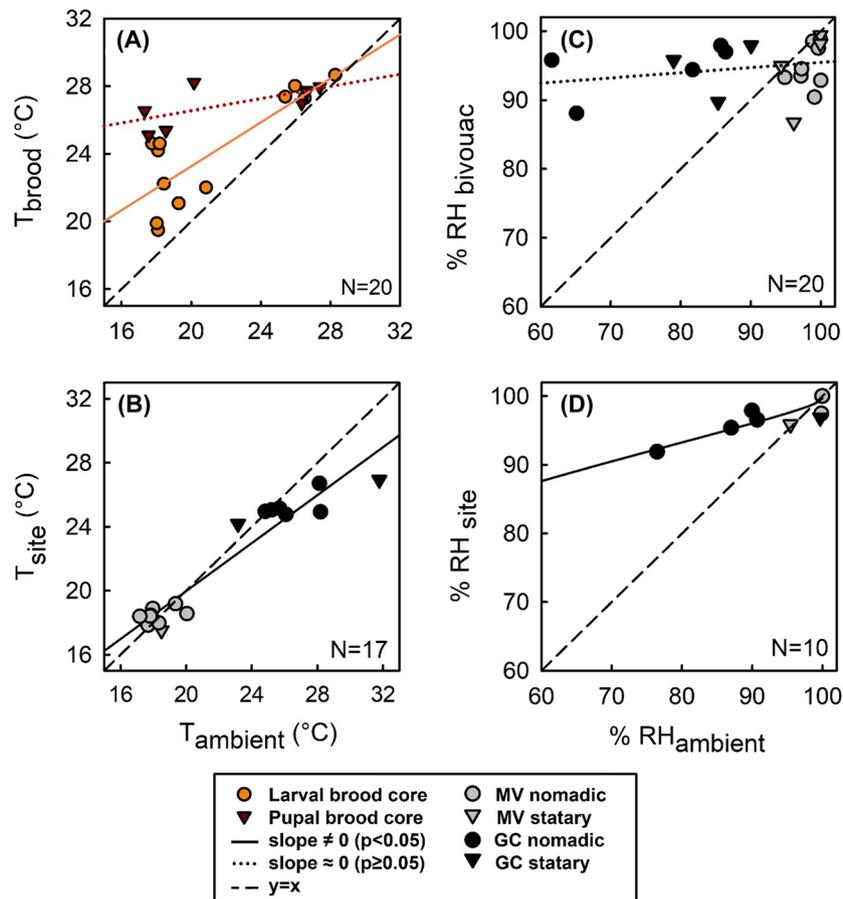
Figure 2. (A) The effect of ambient temperature and brood developmental stage on relative thermoregulation versus thermoconformation. The pupal trend line was not significantly different from 0, indicating strict thermoregulation. (B) Relationship between ambient and empty bivouac site temperature indicating imperfect site-selection associated thermoregulation. (C) Relationship between ambient and bivouac relative humidity indicating strict thermoregulation. The trend was not significantly different from 0, indicating strict hygroregulation. (D) Relationship between ambient and empty bivouac site relative humidity. All points are bivouac means. Grey and black symbols denote high and low elevation sites respectively.

± 5.53% across all sites and ambient conditions sampled. Relative humidity never fell below 86% in any part of any measured bivouac.

Metabolic warming versus site-selection

Metabolic warming of bivouacs increased as ambient temperature decreased at high elevations (Fig. 3; $F = 22.9$, $df = 14$, $p < 0.001$, $R^2 = 0.59$, $T_{warm} = 15.26 - 0.47 \times T_a$). To check whether this pattern was affected by changes in ambient temperature between bivouac data collection days and subsequent site measurement days, we also re-ran the linear regression on a subset ($n = 13$) of bivouac data points for which all temperatures for the bivouacs and the empty sites were time-of-day matched. This analysis yielded a similar relationship between ambient temperature and estimated metabolic heat added by ant presence ($F = 10.50$, $df = 11$, $R^2 = 0.44$, $p = 008$; $T_w = 13.34 - 0.40 \times T_a$). All mean bivouac temperatures were greater than or equal to their corresponding mean empty site temperatures,

providing no evidence of net cooling caused by the ants (Fig. 3; $n = 22$).

Evacuated bivouac site temperature decreased significantly with ambient temperature (Fig. 2B; $F = 142.3$, $df = 14$, $p < 0.001$, $R^2 = 0.90$, $T_{site} = 5.15 + 0.74 \times T_a$), but 95% confidence intervals of the slope did not include 1 (slope $= 0.74 \pm 0.12$), suggesting some degree of imperfect passive thermoregulation was contributed by bivouac site selection by colonies. Relatively warm sites were selected when ambient temperature was low (at high elevations), and relatively cool sites were selected when ambient temperature was high (at low elevations). Humidity of evacuated bivouac sites varied strongly with ambient humidity (Fig. 2D; $F = 15.12$, $df = 8$, $p < 0.005$, $R^2 = 0.61$, $RH_{site} = 70.52 + 0.28 \times RH_a$), but with a slope similarly less than 1 (slope $= 0.28 \pm 0.22$), suggesting imperfect hygroregulation in the form of bivouac site selection. In all cases, humidity of evacuated bivouac site was greater than ambient, but the difference between ambient and bivouac site humidity was far greater in the lowland dry forests of Guanacaste.
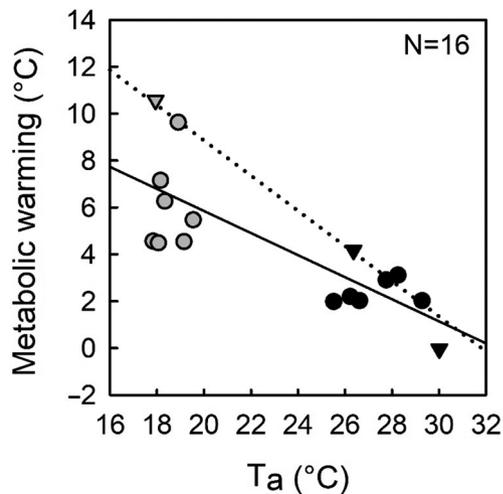
Figure 3. The relationship between estimated metabolic warming and ambient temperature. Black symbols are bivouacs measured in low elevation Guanacaste, grey symbols are bivouacs measured in high elevation Monteverde. The solid line is the trend line of all data. The dotted line is the estimated amount of warming needed to maintain a homeostatic bivouac temperature of 28°C, as was previously thought to be the norm.

Of 103 total bivouacs surveyed, 24.3% were exposed, 28.2% were sheltered, and 47.6% were enclosed. There was no effect of either colony phase or elevation on proportion of bivouacs that were exposed, sheltered or enclosed (Fig. 4; $X^2 = 2.85$, df = 6, p = 0.83). However, bivouacs were more frequently subterranean in low-elevation Guanacaste compared to Monteverde (Fig. 4; $X^2 = 10.16$, df = 1, p = 0.001). Frequency of subterranean bivouacs did not differ between nomadic and statary phases ($X^2 = 0.02$, df = 1, p = 0.876).

Colony-level traits and brood depth

In this study, individual colonies of army ants were often observed across emigrations, forming multiple bivouacs in different sites. Colony ID was not a significant predictor of internal bivouac conditions or empty bivouac site (site selection) conditions (p > 0.05 for all models), and so Colony ID was excluded as a predictor in the main analyses presented here. Notably, this also indicates that factors associated with colony ID such as age, colony personality, and colony size were therefore not major factors in the patterns presented here. Depth of the most superficial brood did not significantly differ across sites or brood type (ANOVA, $F_{3, 29} = 1.92$, p = 0.148), suggesting that the demonstrated difference between brood temperatures within the bivouac was not a passive result of deeper placement of pupae within the bivouac.

Discussion

Army ant bivouacs functioned as flexible endothermic superorganisms, warming brood with metabolic heat, but
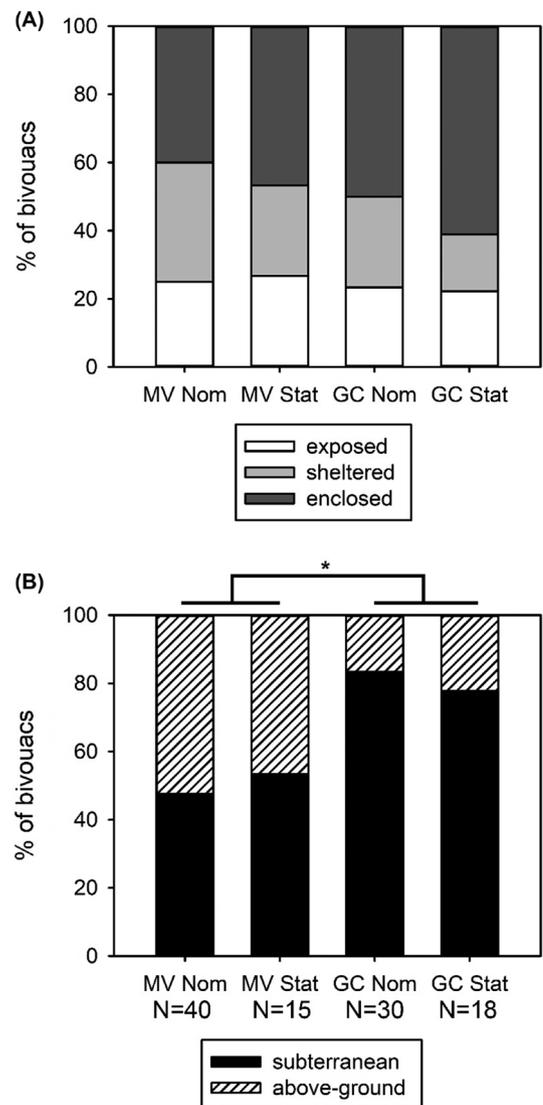
Figure 4. (A) Frequency of bivouacs with three levels of exposure. (B) Frequency of bivouacs above versus below ground across site (MV = Monteverde/high elevation, GC = Guanacaste/low elevation) and colony phase (nomadic versus statary) for 103 bivouacs surveyed in 2015 and 2016.

changing the amount of warming in response to environmental and developmental factors. Bivouacs engaged in brood age-dependent facultative homeothermy to cope with the environmental pressure of low ambient temperatures at high elevations. Contrary to previous assertions, this means that bivouacs were not strict thermoregulators throughout the colony cycle, but rather, functioned with adaptively plastic thermoregulatory strategies. Our data suggest that bivouacs modulated their heat production depending on costs and benefits set by environmental conditions and by the different physiological needs of developing brood. Flexibility in army ant larval development allowed colonies to thermoconform during nomadic phases at high elevations. This colony-level strategy resembles those employed by vertebrates adapted

to highly variable climates (Hayden and Lindberg 1970, Tracy 1977, Puchalski et al. 1988, Bauwens et al. 1999). Collective brood-cooling strategies have also been described for honey bees at high latitudes as an adaptation to seasonally adverse conditions (Winston 1991), and carpenter ants to combat diel variation in temperature (Roces and Núñez 1989). However, unlike these examples high-elevation army ant colonies remained fully active while coping with non-transient low temperatures. This is different from adopting a dormancy-like strategy to deal with temporary adverse conditions. High elevation bivouacs relax thermoregulation during the thermally flexible larval phase and increase warming during the pupal phase as a strategy to survive aseasonally low temperatures associated with tropical high elevations. This strategy is likely an important factor which allows for colonies to be aseasonally active and efficient in a wide variety of thermal environments.

In relatively dry and hot lowland Guanacaste, mean ambient temperature frequently exceeded the putative thermoregulation window of 28.5 ± 1°C for *E. burchellii* bivouacs (Franks 1989), with mean forest floor temperatures as high as 30.5°C in the immediate vicinity of bivouacs. Although bivouacs did not always maintain a narrow homeostatic thermal range across elevations, our data show that bivouac brood cores were rarely hotter than 28–29°C across brood developmental cycles and elevations. The 28–29°C temperature range may be at or near the upper limit for normal brood development in *E. b. parvispinum,* rather than representing a target or ideal developmental temperature window.

Brood developmental stage differences

Hymenopteran pupae are generally more tightly thermoregulated than larvae (Kronenberg and Heller 1982, Ishay and Barenholz-Paniry 1995, Luczynski et al. 2007, Bennett et al. 2015), likely due in part to temperature-sensitive neurological development occurring during the pupal stage (Farris et al. 1999, Jones et al. 2005). Army ants appeared to show differences in thermal flexibility between larval and pupal stages. Support of this is both shown by the tendency of pupal bivouacs to be more tightly thermoregulated than larval bivouacs, and by the tendency of transitional bivouacs (housing both larval and pupal brood) to resemble pupal bivouacs by more tightly thermoregulating the brood core.

Some ant species separate brood stages spatially in order to cater to differences in brood needs (Franks and Sendova-Franks 1992), while army ants separate brood thermoregulation temporally via developmental synchrony. The evolution of developmental synchrony in army ants has been previously assumed to be the result of selection pressure on colony mobility, foraging behavior and nutrition (Schneirla 1971, Kaspari et al. 2011). Our data suggest there is also a thermal benefit of brood synchrony at high elevations. When ambient temperature is low, colonies may conserve energy by reducing collective metabolic heating during the typically 16-d long nomadic phase, when colonies only cater to the thermal needs of larvae. This nomadic phase benefit may partially offset the energetic costs of large increases in metabolic heating of pupae at high elevations. This energy-conservation strategy bears loose resemblance to fire ant colonies, which are hypothesized to reduce colony temperature during times of colony famine to reduce energetic expenditure (Porter and Tschinkel 1993).

The effect of low temperatures on army ant larval physiology remains unstudied. Workers of other ant species have been found to selectively expose larvae to cold temperatures when food availability is low in order to slow brood metabolic demand when there is less food available (Roces and Núñez 1989, Porter and Tschinkel 1993). Decreased temperatures during development increase time to maturity among many insects (Williams 1946, Porter 1988, Abril et al. 2010). We could not measure enough full-length developmental cycles of the colonies in this study to test whether high elevation colonies have slower development time as a repercussion of having cooler larvae, but lengthened colony cycles, particularly nomadic phases, are a possible cost of facultative endothermy at high elevations.

Metabolic warming versus site selection effects

The magnitude of net metabolic warming performed collectively by ants within bivouacs was highly responsive to ambient temperature and increased at cool high elevation sites. Across the ambient temperature range measured in this study, mean bivouac temperature was modified from that of the empty bivouac site by as little as 0°C and as much as 10°C. The negligible net warming attributable to ant presence in some lowland bivouacs does not support the prediction that army ants have a baseline obligate warming load which cannot be mitigated by the colony (Franks 1989). Mechanisms involved in this responsive and variable net warming might include plastic modification of bivouac density or convection of the individual workers composing the bivouac, however these behaviors were not explored in this study. While bivouacs were able to substantially reduce their net warming, we observed no signs of net cooling attributable to ant presence even when ambient forest floor temperature was high. This suggests that passive mechanisms such as site selection are the major driver for bivouac cooling in environments where mean ambient temperature is greater than 29°C.

We show support for the hypothesis that bivouac site selection buffers elevational variation in temperature, previously demonstrated at high elevations (Soare et al. 2011). Selected lowland bivouac sites were on average cooler than ambient, while bivouac sites warmer than ambient were selected at high elevations. Though site selection did contribute to bivouac warming at high elevations in our study, the warming effect was small relative to the larger role that worker metabolic heating played in overall bivouac warming. More striking was the substantial contribution of cool bivouac site selection when temperatures were high in lowland Guanacaste. Cool bivouac site selection is likely a major factor in colony avoidance of extreme ambient heat events in lowland seasonal dry forests.

Our survey did not support the hypothesis that bivouacs are more exposed and aboveground at low elevations to enable convective cooling (Soare et al. 2011). Bivouacs of *E. b. parvispinum* in high elevation Monteverde were less likely to be underground than in lowland Guanacaste, and bivouacs were seldom exposed regardless of elevation. These differences in bivouacking preference may be a fixed trait in *E. b. parvispinum*, as it is a monophyletic lineage that is the sister to all other *E. burchellii* subspecies (Winston et al. 2017), often occupying more thermally variable (seasonal) environments (Watkins 1976). Lowland secondary forests of Guanacaste had larger diel fluctuations in ambient relative humidity and temperature than did Monteverde. Soil buffering plays an important role in shielding underground bivouacs of other army ants from extreme temperatures associated with diel thermal fluctuations (Baudier and O'Donnell 2016), and may be used facultatively by *E. b. parvispinum* for this purpose. The tendency to bivouac belowground in lowland Guanacaste may better shield from desiccation during daytime heat spikes during which desiccation is also a potential risk.

Hygroregulation

Bivouacs in dry and hot Guanacaste kept the brood similarly moist as in high elevation cloud forest habitats, despite there being almost 50% difference in daily relative humidity lows between the two sites. Much of this hygroregulation appears to be accomplished via moist site selection, but the strictest homeostatic humidities of 93% and above were only observed when ants were present, suggesting that the microclimate of the bivouac itself contributes to the hygroregulation of brood as well.

Non-climatic factors

Although the focus of this study has been the climatic factors associated with bivouac homeostasis, there are several other phase-dependent differences between nomadic and statary bivouacs which are worth discussing in the new framework of these findings. First, unlike statary bivouacs, nomadic bivouacs maintain larval brood that must be fed. Nomadic bivouacs therefore bear the burden of increased raiding, emigration, and brood-feeding behaviors, which are all metabolically driven. When raid parties depart from the bivouac, overall bivouac size decreases due to the departure of the ants composing outer layers of the bivouac (unpubl.). At high elevations this might be predicted to cause a reduction in the ability of nomadic bivouacs to thermoregulate as a passive consequence of the need to forage. However, this does not appear to be the case, given that frequently-foraging transitional bivouacs containing both pupal and larval broods were capable of strict thermoregulation similar to statary pupal bivouacs. Similarly, these results suggest that the act of feeding larval broods does not inherently compromise the thermoregulatory ability of the bivouac, since mixed-brood bivouacs were able to maintain homeostasis while also feeding larvae. The act of emigration, however, intermittently exposes brood (mostly larvae) to low-temperature ambient conditions at night (O'Donnell et al. 2009). The developmental effects of emigration-associated thermal variation were not investigated in this study but have the potential to be an important selective pressure for larval ability to thrive at low temperatures.

Climate change implications

Dry forest populations of *E. b. parvispinum* in Guanacaste may particularly be at risk of extirpation with increased mean temperatures, as evidenced by their inability to actively cool bivouacs, and their apparent reliance on availability of cool bivouac sites in these environments. As temperatures in lowland forests increase, availability of cool potential bivouac sites will likely be reduced. Conversely, at high elevations increasing temperatures may trigger upwards elevational shifts into habitats previously uninhabited by forest-floor carpet raiding species, facilitated by increase in available warm bivouac sites and higher environmental temperatures overall.

Along with altering a single species' interaction with its abiotic environment, directional climate change also changes interspecific interactions in biological communities (Diamond et al. 2016). This is especially pertinent in communities where army ants occur, as *Eciton burchellii* is host to the most complex set of animal associations known to science (Rettenmeyer et al. 2011). Inquilines living within bivouacs can be more thermally sensitive to extreme temperature than their foraging ant hosts (Baudier and O'Donnell 2016). These complex communities are therefore at risk of both direct negative effects due to extirpation of *E. b. parvispinum* from its historic range but may also be susceptible to costs incurred prior to extirpation, depending on specific breadths of thermal performance. Conversely, the expansion of surface-raiding army ants to higher elevations within Costa Rica would place a major predatory pressure on surface-dwelling insect communities and may also introduce in-tow a variety of ant-following avian kleptoparasites to ecosystems previously void of such species (O'Donnell 2017). Sky-island insect communities having evolved in lieu of these predation pressures could be irreversibly altered as a consequence.

References

Abril, S. et al. 2010. Effect of temperature on the development and survival of the Argentine ant, *Linepithema humile*. – J. Insect Sci. 10: 97.

Angilletta, M. J. 2009. Thermal adaptation: a theoretical and empirical synthesis. – Oxford Univ. Press.

Angilletta Jr, M. J. et al. 2002. The evolution of thermal physiology in ectotherms. – J. Therm. Biol. 27: 249–268.

Baudier, K. M. and O'Donnell, S. 2016. Structure and thermal biology of subterranean army ant bivouacs in tropical montane forests. – Insectes Soc. 63: 467–476.

Baudier, K. M. et al. 2018a. Extreme insolation: climatic variation shapes the evolution of thermal tolerance at multiple scales. – Am. Nat. 192: 347–359.

Baudier, K. M. et al. 2018b. Data from: Plastic collective endothermy in a complex animal society (army ant bivouacs: *Eciton burchellii parvispinum*). – Dryad Digital Repository, <http://dx.doi.org/10.5061/dryad.vk13np4>.

Bauwens, D. et al. 1999. Field body temperatures, activity levels and opportunities for thermoregulation in an extreme microhabitat specialist, the girdled lizard (*Cordylus macropholis*). – J. Zool. 249: 11–18.

Becher, M. A. et al. 2009. Pupal developmental temperature and behavioral specialization of honeybee workers (*Apis mellifera* L.). – J. Comp. Physiol. A 195: 673–679.

Bennett, M. M. et al. 2015. Exposure to suboptimal temperatures during metamorphosis reveals a critical developmental window in the solitary bee, *Megachile rotundata*. – Physiol. Biochem. Zool. 88: 508–520.

Cook, C. N. et al. 2016. Larvae influence thermoregulatory fanning behavior in honeybees (*Apis mellifera* L.). – Insectes Soc. 63: 271–278.

Diamond, S. E. et al. 2016. Climatic warming destabilizes forest ant communities. – Sci. Adv. 2: e1600842.

Ellis, M. B. et al. 2008. Hygropreference and brood care in the honeybee (*Apis mellifera*). – J. Insect Physiol. 54: 1516–1521.

Farris, S. et al. 1999. Larval and pupal development of the mushroom bodies in the honey bee, *Apis mellifera*. – J. Comp. Neurol. 414: 97–113.

Franks, N. R. 1989. Thermal regulation in army ant bivouacs. – Physiol. Entomol. 14: 397–404.

Franks, N. R. and Sendova-Franks, A. B. 1992. Brood sorting by ants: distributing the workload over the work-surface. – Behav. Ecol. Sociobiol. 30: 109–123.

Gotwald Jr, W. H. 1995. Army ants: the biology of social predation. – Cornell Univ. Press.

Groh, C. et al. 2004. Synaptic organization in the adult honey bee brain is influenced by brood-temperature control during pupal development. – Proc. Natl Acad. Sci. USA 101: 4268–4273.

Hayden, P. and Lindberg, R. 1970. Hypoxia-induced torpor in pocket mice (genus: *Perognathus*). – Comp Biochem. Physiol. 33: 167–179.

Heinrich, B. 1975. Thermoregulation in bumblebees. – J. Comp. Physiol. B 96: 155–166.

Human, H. et al. 2006. Do honeybees, *Apis mellifera scutellata*, regulate humidity in their nest? – Naturwissenschaften 93: 397–401.

Ishay, J. S. and Barenholz-Paniry, V. 1995. Thermoelectric effect in hornet (*Vespa orientalis*) silk and thermoregulation in a hornet's nest. – J. Insect Physiol. 41: 753–759.

Jackson, W. B. 1957. Microclimatic patterns in the army ant bivouac. – Ecology 38: 276–285.

Jay, S. C. 1963. The development of honeybees in their cells. – J. Apic. Res. 2: 117–134.

Jones, J. C. and Oldroyd, B. P. 2006. Nest thermoregulation in social insects. – Adv. Insect Physiol. 33: 153–191.

Jones, J. C. et al. 2004. Honey bee nest thermoregulation: diversity promotes stability. – Science 305: 402–404.

Jones, J. C. et al. 2005. The effects of rearing temperature on developmental stability and learning and memory in the honey bee, *Apis mellifera*. – J. Comp. Physiol. A 191: 1121–1129.

Kaspari, M. et al. 2011. Predation and patchiness in the tropical litter: do swarm-raiding army ants skim the cream or drain the bottle? – J. Anim. Ecol. 80: 818–823.

Kronenberg, F. and Heller, H. C. 1982. Colonial thermoregulation in honey bees (*Apis mellifera*). – J. Comp. Physiol. B 148: 65–76.

Kumar, A. and O'Donnell, S. 2009. Elevation and forest clearing effects on foraging differ between surface – and subterranean – foraging army ants (Formicidae: Ecitoninae). – J. Anim. Ecol. 78: 91–97.

Lazaridis, M. 2011. First principles of meteorology and air pollution. – Springer.

Li, Z. et al. 2016. Drone and worker brood microclimates are regulated differentially in honey bees, *Apis mellifera*. – PLoS One 11: e0148740.

Lindauer, M. 1955. The water economy and temperature regulation of the honeybee colony. – Bee World 36: 81–92.

Liu, Y. et al. 2005. Effects of temperature and nutrition on juvenile hormone titers of *Coptotermes formosanus* (Isoptera: Rhinotermitidae). – Ann. Entomol. Soc. Am. 98: 732–737.

Luczynski, A. et al. 2007. Influence of cold storage on pupal development and mortality during storage and on post-storage performance of *Encarsia formosa* and *Eretmocerus eremicus* (Hymenoptera: Aphelinidae). – Biol. Control 40: 107–117.

MacArthur, R. H. 1972. Geographical ecology: patterns in the distribution of species. – Princeton Univ. Press.

O'Donnell, S. 2017. Evidence for facilitation among avian army-ant attendants: specialization and species associations across elevations. – Biotropica 49: 665–674.

O'Donnell, S. et al. 2009. Species and site differences in Neotropical army ant emigration behavior. – Ecol. Entomol. 34: 476–482.

O'Donnell, S. et al. 2011. Elevational and geographic variation in army ant swarm raid rates. – Insectes Soc. 58: 293–298.

Penick, C. A. and Tschinkel, W. 2008. Thermoregulatory brood transport in the fire ant, *Solenopsis invicta*. – Insectes Soc. 55: 176–182.

Penick, C. A. et al. 2017. Beyond thermal limits: comprehensive metrics of performance identify key axes of thermal adaptation in ants. – Funct. Ecol. 31:1091–1100.

Porter, S. D. 1988. Impact of temperature on colony growth and developmental rates of the ant, *Solenopsis invicta*. – J. Insect Physiol. 34: 1127–1133.

Porter, S. D. and Tschinkel, W. R. 1993. Fire ant thermal preferences: behavioral control of growth and metabolism. – Behav. Ecol. Sociobiol. 32: 321–329.

Puchalski, W. et al. 1988. Photoperiod, temperature and melatonin effects on thermoregulatory behavior in Djungarian hamsters. – Physiol. Behav. 42: 173–177.

Rettenmeyer, C. W. 1963. Behavioral studies of army ants. – Univ. Kansas Sci. Bull. 44: 281–465.

Rettenmeyer, C. W. et al. 2011. The largest animal association centered on one species: the army ant *Eciton burchellii* and its more than 300 associates. – Insectes Soc. 58: 281–292.

Roces, F. and Núñez, J. A. 1989. Brood translocation and circadian variation of temperature preference in the ant *Camponotus mus*. – Oecologia 81: 33–37.

Ruf, T. and Geiser, F. 2015. Daily torpor and hibernation in birds and mammals. – Biol. Rev. 90: 891–926.

Schneirla, T. C. 1971. Army ants: a study in social organization. – W. H. Freeman.

Schneirla, T. C. et al. 1954. The bivouac or temporary nest as an adaptive factor in certain terrestrial species of army ants. – Ecol. Monogr. 24: 269–296.

Soare, T. W. et al. 2011. Choice of nest site protects army ant colonies from environmental extremes in tropical montane forest. – Insectes Soc. 58: 299–308.

Teles da Silva, M. 1977. Behaviour of the army ant *Eciton burchelli* Westwood (Hymenoptera: Formicidae) in the Belém region. I. Nomadic-statary cycles. – Anim. Behav. 25: 910–923.

Teles da Silva, M. 1982. Behaviour of army ants *Eciton burchelli* and *E. hamatum* (Hymenoptera, Formicidae) in the Belem region III. Raid activity. – Insectes Soc. 29: 243–267.

Tracy, C. R. 1977. Minimum size of mammalian homeotherms: role of the thermal environment. – Science 198: 1034–1035.

Watkins, J. F. 1976. The identification and distribution of New World army ants (Dorylinae: Formicidae). – Markham Press Fund of Baylor Univ. Press.

Williams, C. M. 1946. Physiology of insect diapause: the role of the brain in the production and termination of pupal dormancy in the giant silkworm, *Platysamia cecropia*. – Biol. Bull. 90: 234–243.

Winston, M. E. et al. 2017. Early and dynamic colonization of Central America drives speciation in Neotropical army ants. – Mol. Ecol. 26: 859–870.

Winston, M. L. 1991. The biology of the honey bee. – Harvard Univ. Press.

Supplementary material (Appendix ECOG-04064 at <www.ecography.org/appendix/ecog-04064>). Appendix 1.